

# Screening for resistance to sigatoka (*Pseudocercospora fijiensis*)

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## Introduction

Banana, and especially the cooking type known as East Africa highland banana, is a vital staple food and income crop for over 80 million people in East Africa. However, productivity has been declining, partly due to diseases such as Sigatoka leaf diseases (Kimunye *et al.*, 2020).

Sigatoka disease of banana is a foliar disease caused by fungi of the genus *Pseudocercospora* (previously called *Mycosphaerella*). Species associated with Sigatoka disease include; *P. fijiensis*, causal agent of black Sigatoka, *P. musicola* of yellow Sigatoka (Stover and Simmonds, 1987), and *P. eumusae* of Eumusae leaf spot (Carlier *et al.*, 2000), Chang *et al.*, 2016). Yellow Sigatoka symptoms are characterised by oval to round necrotic lesions, which first appear pale yellow on the lower surface of the leaf, and this differentiates it from black Sigatoka at early stages of lesion development (e.g., Stages 1 and 2).

Damage caused by *Pseudocercospora* species is manifested as necrotic leaf lesions that reduce the functional leaf area and photosynthetic capacity, resulting in reduced crop yield and fruit quality leading to banana yield losses ranging from 20-50% (Carlier *et al.*, 2000). Long-distance spread of the pathogen is through infected planting material (suckers) and leaves, which are often used as packaging materials in developing countries. Disease dispersal and development is favoured by high moisture and humidity and the disease is distributed in all farms and in all cultivars in Banana growing regions and districts of East Africa (Kimunye *et al.*, 2020).

Sigatoka disease is managed on farms through pruning infected leaves, reducing plant density and planting barriers/wind breakers around plantations. However, these methods are labour intensive and are not very effective in controlling the disease. Biocontrol through use of biopesticides has been tried however, biopesticides are very slow and inefficient. Use of resistant cultivars through breeding is the most cost effective and sustainable solution to manage sigatoka. Breeding for resistant cultivars requires identification of resistant genotypes that are used as parents and how this resistance is passed onto the progeny. With this information, we can now find out how to introgress resistance into susceptible genotypes.

The purpose of this SOP is to provide guidance for the breeding program of bananas in assessment of genetic resistance to sigatoka disease.

## Materials and methods

### Plant materials

- 1) TC generated plant material including:
  - a) Test genotypes (Parental genotypes and newly developed hybrids)
  - b) Resistant checks:
    - i. Calcutta 4
  - c) land race controls
    - i. TM-28 OBINO LEWAI
    - ii. Mchare
    - iii. Mbwazirume

### Other materials (For screenhouse evaluations)

1. Sterile forest soil
2. Sterile sand
3. 13-liter plastic pots
4. Watering cans
5. Sigatoka cultures

### Experimental design

A partially replicated (P-Rep) design for both screen house and field evaluations will be adopted. The experiment on screening the banana genotypes for resistance to sigatoka disease will be carried out in the field where natural infestation is highest.

### Parental genotypes

Partially replicated experimental design (P-Rep) with three blocks will be adopted with each parental genotype occurring in duplicate and the checks in triplicate for the entire experimental set up. Each plot will constitute three plants per genotype and spreader rows will be included every after two rows of parental genotypes including checks and also as borders. P-rep designs are useful when plant materials and space are limiting factors. In fact, P-rep

2829-62	222K-1	PITA 7 (1 1154K-1	D0128	TMB2x 31	Pizang Lilin	TM-28 O	2905	Mbwazirume	Mbwazirume	716	376K-7	TM-28 O	TMB2x 31	PITA 1 (54	402	Calcutta 4	PITA 17	293643	
2829-62	222K-1	PITA 7 (1 1154K-1	D0128	TMB2x 31	Pizang Lilin	TM-28 O	2905	Mbwazirume	Mbwazirume	716	376K-7	TM-28 O	TMB2x 31	PITA 1 (54	402	Calcutta 4	PITA 17	293643	
2829-62	222K-1	PITA 7 (1 1154K-1	D0128	TMB2x 31	Pizang Lilin	TM-28 O	2905	Mbwazirume	Mbwazirume	716	376K-7	TM-28 O	TMB2x 31	PITA 1 (54	402	Calcutta 4	PITA 17	293643	
1297-3	Mchare	401K-1	Kunnan	PITA 8 (1 5610S-1	PITA 18 (1	TMB2x 31	Mchare	PITA 14 (1	Maleb	29275S-4	PITA 4 (1	Truncata	TMB2x 54	Kokopo	Galeo	201071K	Enirabahima		
1297-3	Mchare	401K-1	Kunnan	PITA 8 (1 5610S-1	PITA 18 (1	TMB2x 31	Mchare	PITA 14 (1	Maleb	29275S-4	PITA 4 (1	Truncata	TMB2x 54	Kokopo	Galeo	201071K	Enirabahima		
1297-3	Mchare	401K-1	Kunnan	PITA 8 (1 5610S-1	PITA 18 (1	TMB2x 31	Mchare	PITA 14 (1	Maleb	29275S-4	PITA 4 (1	Truncata	TMB2x 54	Kokopo	Galeo	201071K	Enirabahima		
1201K-1	PITA 3 (55	29275S-4	Calcutta 4	25291-34	PITA 11 (26	SH 3217	Kazazka	25447-37	Guyod	IITA hybr	25374S-11	SH 3362	Kwaro	Hung Tu	cv. Rose	PITA 5 (2	1747K-1	Enirabahima	
1201K-1	PITA 3 (55	29275S-4	Calcutta 4	25291-34	PITA 11 (26	SH 3217	Kazazka	25447-37	Guyod	IITA hybr	25374S-11	SH 3362	Kwaro	Hung Tu	cv. Rose	PITA 5 (2	1747K-1	Enirabahima	
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Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	
PITA 17 (4	Pizang Lilin	SH 3217	25374S	1747K-1	201087K-3	2905	TMB2x 31	PITA 11 (2	IITA hybr	Borneo	Mbwazirume	25291-34	1448-1	317K-2	1411K-1	1154K-1	Calcutta 4	Hung Tu	Kazazka
PITA 17 (4	Pizang Lilin	SH 3217	25374S	1747K-1	201087K-3	2905	TMB2x 31	PITA 11 (2	IITA hybr	Borneo	Mbwazirume	25291-34	1448-1	317K-2	1411K-1	1154K-1	Calcutta 4	Hung Tu	Kazazka
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Mwitu Per	402	10963S-1	TMB2x 31	25447-37	TMB2x 31	SH 3142	Maleb	PITA 6 (4	317K-1	Bankii 8	222K-1	376K-1	Calcutta 4	201087K-	246K-1	376K-7	PITA 18 (1	Enirabahima	
Mwitu Per	402	10963S-1	TMB2x 31	25447-37	TMB2x 31	SH 3142	Maleb	PITA 6 (4	317K-1	Bankii 8	222K-1	376K-1	Calcutta 4	201087K-	246K-1	376K-7	PITA 18 (1	Enirabahima	
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PITA 4 (5)	Galeo	660K-1	29275S	TM-28 C	TM-28 OE	Kokopo	Mchare	PITA 2 (5	PITA 12 (1	Pallama	Mchare	Mbwazirume	Zabrina G	TMB2x 8	Pahang	TMB2x 5	Calcutta 4	Enirabahima	
PITA 4 (5)	Galeo	660K-1	29275S	TM-28 C	TM-28 OE	Kokopo	Mchare	PITA 2 (5	PITA 12 (1	Pallama	Mchare	Mbwazirume	Zabrina G	TMB2x 8	Pahang	TMB2x 5	Calcutta 4	Enirabahima	
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Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	Enirabahima	
1201K-1	Kunnan	1297-3	PITA 3	TMB2x 31	Calcutta 4	716	PITA 5 (1	317K-1	201087K-	PITA 2 (1	Pallama	29364S-	201087K-	1448-1	Mbwazirume	1411K-1	PITA 12 (6	PITA 7	317K-2
1201K-1	Kunnan	1297-3	PITA 3	TMB2x 31	Calcutta 4	716	PITA 5 (1	317K-1	201087K-	PITA 2 (1	Pallama	29364S-	201087K-	1448-1	Mbwazirume	1411K-1	PITA 12 (6	PITA 7	317K-2
1201K-1	Kunnan	1297-3	PITA 3	TMB2x 31	Calcutta 4	716	PITA 5 (1	317K-1	201087K-	PITA 2 (1	Pallama	29364S-	201087K-	1448-1	Mbwazirume	1411K-1	PITA 12 (6	PITA 7	317K-2
29275S-4	246K-1	401K-1	Bankii	Truncata	PITA 14 (7	PITA 1 (5	376K-1	TMB2x 6	PITA 6 (4	10963S-1	Mbwazirume	29275S-1	201071K	Mwitu Per	660K-1	Calcutta 4	Kwaro	Enirabahima	
29275S-4	246K-1	401K-1	Bankii	Truncata	PITA 14 (7	PITA 1 (5	376K-1	TMB2x 6	PITA 6 (4	10963S-1	Mbwazirume	29275S-1	201071K	Mwitu Per	660K-1	Calcutta 4	Kwaro	Enirabahima	
29275S-4	246K-1	401K-1	Bankii	Truncata	PITA 14 (7	PITA 1 (5	376K-1	TMB2x 6	PITA 6 (4	10963S-1	Mbwazirume	29275S-1	201071K	Mwitu Per	660K-1	Calcutta 4	Kwaro	Enirabahima	
TM-28 OE	SH 3362	Pahang	365K-1	TMB2x 31	Mchare	1438K-1	TM-28 O	D0128	2829-62	29275S-4	5610S-1	cv. Rose	Calcutta 4	Borneo	Mchare	Guyod	Zabrina G	Enirabahima	
TM-28 OE	SH 3362	Pahang	365K-1	TMB2x 31	Mchare	1438K-1	TM-28 O	D0128	2829-62	29275S-4	5610S-1	cv. Rose	Calcutta 4	Borneo	Mchare	Guyod	Zabrina G	Enirabahima	
TM-28 OE	SH 3362	Pahang	365K-1	TMB2x 31	Mchare	1438K-1	TM-28 O	D0128	2829-62	29275S-4	5610S-1	cv. Rose	Calcutta 4	Borneo	Mchare	Guyod	Zabrina G	Enirabahima	

designs allow for repeated trial evaluations at different locations. The experimental design for the 74 parental genotypes including checks is shown in Figure 1 below. The average efficiency 0.99 indicating that the design is optimal.

**Figure 1.** P-rep lay out for Sigatoka disease screening for the 74 parental genotypes in white background and checks and controls in blue background. The red background represents the spreader rows.

### **Hybrids**

The augmented design resulting into an incomplete block design where standard checks are replicated in each block and test genotypes not replicated will be adopted for the hybrids. Such augmented designs are very useful where very large numbers of hybrids are produced with limited planting materials and reduced space.

### **Evaluations**

1. Tissue culture derived plants of different genotypes are left in the nursery under humid chamber for 4 weeks and hardened under shade in the nursery for an extra four weeks.
2. From nursery, the plants are taken to the screen house or field where they are screened for resistance to sigatoka disease.

### **SCREEN HOUSE EVALUATIONS**

1. Screen house evaluations may sometimes result in resistance phenotypes that are not true-to-type; due to differences in the leaf physiology between *invitro* plantlets and adult plants.
2. Screen house evaluation decreases the effect of the environment in the host-parasite interactions leading to accurate and reproducible data.
3. Screen house evaluation also allows the observation of disease development stages in relation to the development of the plant; all the stages of development of monocyclic infection are reproduced while accounting for the physiology of banana leaves.

### **Mycelial suspension**

1. From an actively growing monosporic culture on PDA, scrap mycelia directly into a sterile mortar with a surgical blade.

2. Grind in 5 ml sterile distilled water with a drop of 0.05% Tween 20 into a fine suspension. Filter the suspension through two layers of cheesecloth to get rid of media debris.
3. Quantify the suspension using a haemocytometer.
4. Alternatively, harvested mycelia can be weighed on a balance, and fragmented by blending in sterile distilled water.

### **Spore suspension**

1. Add 3 ml of sterile distilled water with a drop of 0.05% Tween 20 to a sporulating culture (sporulating colonies appear pinkish).
2. Dislodge the spores by lightly brushing on the surface of the colony.
3. In case mycelial fragments have been dislodged too, sieve the suspension through double layers of cheesecloth.
4. Quantify using a haemocytometer under a microscope and adjust to required concentrations using sterile distilled water.
5. Prior to inoculation, add 1%v/v Triton X-100 to the inoculum suspension to enable mycelia and spores to adhere to the leaf surface.

### **Artificial inoculation of banana plants** (Abadie *et al.*, 2008)

1. Inoculate the youngest leaf using an artist air spray brush (normally used for painting), using a spore suspension of  $1 \times 10^5$  spores /ml.
2. Spray approximately 1 ml of suspension on the lower surface of the youngest leaves using a microsprayer
3. Alternatively, use a painters' brush / camel brush to evenly spread the suspension on the leaf surface.
4. For *P. fijiensis*, inoculate the axial (adaxial and abaxial) part of the leaf, whereas for the other *Pseudocercospora* spp., inoculate the upper leaf area (adaxial).
5. Allow the inoculated area to dry for 1-2 hrs, and then place plants into a humid chamber with 100% humidity for 48-96 hrs.
6. Move the plants into the screen house and monitor plants for infection by studying the inoculated leaves for symptoms on a 5-day interval.

### **Data collection**

For screen house evaluations, first symptoms usually appear 10-15 days after inoculation. The time from the first to final lesions differs from 20-25 days for a susceptible plant and 60-70 days for a resistant cultivar.

Data captured includes:

1. Incubation time.
2. Latent period (time taken by fungus to start producing mature pseudothecia and ascospores)
3. Disease severity after Gauhl scoring scale (Table 1).

4. Symptom evolution time (Time from appearance of first symptoms to development of mature spots).
5. Symptom stages (Stover and Simmonds, 1987)

**Table 1.** Disease Severity after Gauhl scoring scale (Leiva-Mora et al., 2015)

Rating	Description
<b>0</b>	No visible symptoms of the disease
<b>1</b>	Less than 1% (only streaks or up to ten spots of the leaf with
<b>2</b>	disease symptoms)
<b>3</b>	1 to 5% of the leaf area with symptoms
<b>4</b>	6 to 15% of the leaf area with symptoms
<b>5</b>	16 to 33% of leaf area with symptoms
<b>6</b>	34 to 50% Of the leaf area with symptoms
	51 to 100% of the leaf area with symptoms

## FIELD EVALUATION

1. The experimental fields are be established in areas where the disease pressure is high for natural infection.
2. There should be a 2.5 m space between plants in each row and 3 m between rows.
3. The trials should be managed according to the local farmers' agronomic practices. Leaf spot diseases should not be controlled.
4. Proper management practices regarding nutrition, weed control and irrigation should be carried out in the experimental field.
5. The data should be collected for two successive cycles (on the mother plant and first sucker).

The evaluation of resistance begins three months after planting and data is collected every three months at flowering and at harvest. Every test plant, except the extra plants at the ends of rows, should be used for data collection.

The following parameters are used to assess the reaction of banana genotypes to sigatoka disease

1. Total number of standing leaves (NSL),
2. Total number of functional leaves (NFL),
3. Youngest leaf with streaks (YLStr),
4. Youngest leaf spotted (YLS),
5. Disease severity index
  - Disease severity is the amount of leaf area affected by *Pseudocercospora* leaf spots and can be expressed in disease grades or in percentage.
  - Leaves should be graded using Gauhl's modification of Stover's severity score system.
    - 0 Healthy
    - 1 < 1% leaf area affected (streaks and until 10 spots)

- 2 Until 5% of necrotic area
  - 3 Between 6 and 15% of necrotic area
  - 4 Between 16 and 33% of necrotic area
  - 5 Between 34 and 50% of necrotic area
  - 6 > than 51% of necrotic area
- Assessments should be carried out monthly from third month after planting until harvest. The following data should also be recorded:
  - Disease grades should be recorded for each leaf on each test plant. Only upright leaves should be recorded (with petioles upright).
6. After disease severity has been recorded, the infection index for each test plant should be calculated following the formula:
- Disease severity index: =  $[\sum nb / (N-1) T] \times 100$   
 Where, n = number of leaves in each grade  
 b = grade  
 N = number of grades used in the scale  
 T = total number of leaves scored
7. Area under the curve of progress of the disease (Campbell and Madden, 1991)  
 AUDPC =  $S[(x_i + 1 + x_i)(t_{i+1} + t_i)/2]$  where:  
 x<sub>i</sub> = proportion of disease in the i<sup>th</sup> counterparty observation  
 t<sub>i</sub> = time in the i<sup>th</sup> counterparty observation  
 I = from 1 to N
8. Proportion of healthy leaves and Index of non-spotted leaves (INSL). This is estimated as the proportion (as decimal or percentage) of leaves rated in grade 0 of the total leaves assessed. It can be also estimated as an Index of non-spotted leaves (INSL), from YLS values obtained in the assessment by the following formula:  $INSL = (YLS-1)/NL$   
 where: NL: T = total number of leaves scored
9. Disease development time (DDT)
10. Symptom evolution time, estimated by the formula YLStr
11. Transition period from streaks to spots (Churchill, 2011, Pérez-Vicente *et al.*, 2021.)

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