Genotypic variation of Banana response to the fungal pathogen *Mycosphaerella*

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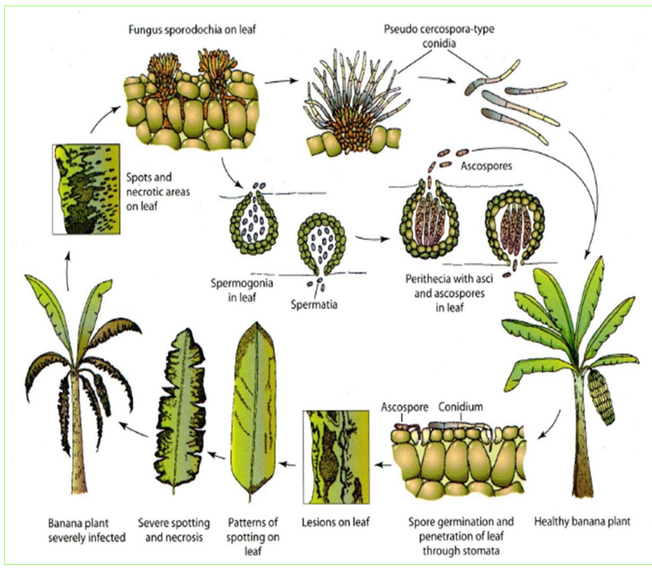
CONTEXT & OBJECTIVES

Most dessert and cooking bananas (*Musa* spp), whether cultivated for local production or for export, are very susceptible to leaf diseases caused by *Mycosphaerella* spp., a hemibiotrophic ascomycete. *M. musicola* affects all production areas worldwide. *M. fijiensis*, the most damaging species, is gradually taking over; it arrived in Martinique in 2010 and this year (2012) in Guadeloupe.

The only control method currently available is frequent fungicide applications. Although such control in the French West Indies (FWI) is supervised as part of a bioclimatic early warning system, some cases of resistance to fungicides have appeared (de Lapeyre *et al.*, 2009). In addition, the use of fungicides pollutes the environment, which is harmful to the tourist industry (important in the FWI) and it remains expensive for producers.

Despite its economic importance (considered as one of 7 most serious biological threats to food security by Pennisi, Science 2010), little is known about the physiological events occurring during the pathogen's life cyle in the plant. To learn more and support the CIRAD breeding program that aims at creating new banana cultivars resistant to *Mycosphaerella* spp., **the objectives** of the study are to:

- **develop a phenotyping tool for easier monitoring of the banana /*Mycosphaerella* interaction,**
- **carry out the first analysis of transcriptome changes in a preliminary RNA-Seq experiment.**

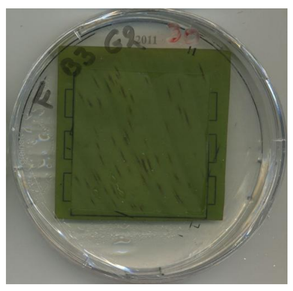


Life cycle of *Mycosphaerella fijiensis*
From Agrios, George N. 2005.

METHODS & RESULTS

a bioassay based on detached leaves phenotyped with an image analysis software

1. To get an easier and reliable quantification of the interaction output with a bioassay based on detached leaves maintained *in vitro* in controlled conditions.

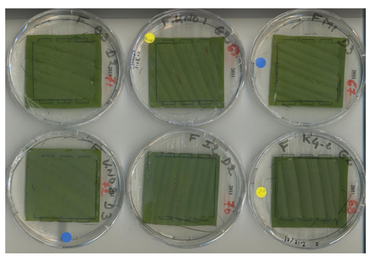


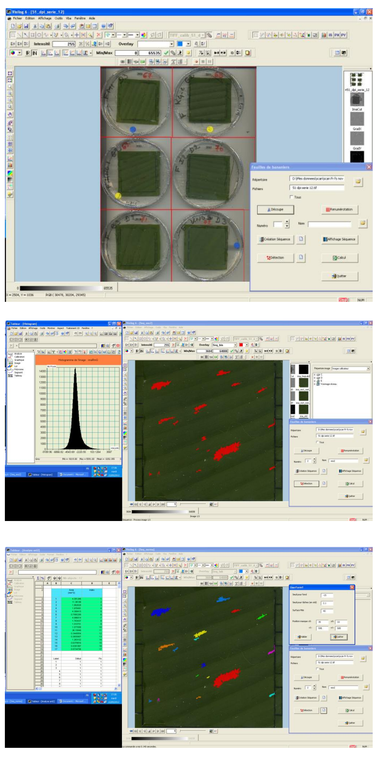
To monitor the whole infection cycle, it was important to develop a specific medium that allows leaf piece survival for as long as 90 days.

Protocol was first established by Abadie *et al.*, 2008 and further improved by:
- modification of survival medium (Agar 4g/l, GA3 5mg/l),
- use of Radium lamps 36W/840,
- use of Greiner petri dishes.

2. To monitor incubation period, infection efficiency and lesion growth rate with an image analysis software.

An image capture with a handheld scanner at a resolution of 300 pixels





An image analysis with the software package Visilog® Noesis (www.noesis.fr) and a banana specific script that allows the automatic:

- detection of leaves pieces,
- recognition of disease areas, (threshold for background, lesion intensity and minima surface can be adapted to each experiment),
- counting of their numbers and surfaces,
- follow up of each disease area through time,
- export to an excel sheet.

References:

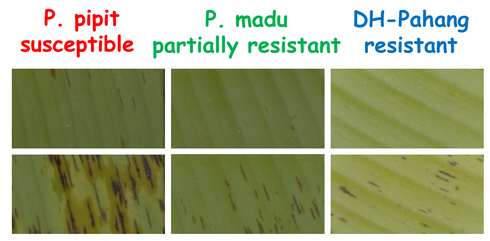
Abadie *et al.*, 2008. Artificial inoculation on plants and banana leaf pieces with *Mycosphaerella spp.*, responsible for Sigatoka leaf spot diseases. Fruits, 63 (5): 319-323.

De Lapeyre *et al.*, 2009. Is chemical control of *Mycosphaerella* foliar diseases of bananas sustainable? Acta Horticulturae, 828: 161-170.

Pennisi, 2010. Armed and Dangerous. Science, 237:804-805.

a first banana / *Mycosphaerella* differential expression analysis from RNA-Seq data

What are the molecular events occuring during the interaction in three accessions with contrasted reactions to *M. fijiensis*?



RNA mock
Detached leaves *in vitro*
Pool 10+16 dpi

RNA *M.fijiensis* inoculated
Spray inoculation of mycelium on detached leaves *in vitro*
Pool 10+16 dpi (no visible symptoms yet)

↓

Illumina GAIIX sequencing
76 bp chemistry

↓

Mapping the reads on the 36 542 banana predicted CDS using SOAP v.2

↓

Read count numbers for each gene model

↓

Statistical analysis: libraries were compared on a one-to-one basis using R package DESeq version 1.5.6.

↓

306 differentially expressed (DE) genes (False discovery rate FDR<0.1) (figure a)

accessions	sample	raw reads (x10⁶reads)	usable reads (x10⁶reads)	mapped reads CDS (x10⁶reads)	%
P. pipit susceptible	mock	30.36	16.40	10.46	64%
	M. fij.	33.07	15.74	10.07	64%
P. madu part.resistant	mock	34.71	17.36	11.39	66%
	M. fij.	31.68	15.52	10.50	68%
DH Pahang resistant	mock	27.10	14.17	10.94	77%
	M. fij.	33.10	14.56	9.60	66%

DH-Pahang resistant

65 % of DE genes down-regulated

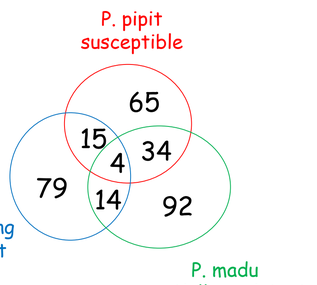
- no upregulation of kinases, WRKY transcription factors or PR genes

- secondary metabolism and carbohydrate metabolism genes are differentially regulated

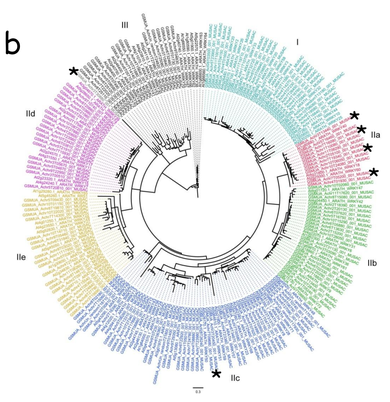
↓

Rapid resistance reaction at earlier time points?

a



b



a, Venn diagram of differentially expressed genes
b, Maximum likelihood phylogenetic tree of banana and Arabidopsis WRKY transcription factors. Stars indicate upregulated genes in the banana /*M. fijiensis* interaction.

P. pipit susceptible (S)

P. madu partially resistant (PR)

67% (S) and 80% (PR) of DE genes are upregulated. Including:

- 8 and 28 receptor-like kinases in S and PR respectively (LRR-RLK, Wall associated kinases (WAKs), Lectin-like kinases) possibly involved in pathogen perception

- banana homologs of Arabidopsis WRKY18/40/60 transcription factors known to be involved in plant defense (WRKY IIa group, figure b)

-pathogenesis-related (PR) genes (PR1, chitinases…)

↓

Basal defense-oriented reprogramming was induced but did not lead to complete resistance.

CONCLUSIONS & PROSPECTS

Phenotyping: The developed protocol allows a better and faster characterization of the different resistance components which are needed for analyses of the genetic bases of banana resistance and *Mycosphaerella* agressiveness traits.

RNASeq: This survey gave the first insights on molecular events in the banana/*M. fijiensis* interaction and has to be completed by additional sequencing (including biological replicates) and by QRT-PCR validation.