## TR4 resistant GAL Induced Mutation Breeding



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# **Pre-set objectives**

- Restrict our resistance to Cavendish or plantains
- Fully resistant not tolerant
- Stable and sustainable
- Appropriate for the export market
- Meet yield and quality criteria of the export market
- Expedite the development



# Setting up the parameters of a field trial

Parameters:

- ✓ Pre-screening in a greenhouse after inoculation
- ✓ Choice of an infected region for a field-trial
- ✓ Duration of the field trial  $R_0 R_{3(70-85 \text{ wks})}$







✓ Proper labelling



# Why GAL?

- $\checkmark$
- Excellent yield ~28 Kg @ R<sub>0</sub> Stress tolerant (water and heat) Quick return High quality bunch Easy to manage







# **Strategy** – Induction of HTP genetic diversity





#### Elite clone







#### Library of mutants

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## **Strategy** - Activation of retro transposable elements



# Levels of validation – all levels



#### Resistance:

- Molecular proof of induced mutagenesis
- Ex Vitro Greenhouse inoculation experiment (University of Wageningen)
- Whole genome sequencing Comparing resistant and Wt plants in relevant QTLs
- Field trials Screening in 3 heavily infected plantations in the Philippines
- Field trial in Infected plantation in La Guajira
  Trial will start in Q 3 of 2022

#### Agrotechnical parameters:

- Yield components Bunch weight
  - Number of fingers
  - Cycling time
- Fruit quality -
- Proper ripening
- Normal Green life High Brix value



## Mechanism - In-vitro Activation of Re/Transposons

Southern blot hybridization Genomic DNA probed with Reverse Transcriptase



De-methylated 3 times In 24 cycles of In vitro transfers



## **Screening System** - resistance to fusarium wilt (Race 1/TR4)









Grand Naine

Gros Michel



#### **Summary** - Inoculation experiments

At the University of Wageningen Prof. Kema, Dr Fernando Garcia

Batch	Number of	Number of	Percent
	accessions	asymptomatic	asymptomatic
	tested	plants	plants
1	4500	153	3.4%
2	5200	262	5.0%





**415 selected lines** 

Professor Gert Kema

Dr. Fernando Garcia



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## Case 1- "GAL" TR4 Resistance phase I



#### 153 plants resistant/tolerant



#### 3947 plants susceptible











APART

# Yield and Quality - The Philippines field trial

plot	Number of plants	Number of asymptomatic plants	Percent asymptomatic plants
1	3000	2985	99.5%
2	4500	4363	96.9%

#### Control











FAT

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EED APART









confidential

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The resistant GAL (right) and Control (Williams on the left) were grown in a field heavily infected with TR4 for approximately 4 years



# Further validation - Whole Genome Sequence analysis



Co-mapping R landing pad and R-Genes cluster

Assembly Data

Sequencing Raw Data

variety	Scaffold #	Assembly size	N50
GAL	36,671	1,509,245,576bp	8,627,917bp
Asymptomatic clone	414,835	1,637,131,567bp	6489bp

variety	sequencing technology	depth (coverage)
GAL	2 <sup>nd</sup> generation illumina	X223
GAL	3 <sup>nd</sup> generation illumina	X26
GAL	RNA-Seq	X137
Asymptomatic clone	2 <sup>nd</sup> generation illumina	X84

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## Breeding for Short Valery Resistant to TR4





height



# **Case 2** - "DR-2"



#### Of 12000 clones 191 were asymptomatic



## **Next Phase** - Validation and mother plantation in La Guajira

Time lines: (in weeks)

Activity	Time in weeks	Responsibility	Observers
Import from the Netherlands (weeks 36-37	T <sub>0</sub> - T <sub>2</sub>	Rahan Meristem Israel/PlantSphere	ICA/ MC
Propagation and rooting in Colombia	$T_2 - T_{30}$ Yanai please verify	MC	ICA
Hardening	T <sub>31</sub> — T <sub>39</sub> <sub>Yanai</sub> please verify	MC	ICA
Bagging	<mark>T<sub>39</sub> − T<sub>45</sub></mark> <sub>Yanai</sub> please verify	MC	
Field planting	T <sub>46</sub>	Tecbaco RM	
First report	T <sub>78</sub>	Tecbaco RM	
Second report	T <sub>81</sub>	Tecbaco RM	
Final report	T <sub>106</sub>	Tecbaco RM	





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