

Use of cDNA-AFLP to study the defence-related gene expression in bananas (*Musa* spp.), inoculated with *Colletotrichum musae* responsible of crown rot

61st International Symposium on Crop Protection

Ghent, Belgium

May 19, 2009



Identification of pre- and post-infection genes potentially implied in quantitative banana response to crown rot disease

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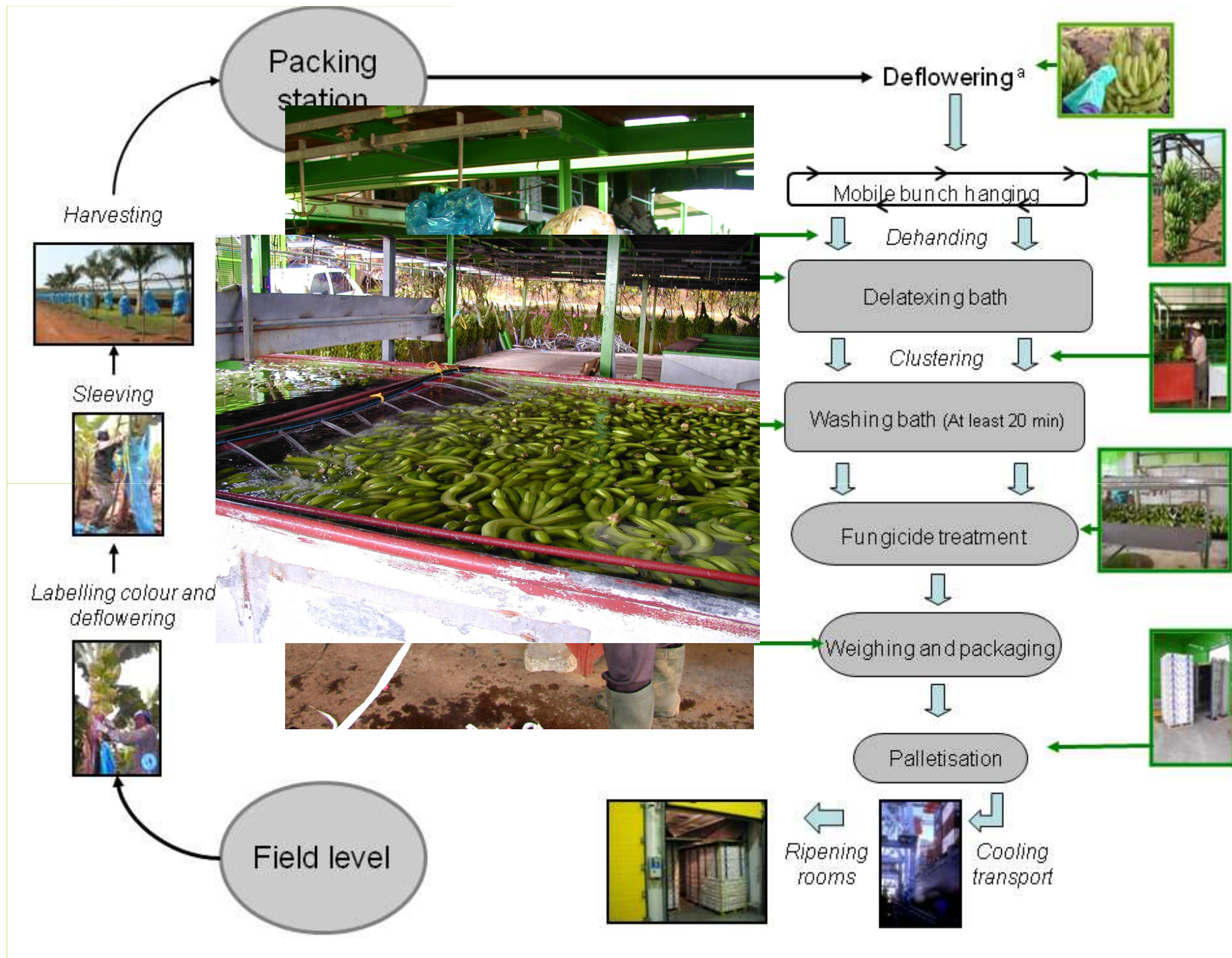
Crown rot disease



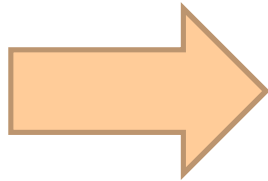
Crown rot disease

- Banana post-harvest disease
- All producing countries
- The main post-harvest disease
- Losses of up 86% in non chemically area
- First symptoms are generally only noted after maritime shipping
- Parasitical complex
 - *Colletotrichum musae*





Crown rot disease



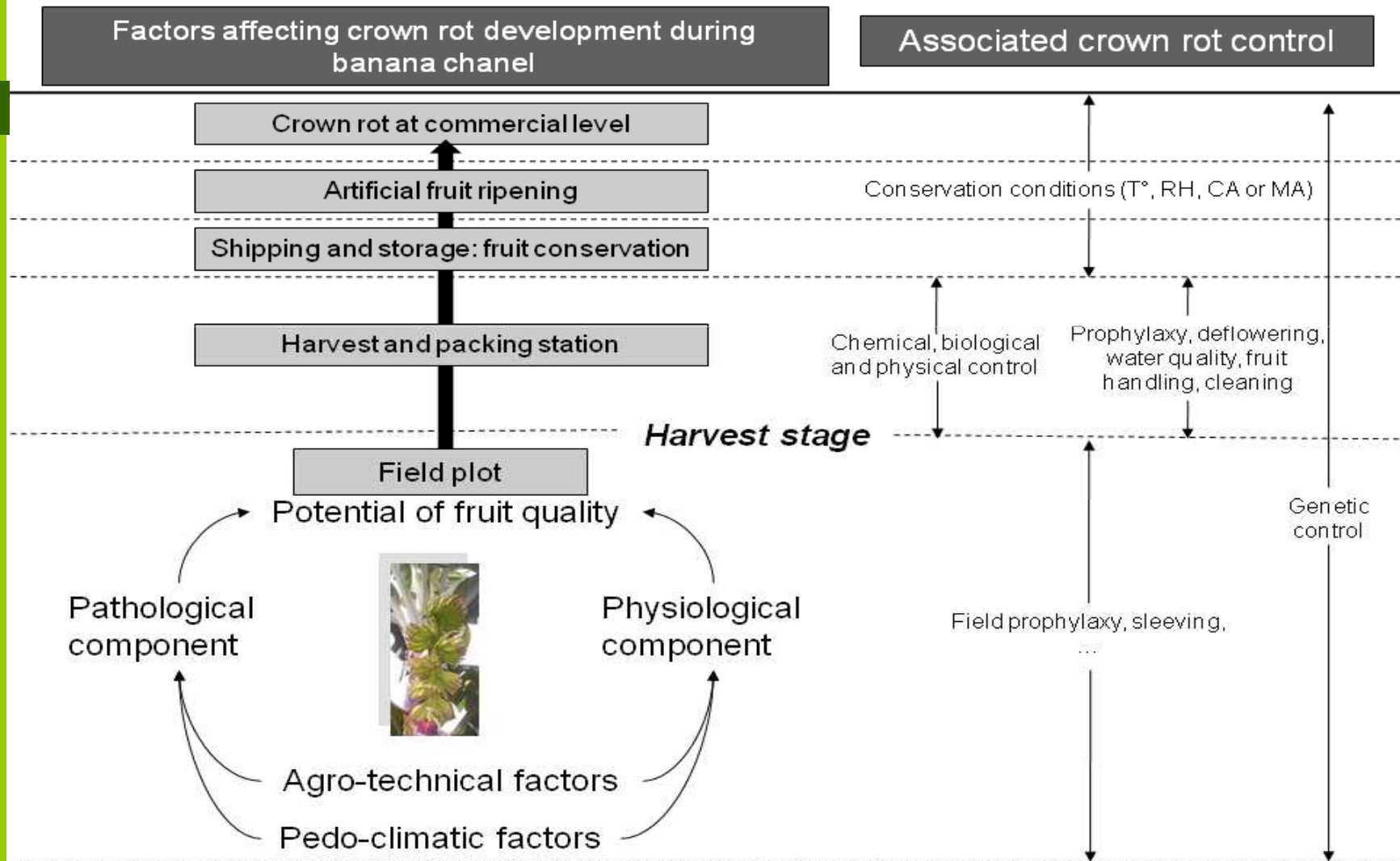
Negative impact on the market value of bananas



Incidence variation



Incidence variation



Aims

Determining the genetic bases which influence
the banana crown susceptibility level to *C.*
musae



Materials and methods



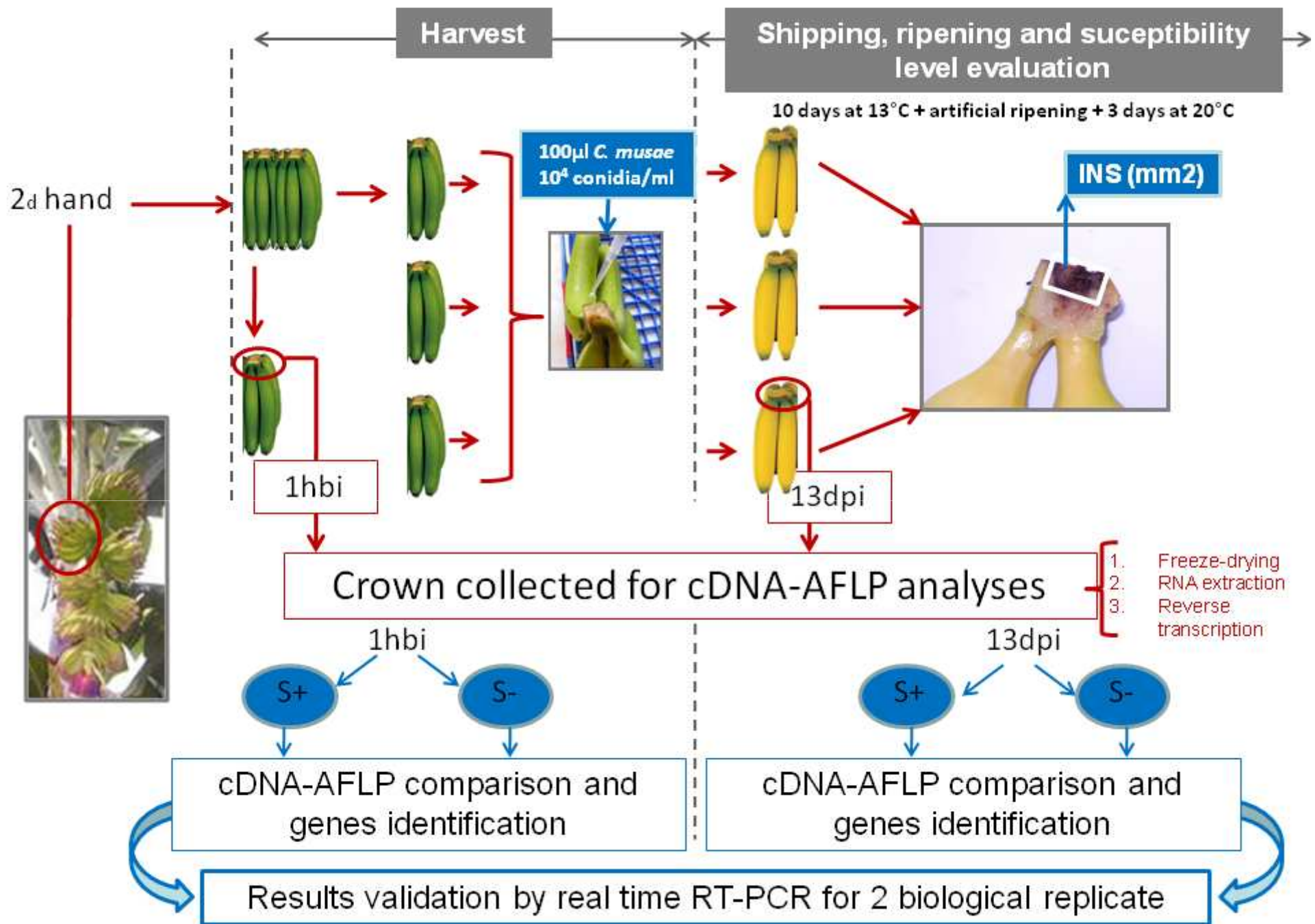
Materials and methods

- Studying gene expression by mRNA profil comparison (cDNA-AFLP) between bananas showing 2 susceptibility levels



Pre- and post-infection

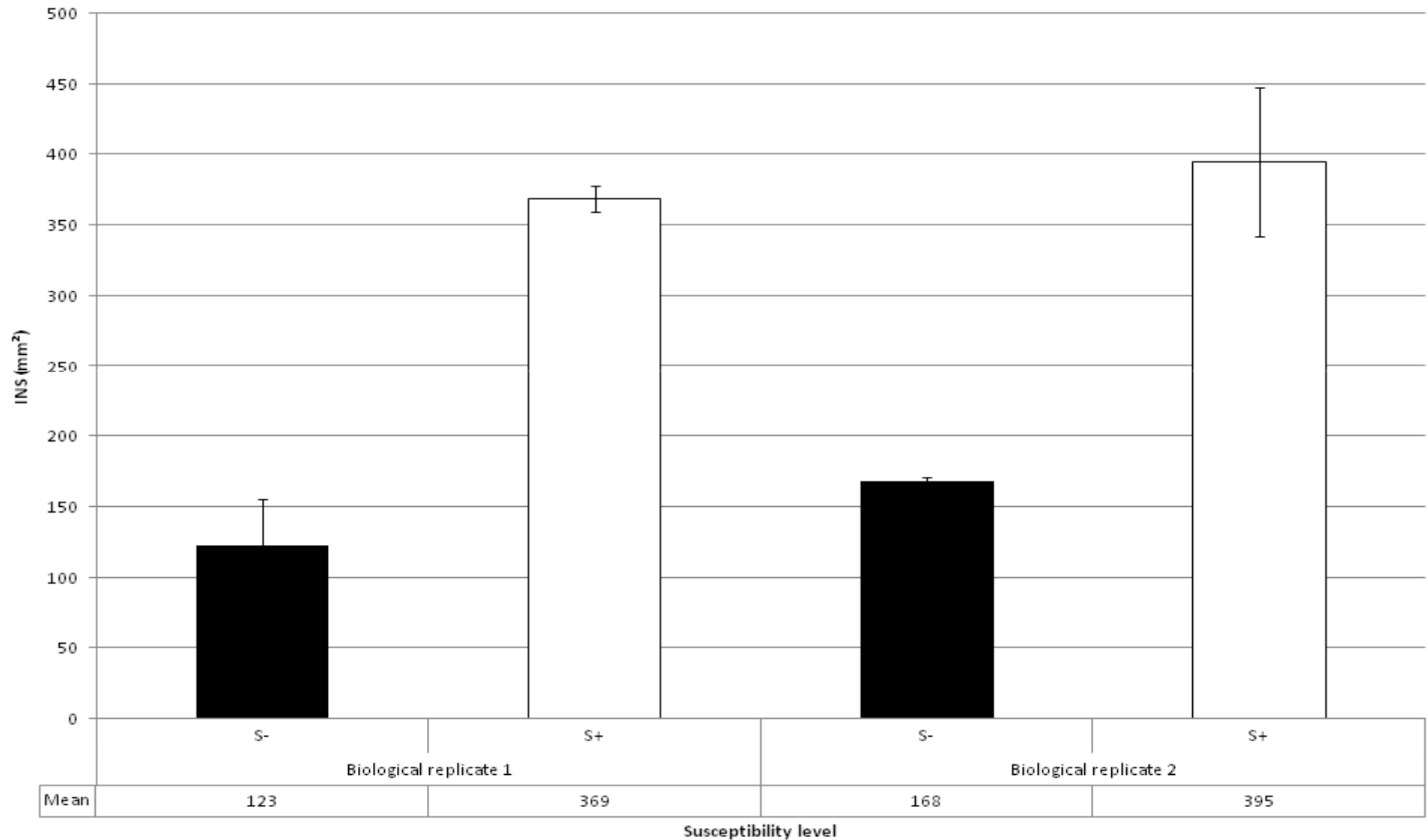




Results

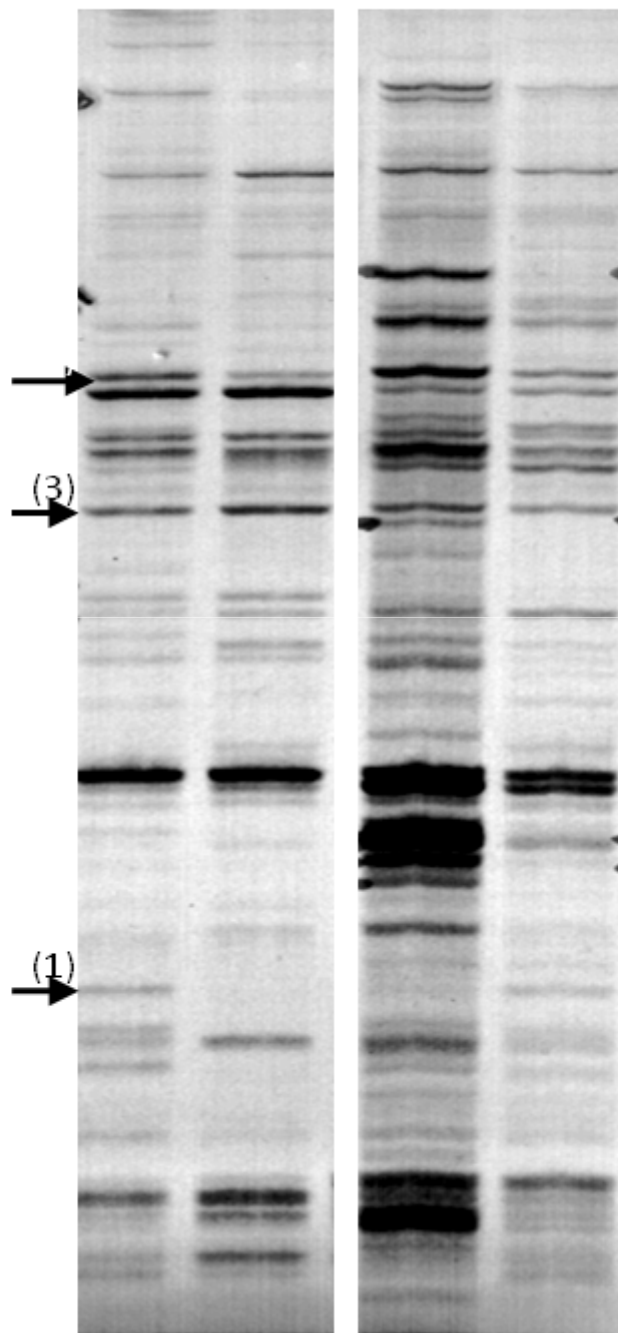


Susceptibility level evaluation



Eco RI+cc/Mse I+ag

S- (1hbi) S+ (1hbi) S- (13dpi) S+ (13dpi)



cDNA-AFLP results (1hbi)

Expression profil (1hbi)	Number
cDNA fragments displayed (100-450bp) in S ⁻ and S ⁺	822 and 683
Differentially expressed fragments	157
Upregulated in S ⁻	62
Downregulated in S ⁻	95
TDFs selected and excised	32
TDFs successfully recovered from the gels, reamplified, cloned and sequenced	16
Non redundant significant similarities with the database after analysis	15
Selected TDF's for real time RT-PCR confirmation	7
cDNA-AFLP results confirmation by real time RT-PCR / Biological replicate 1	7
cDNA-AFLP results confirmation by real time RT-PCR / Biological replicate 2	6



cDNA-AFLP results (13dpi)

Expression profil (13dpi)	Number
cDNA fragments displayed (100-450bp) in S ⁻ and S ⁺	811 and 845
Differentially expressed fragments	286
Upregulated in S ⁻	166
Downregulated in S ⁻	120
TDFs selected and excised	99
TDFs successfully recovered from the gels, reamplified, cloned and sequenced	62
Non redundant significatives similarities with the database after analysis	31
Selected TDF's for real time RT-PCR confirmation	21
cDNA-AFLP results confirmation by real time RT-PCR / Biological replicate 1	17
cDNA-AFLP results confirmation by real time RT-PCR / Biological replicate 2	5



cDNA-AFLP results confirmed by real-time RT-PCR (2 biological replicates)

Annotation	Isolation stage	Regulation level	
		1hbi	13dpi
Protein Kinase	13dpi	+2	+4
Dual specificity phosphatase	13dpi	+4	+4
Ubiquitin ligase	13dpi	+2	+3
Ubiquitin carboxyl-terminal hydrolase	13dpi	+1; +1	+2
Serine carboxypeptidase	13dpi	+2	+3
Glicolipid-transfer protein			
Glicolipid-transfer protein			
Cellulose synthase			
CAF1			
Dopamine-β-hydroxylase			
Hypothetical protein			

Signaling pathway

- Leads to reversible proteins phosphorylation/dephosphorylation

- They are triggered by an array of stimuli and target a broad range of downstream effectors

Regulating various processes

Defense response: it is well know but very complex

cDNA-AFLP results confirmed by real-time RT-PCR (2 biological replicates)

Annotation	Isolation stage	Regulation level	
		1hbi	13dpi
Protein Kinase	1hbi	+4	+4
Dual specificity phosphatase	1hbi	+4	+4
Ubiquitin ligase	1hbi	+3	+3
Ubiquitin carboxyl-terminal hydrolase	13dpi	+1	+2
Serine carboxypeptidase	13dpi	+2	+3

Proteolytic pathway

To regulate the fate of proteins

- Housekeeping role
- Remove abnormal, non-functional or short-lived protein
- Release amino acids for recycling
- Play a role in the regulation of biological processes (affecting transcription factors, signal transduction)



Such those mediating response to pathogens

cDNA-AFLP results confirmed by real-time RT-PCR (2 biological replicates)

Annotation	Isolation stage	Regulation level	
		1hbi	13dpi
Protein Kinase	1hbi	+ 2	+ 4
Dual specificity phosphatase	Belong to the lipid-transfer protein		+ 4
Ubiquitin ligase	1hbi	- 2	+ 3
Ubiquitin carboxyl-terminal hydrolase	13dpi	-1; +1	+2
Serine carboxypeptidase	13dpi	+2	+3
Glicolipid-transfer protein	PR-14	+ 3	+ 3
Glicolipid-transfer protein		+ 1	+ 3
Cellulose synthase		-2	-2
CAF1	13dpi	-1; +1	+2
Dopamine-β-hydroxylase	1hbi	+ 1	+ 4
Hypothetical protein	1hbi	- 4	- 4

cDNA-AFLP results confirmed by real-time RT-PCR (2 biological replicates)

Annotation	Isolation stage	Regulation level	
		1hbi	13dpi
Protein Kinase	1hbi	+ 2	+ 4
Dual specificity phosphatase	1hbi	- 4	+ 4
Ubiquitin ligase	1hbi	- 2	+ 3
Ubiquitin carboxyl-terminal hydrolase	13dpi	-1; +1	+2
Serine carboxypeptidase	13dpi	+2	+3
Glicolipid-transfer protein	1hbi	- 1	+ 3
Glicolipid-transfer protein	13dpi	- 1	+ 3
Cellulose synthase	13dpi	-2	-2
CAF1	13dpi	-1; +1	+2
Dopamine- β -hydroxylase	1hbi	+ 1	+ 4
Hypothetical protein	1hbi	- 4	- 4

Cellulose synthase

- Downregulated in less susceptible
 - Surprising result (cellulose are cell-wall component)
 - Activating of lignin synthesis?



cDNA-AFLP results confirmed by real-time RT-PCR (2 biological replicates)

Annotation	Isolation stage	Regulation level	
		1hbi	13dpi
Protein Kinase	1hbi	+ 2	+ 4
Dual specificity phosphatase	1hbi	- 4	+ 4
Ubiquitin ligase	1hbi	- 2	+ 3
Ubiquitin carboxyl-terminal hydrolase	13dpi	-1; +1	+2
Serine carboxypeptidase	13dpi	+2	+3
Glicolipid-transfer protein	1hbi	- 1	+ 3
Glicolipid-transfer protein	13dpi	- 1	+ 3
Cellulose synthase	13dpi	-2	-2
CAF1	13dpi	-1; +1	+2
Dopamine- β -hydroxylase	1hbi	+ 1	+ 4
Hypothetical protein	1hbi	- 4	- 4

CCR4-associated factor 1 (CAF1)

- In our study, CAF1 over-expression is related with a less susceptibility level
- Biochemical and physiological functions are not clearly established
- Implication in regulating plant growth and defence response was suggested
- The constitutively up-regulating of multiple PR genes (PR1, PR2, PR6, ...) are related to an over-expression of the CAF1 genes



cDNA-AFLP results confirmed by real-time RT-PCR (2 biological replicates)

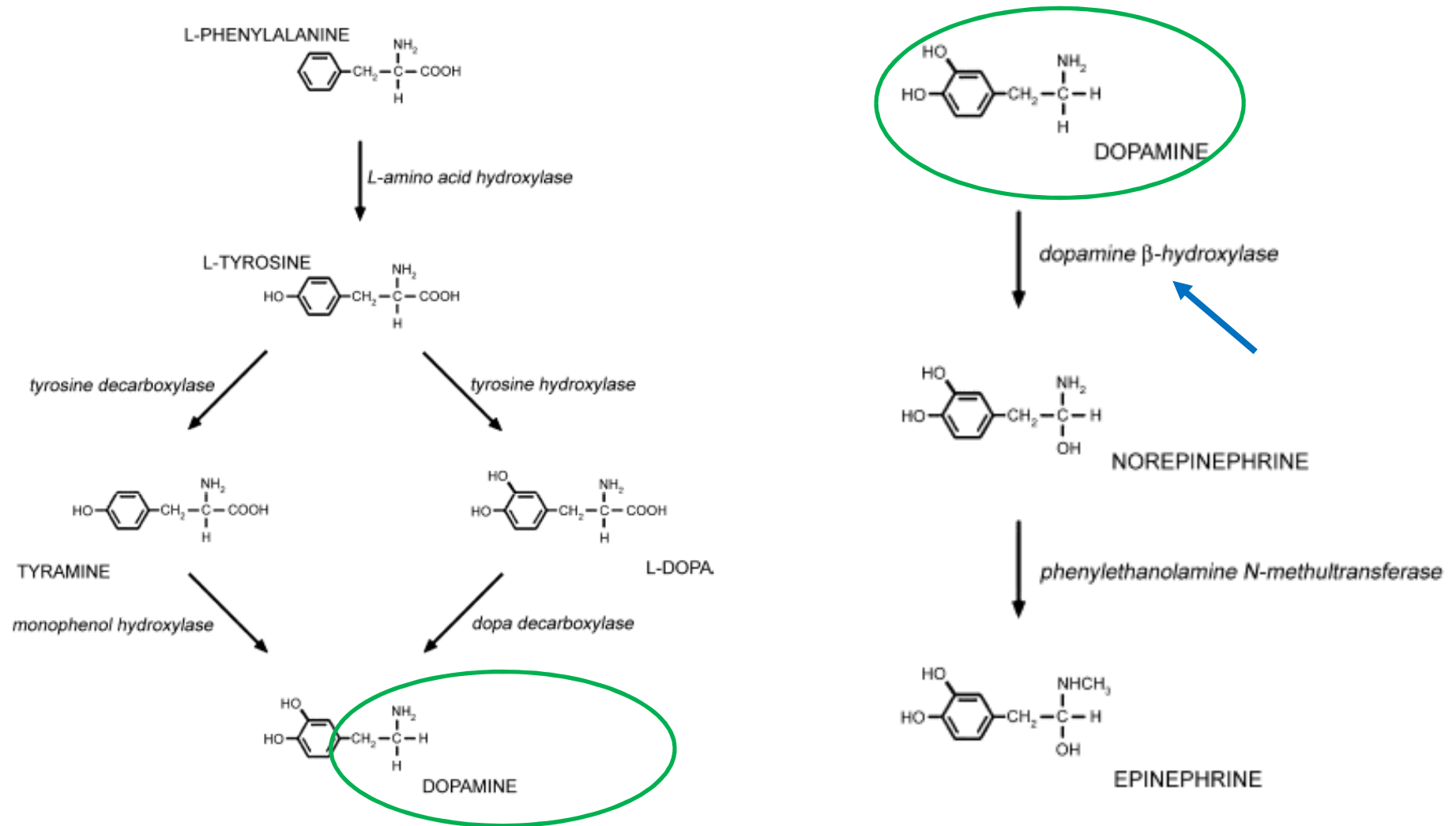
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Dual specificity phosphatase	1hbi	- 4	+ 4
Ubiquitin ligase	1hbi	- 2	+ 3
Ubiquitin carboxyl-terminal hydrolase	13dpi	-1; +1	+2
Serine carboxypeptidase	13dpi	+2	+3
Glicolipid-transfer protein	1hbi	- 1	+ 3
Glicolipid-transfer protein	13dpi	- 1	+ 3
Cellulose synthase	13dpi	-2	-2
CAF1	13dpi	-1; +1	+2
Dopamine-β-hydroxylase	1hbi	+ 1	+ 4
Hypothetical protein	1hbi	- 4	- 4

Dopamine- β -hydroxylase

- Catecholamine synthesis pathway



Fig. 1. Plant catecholamine synthesis pathway.



Dopamine- β -hydroxylase

- Catecholamines, derivatives and precursors
 - Functions very complex
 - Influence many aspects of plant physiology
 - Oxidative status
 - Regulation of plant growth and development
 - Sugar metabolism regulation
 - Flowering
 - Active compound in plant response to stress



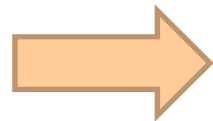
Dopamine- β -hydroxylase

- Catecholamine/plant response to stress
 - Mechanisms?
- Catecholamine and banana resistance to stress
 - Dopamine and oxidation products have already frequently suggested to be implied in banana resistance mechanisms



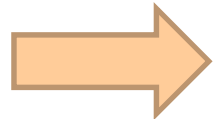
Conclusions

- Signaling pathway and proteolytic machinery



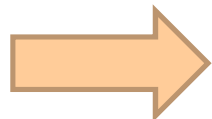
Not surprising

- Glycolipid-transfer protein



Belong to the well-know PR14

- CAF1; Cellulose synthase and Dopamine hydroxylase



Interesting ways of research but further investigations are required



Conclusions

Because plant defense responses are invariably multicomponent in nature, it is not easy to define which components are both necessary and sufficient to confer protection

Our results allow to suggest some hypothesis but further investigations are needed



Thank you for your attention

