Towards more efficient nematode resistance screening in banana: progress and challenges

Kanan Saikai, Happyness Justine, James Kisaakye, Joseph Kisitu, Danny Coyne

icipe

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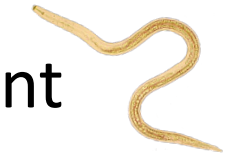
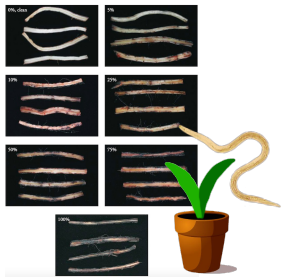
NemAfrica
Knowledge and technical capacity for nematology in Africa

Today’s talk is all about nematodes…

Problem statement 

Rapid screening - *in vitro* 

Rapid screening – macropropagated plants 

Parental line screening 

Nematode pests as constraints to banana production.

Banana plants toppled over

Root necrosis & death

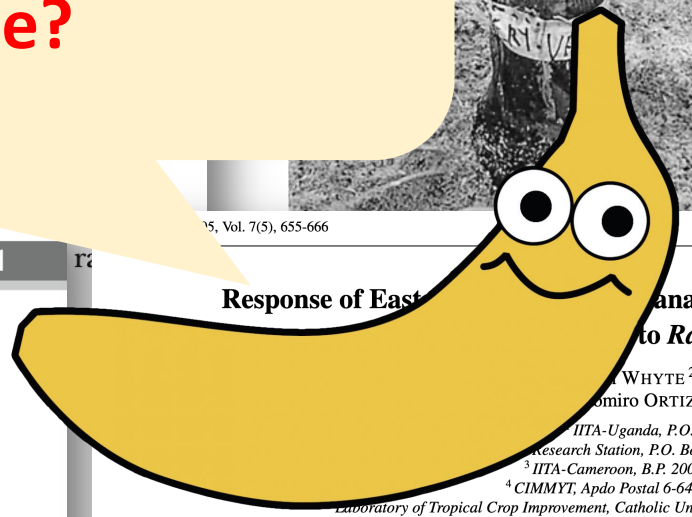
Infected seed

Photo credit: Dr. Danny Coyne

Current nematode screening needs improvements.

More accurate, high throughput screening system is urgently needed!

Can we develop a screening method that can replace the current one?



Evaluation of a method to simultaneously screen *Musa* germplasm against multiple nematode species

D.L. Coyne and A. Tenkouano

Evaluation method

There is no doubting the importance of nematode pests as constraints to *Musa* production (Gowen *et al.* 2005). However, emphasis on plant-parasitic nematodes affecting *Musa* has focused on the epidemiology, management and identification of resistance against *Radopholus similis* (Cobb) Thorne. Evidence is becoming increasingly clear however, that

Severn-Ellis *et al.* 2003). Using such methods, several *Musa* genotypes with resistance to *R. similis* (Pinochet 1996) have been identified. However, the process of identifying resistance remains time consuming.

The ability to rapidly screen many landraces or genotypes developed by breeders would make the process more

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Response of East ananas and hybrids to *Radopholus similis*

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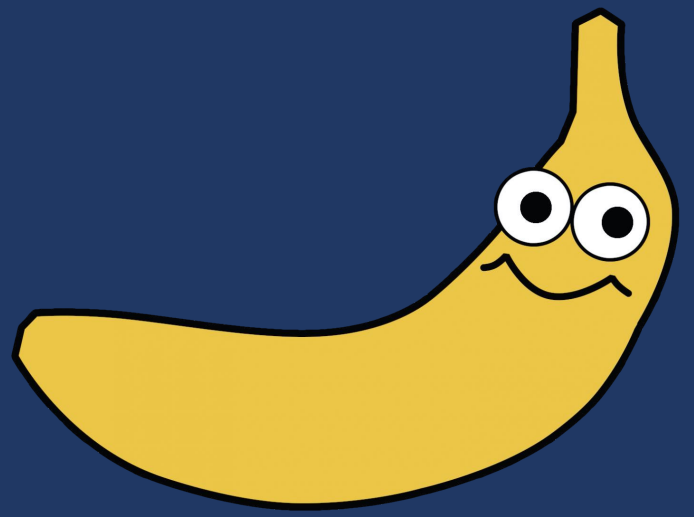
Summary – The burrowing nematode, *Radopholus similis*, is a serious threat to sustainable banana production worldwide. A promising way of controlling nematodes is through the development and deployment of resistant cultivars. This usually involves crossing triploid cultivars with fertile diploids to produce tetraploids that generally display greater male and female fertility. Selected tetraploids are then crossed with improved diploids to produce sterile secondary triploids. This study evaluates the host response of the most commonly

Develop more time-efficient, reliable and high throughput screening system for nematode resistance in banana.

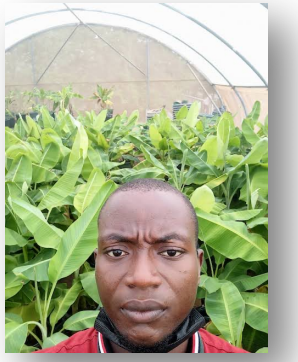


How do we approach?

In vitro system

Joseph Kisitu
(Uganda)

Macro-propagated plant system



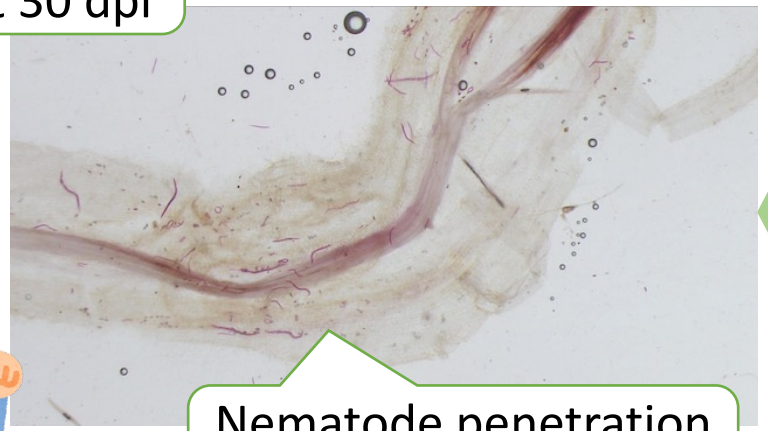
Emmanuel Olajide
(Nigeria)

Rapid screening for nematode resistance using *in vitro* system

In vitro system using river sand saturated with MS media.

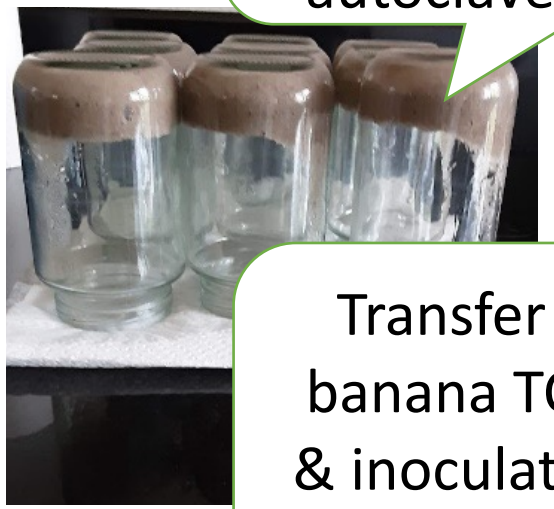
Sterile river sa

Nematode reproduction at 30 dpi

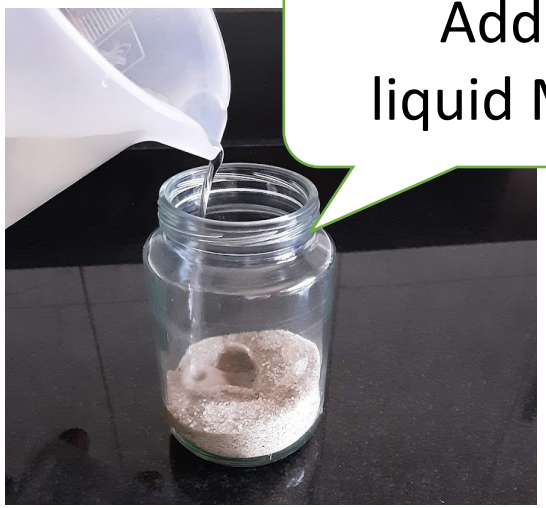


Nematode penetration at 7dpi

Transfer banana TC & inoculate 50 *R. similis*

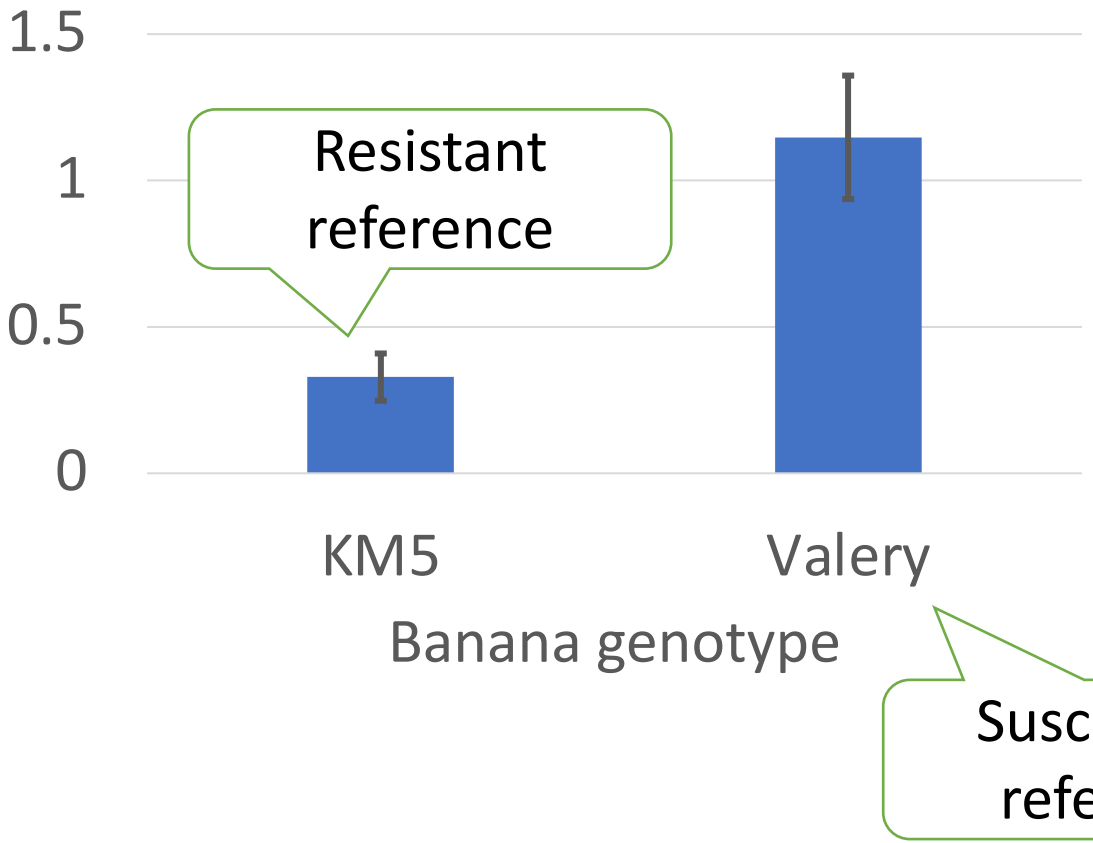


Absorb off excess MS , cover and autoclave



Add liquid MS

Nematode damage was observed at 2 weeks.



Nematode der

0 = 0% damage

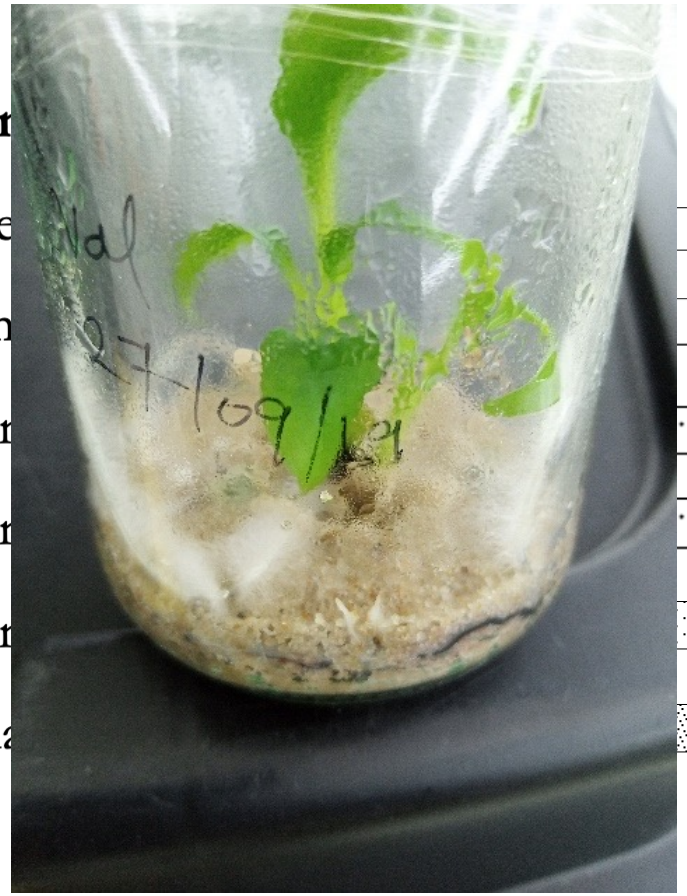
1 = 1-20% dam

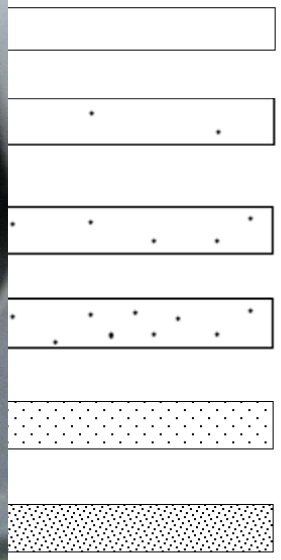
2 = 21-40% dar

3 = 41-60% dar

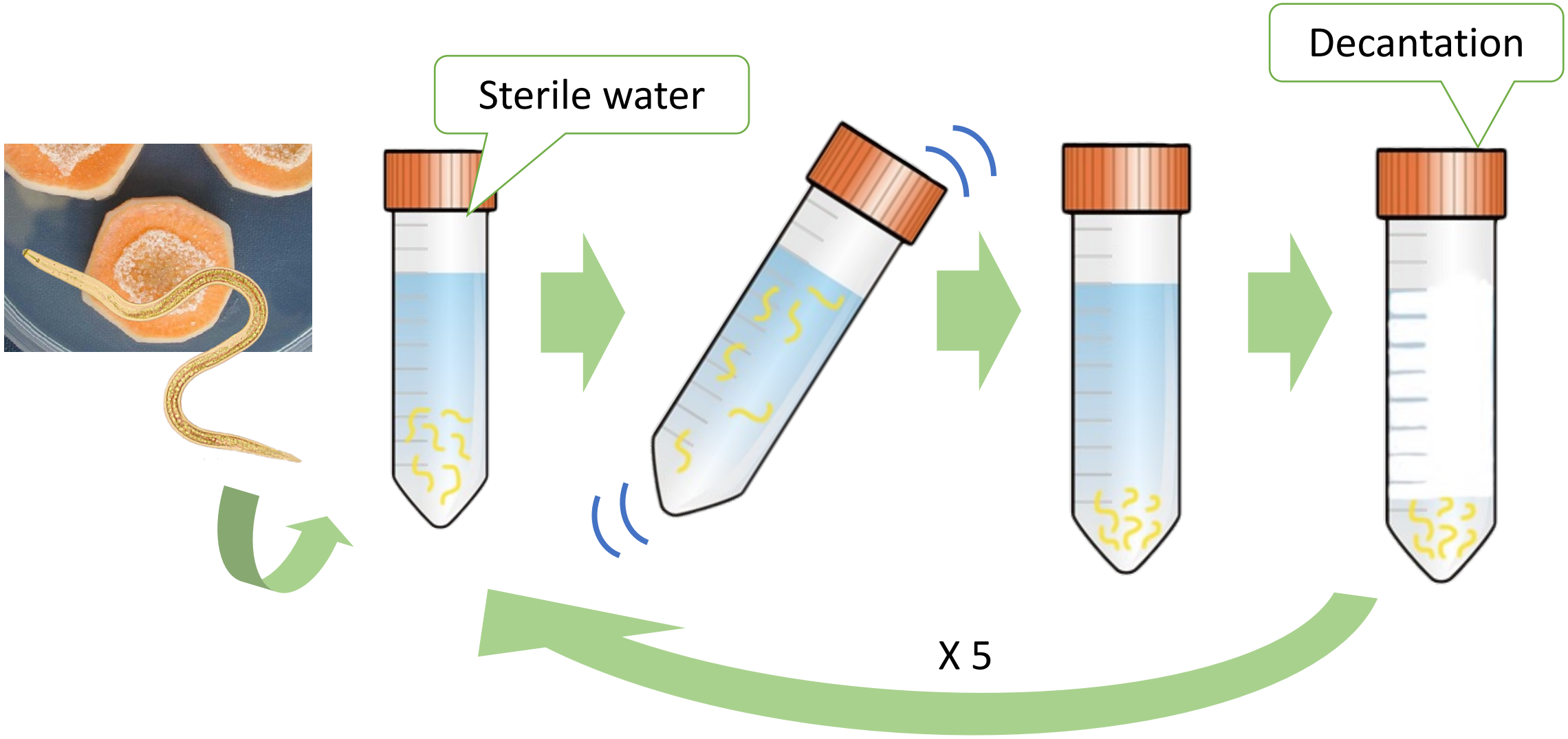
4 = 61-80% dar

5 = 81-100% da



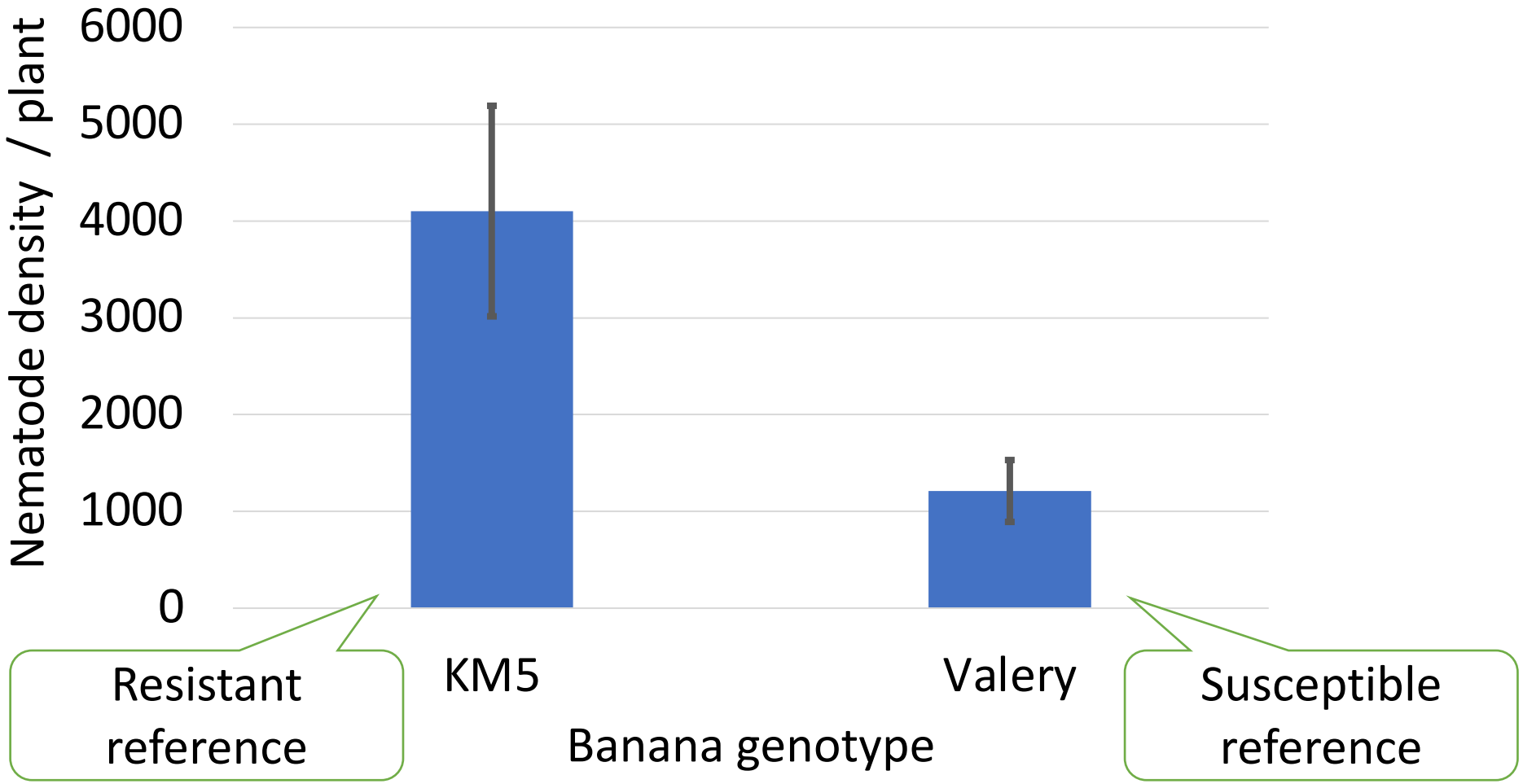


Nematode sterilization protocol was developed to prevent contaminations.



The system is working without contaminations, but need further validation.

At **8 weeks** after inoculation



The *in vitro* system is now working but what's next?

- Need to find better resistance reference.
- Better nematode assessment at 2 weeks (count the actual nematode number in roots)

Quick update….
Joseph Kisitu is presenting the work at ICN in France.

IITA Transforming African Agriculture CGIAR

Towards High Throughput Screening of Banana for Nematode Resistance

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[1] International Institute of Tropical Agriculture (IITA) Uganda, [2] International Institute of Tropical Agriculture (IITA) Kenya, [3] International Centre of Insect Physiology and Ecology (*icipe*), Kenya

Introduction

Banana (*Musa spp.*) is an important food and cash crop globally and a staple food in the Greatlakes region of Africa. Owing to several biotic constraints to production, banana yield has followed a declining trend, with a 4.6% reduction between 2012 and 2019. Plant parasitic nematodes have been sighted as the key pests constraining banana production. Yield losses of up to 50% have been attributed to the root burrowing nematode *Radopholus similis*. Breeding effort to address this challenge produces thousands of banana lines which can hardly be accommodated in the existing protocols for nematode phenotyping. The current study explored the possibility of developing a high throughput bioassay using an *in-vitro* phenotyping approach by modifying the conventional Murashige and Skoog (MS) media to optimize plant growth and provide near natural conditions for contamination free nematode infestation.

Objectives

General

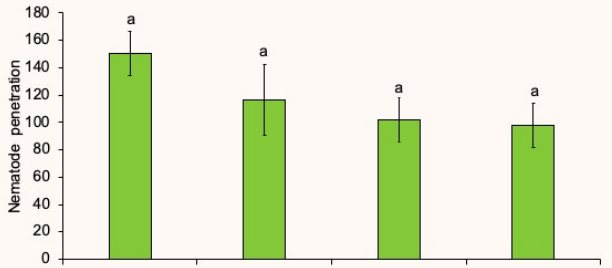
- To develop a high throughput screening method against banana nematodes

Specific

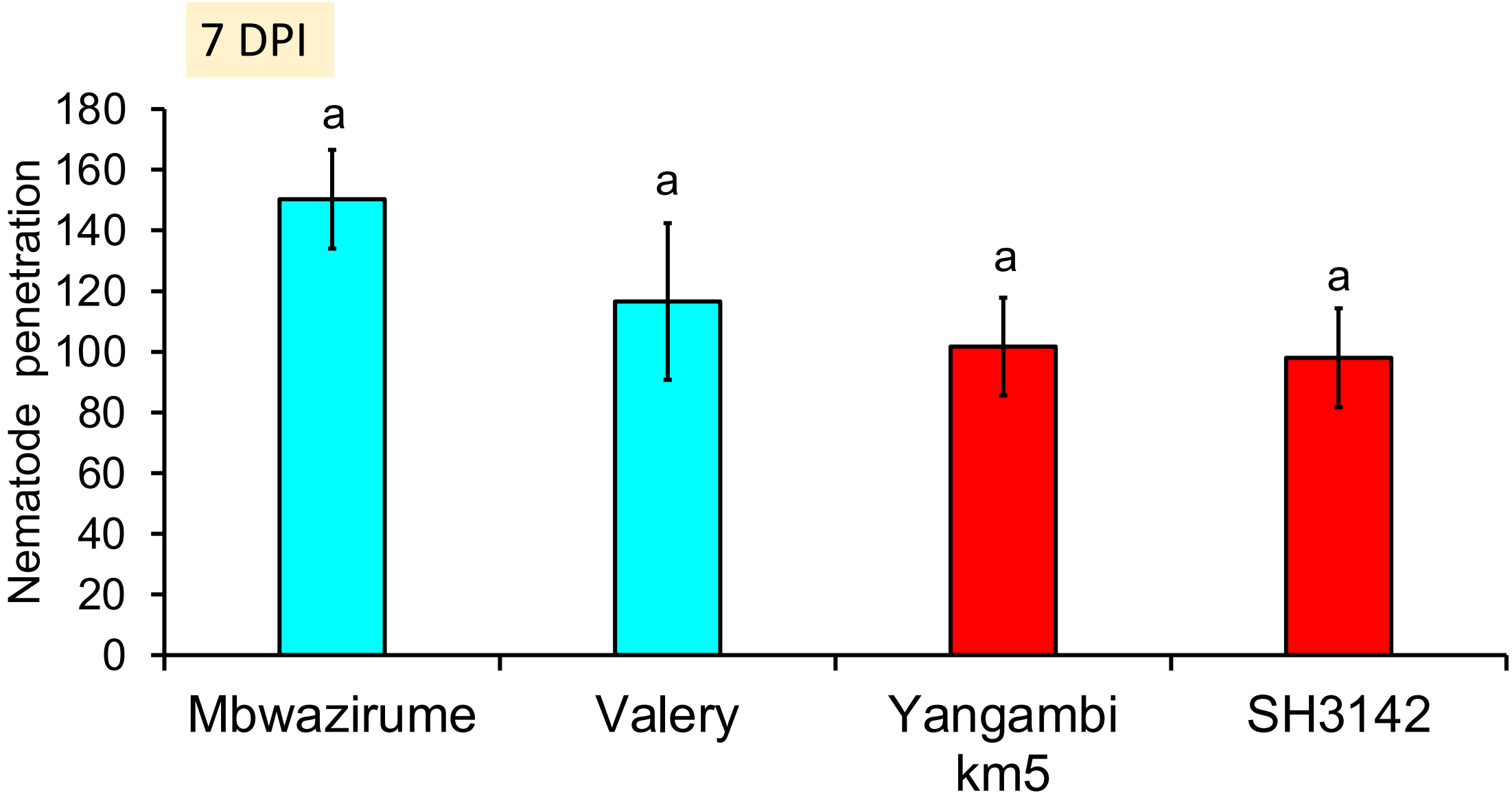
- Achieve optimal *R. similis* penetration into TC banana plants maintained on MS-infused sand media.

Results and Discussion

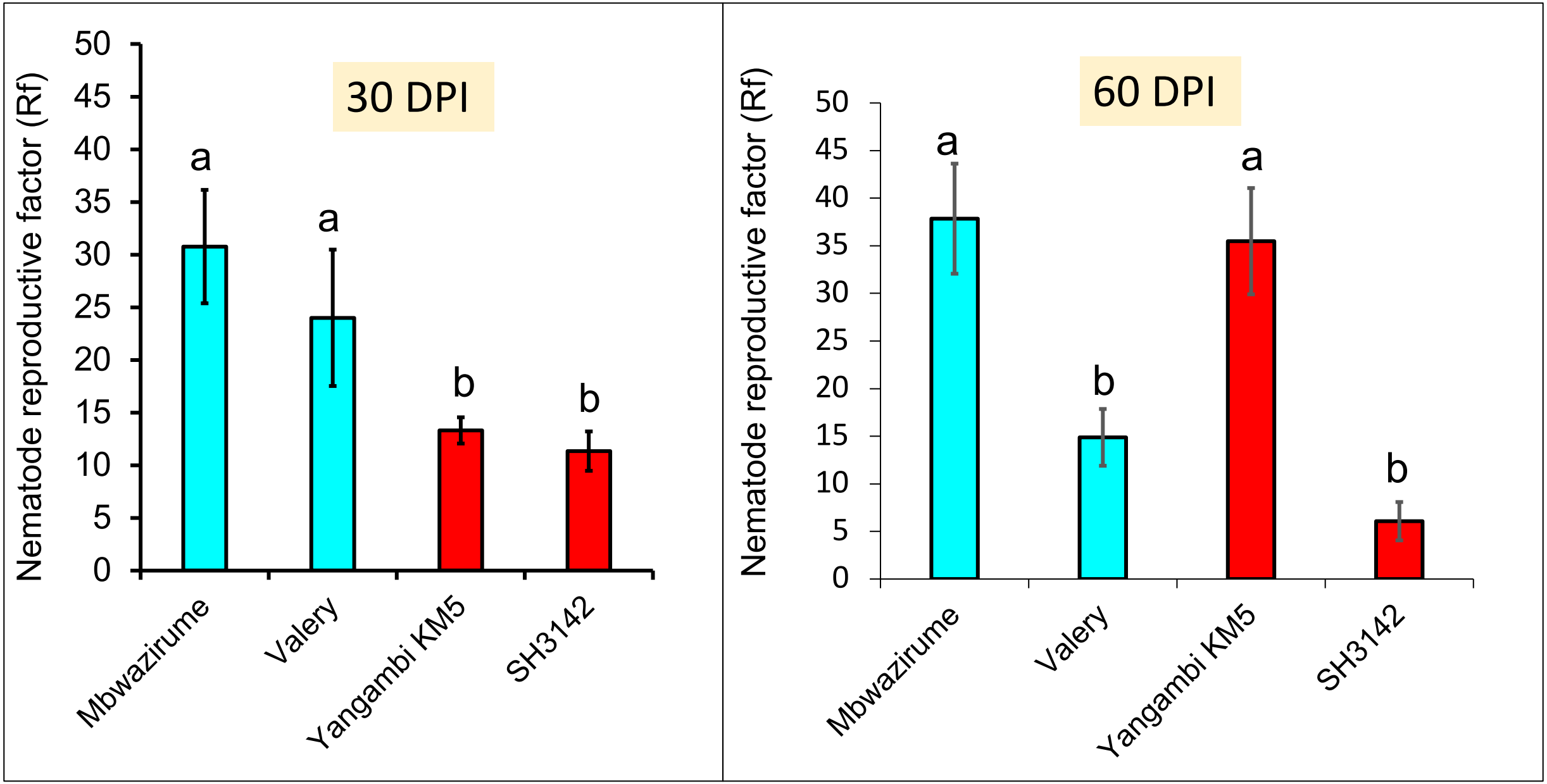
There was no significant differences in the number of *R. similis* penetration into banana roots at day 14. Nematode penetration at 14 days (Fig 2.) generally correlated with reproduction at 30 days (Fig 3.) after nematode inoculation. The banana cultivar SH3142 significantly suppressed nematode reproduction (Figure 3.) relative to the susceptible cultivar Mbwazirume at 30 and 60 days after nematode inoculation. Prolonged exposure of Yangambi KM5 rendered this cultivar susceptible. Based on the observed results, SH3142 is a better resistant reference cultivar compared to Yangambi KM5



Quick update….



Quick update….



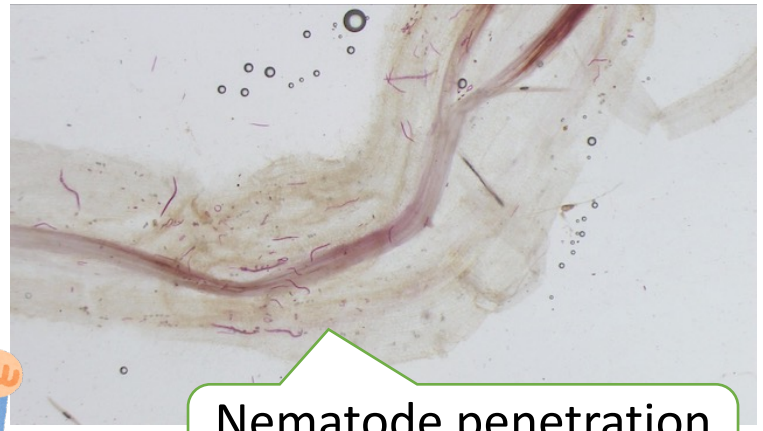
Rapid screening for nematode resistance using macro-propagated plantlets

Macro-propagation offers disease free materials at cheaper cost

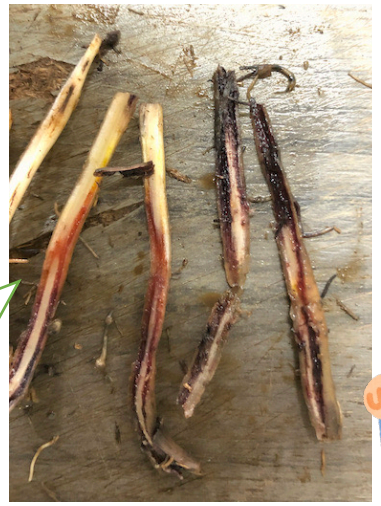
At a three leaf stages

Transplant to SAP media, inoculate with 200 *R. similis*

Agbagba	**Susceptible**
Yangambi KM5	**Resistant**
Pisang lilin	**Resistant**
SH 3142	**Resistant**
Pisang jari buaya	**Resistant**



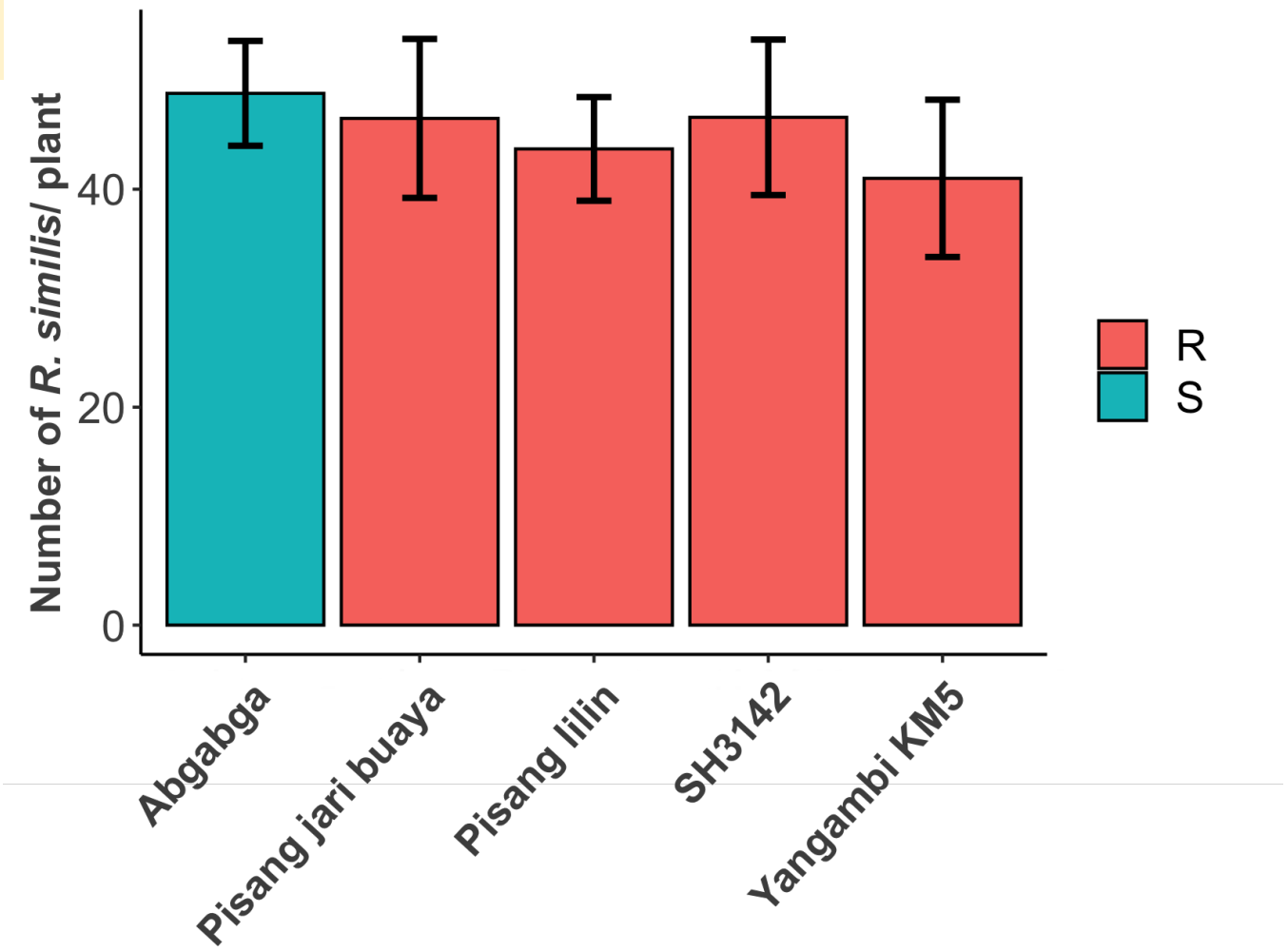
Nematode penetration at 7dpi



At 30 & 60 dpi, Necrosis rating, nematode reproduction, plant growth parameters

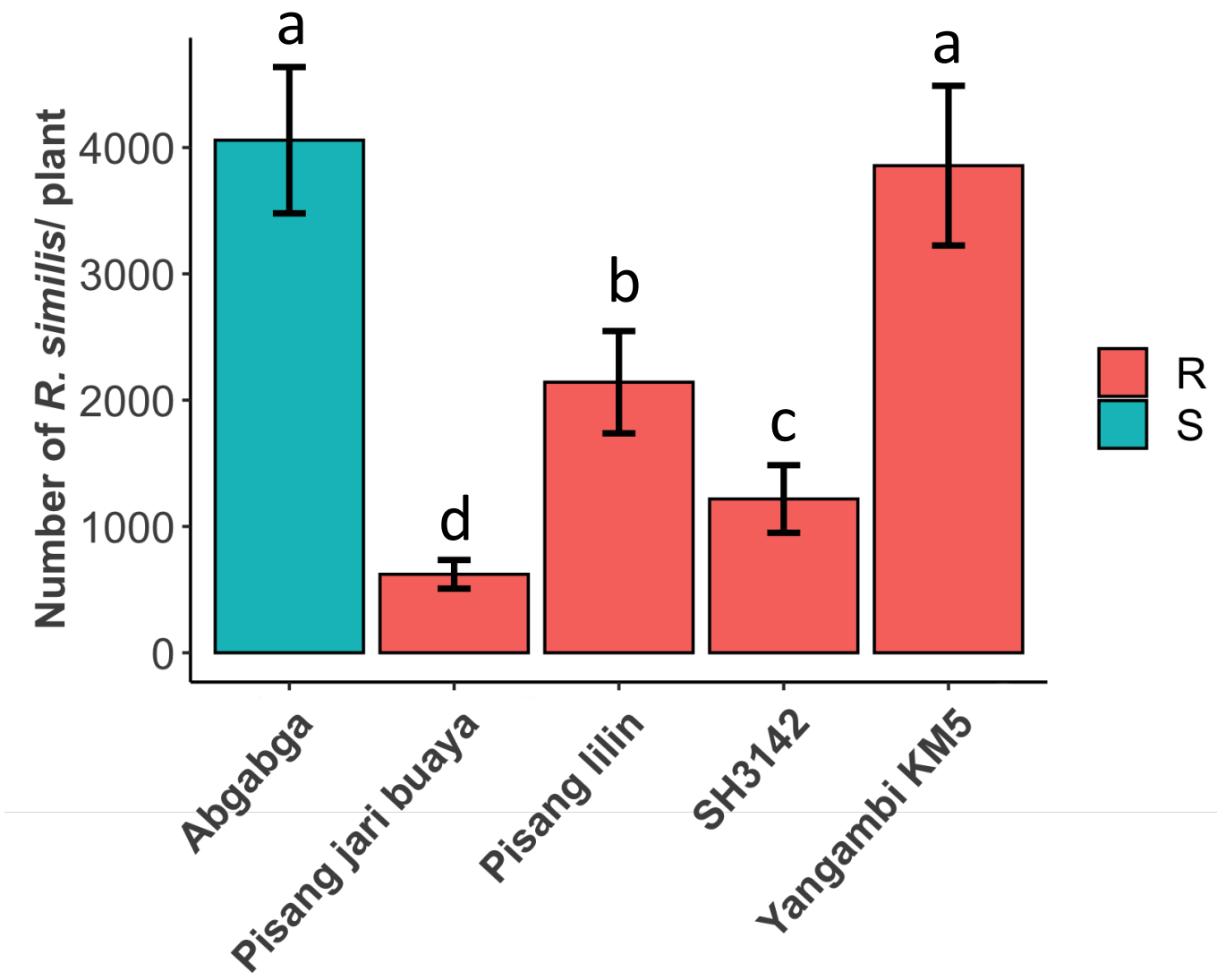
Once again, penetration ≠ reproduction.

7 DPI



Once again, penetration ≠ reproduction.

60 DPI



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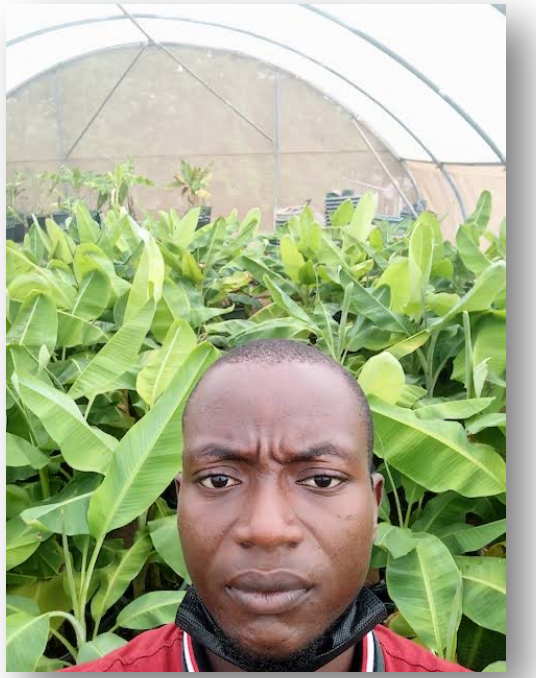


Emmanuel Olajide [1,3], **Laura Cortada** [3], **Delphine Amah** [1], **Danny Coyne** [3,4], **Wim Bert** [3], **Rony Swennen** [2,5] **and Yao A. Kolombia** [1]

[1] International Institute of Tropical Agriculture (IITA), IBADAN, Nigeria, [2] IITA, Kampala, Uganda, [3] Nematology Research Unit, Department of Biology, Ghent University, Belgium, [4] IITA, ICIPE Campus, Nairobi, Kenya, [5]Laboratory of Tropical Crop Improvement, Department of Biosystems, KU Leuven

Characterization of plantain *cv.* Agbagba infection with *Radopholus similis* and *Meloidogyne* spp. using macropropagated plantlets

Background

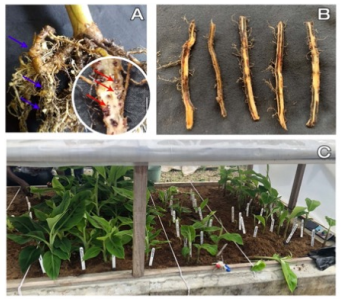
Plant-breeding programmes, especially those for banana and plantain, are time-consuming. Therefore, a better and fast screening process is needed to detect nematode resistant phenotypes. For resistance screening, the biggest bottleneck is growing clean planting materials quickly and for a relatively low cost. Recently, a rapid and cheap method of generating clean planting material (macropropagation) is making its way into mainstream adoption (Ntamwira *et al* (2017); Njau *et al* (2011), and has prompted the need to conduct host plant response to plant-parasitic nematodes (PPNs) using macropropagated plantlets.

Materials and Methods

The goal of this study was to determine the most suitable time to evaluate plantain germplasm for host resistance to PPNs under screenhouse conditions, using macropropagated plantlets. Acclimatized macropropagated plantain cv Agbagba plantlets were inoculated with 1000 mixed life-stages (no eggs) of *Radopholus similis* and 1000 *Meloidogyne* spp. juveniles in 2-litre nursery bags and assessed at 30, 60 and 90 days post-inoculation (dpi).

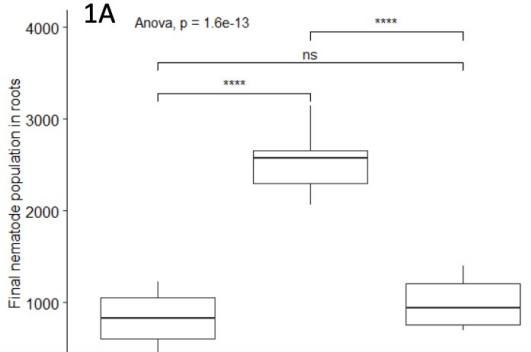
Findings

- Optimal evaluation of *R. similis* and *Meloidogyne* spp. occur at different time points (fig 1A-B, 2A-B)
- For *Meloidogyne* spp., nematode population can be evaluated 90 days post nematode inoculation (fig 2A)
- *R. similis* evaluation in 2-L nursery bags using macropropagated plantlets should be performed at 60 post nematode inoculation (fig 1A)
- It is important to evaluate *R. similis* when the nematode population is at peak and before too much damage is done.

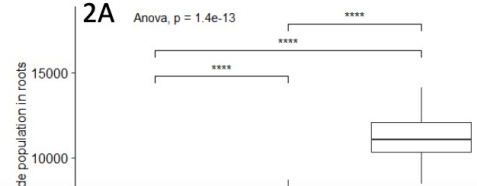


Plantain *cv* Agbagba infected roots showing (A) galled roots = (blue arrows), females = (red arrows) of *Meloidogyne* spp. infection (B) Lesion damage caused by *R. similis* (C) Macropropagated plant material

Results

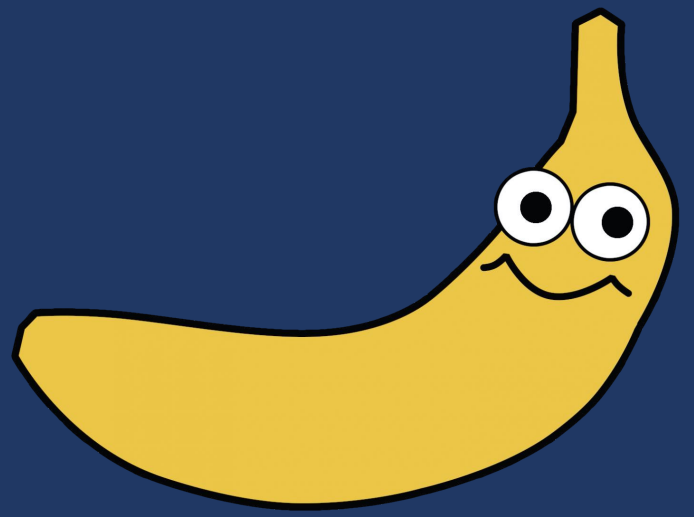


Results



Need to validate the compatibility to the field conditions.

Screen parental banana lines for resistance against *R. similis.*



Preliminary, forest soil, sand and cocopeat were evaluated for a suitable media.

Forest soil

Close to
the farm conditions

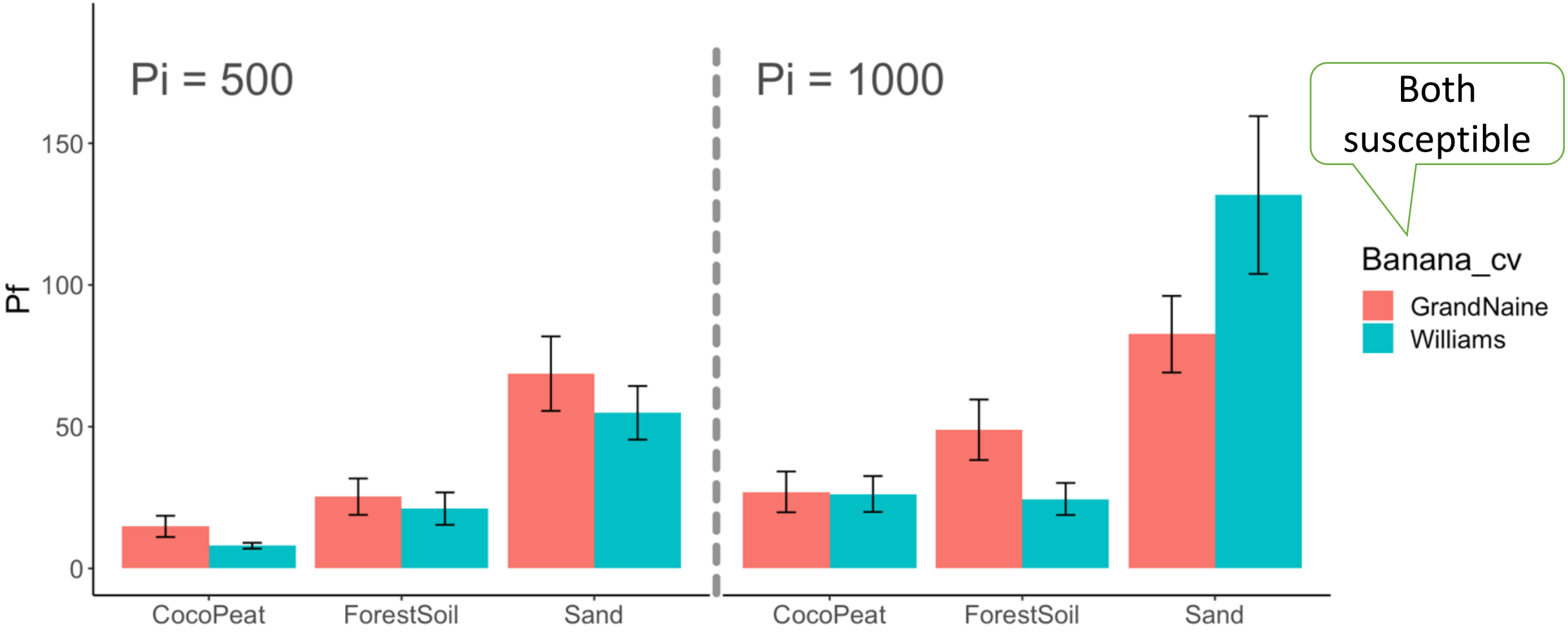
Sand

Nematode infection ↑
Processing is easy

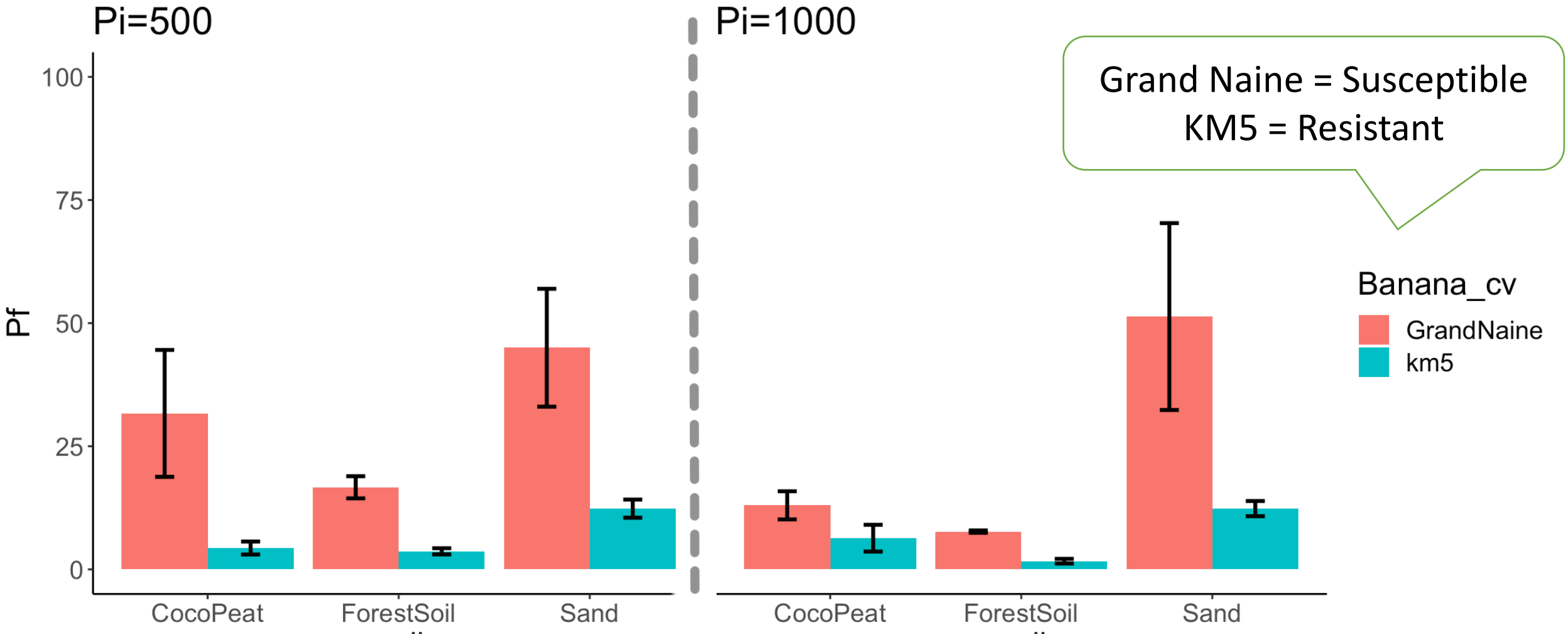
Cocopeat

Uniform plant growth

Sand had the highest nematode infection in Kenya.



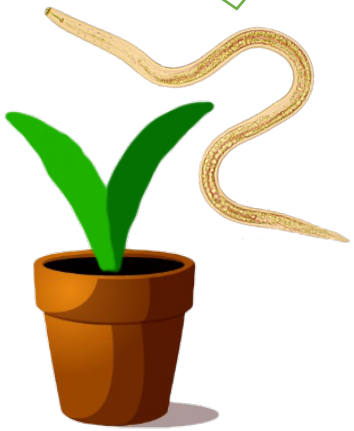
Resistant cultivar had lower nematode infection across the tested media type.



72 parental lines will be evaluated in Tanzania and Ug

Humid chamber for 4 wks
Weaning for 4 wks

Inoculate
With 1000 *R. similis*



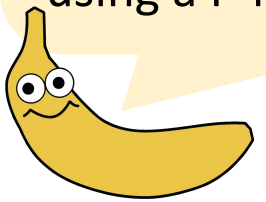
Bottom application of water/Fertilizer

Susceptible check

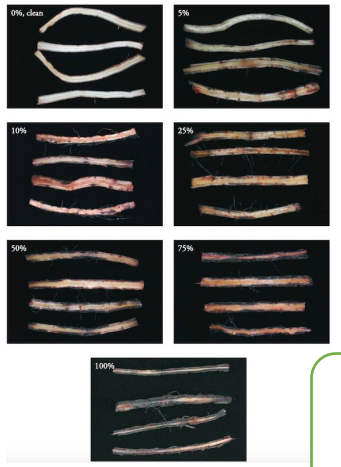
Check nematode infection at 8 wks

First trial is starting soon at both locations!

Tanzania = 29 genotypes
Uganda = 40 genotypes
using a P-rep experimental design.



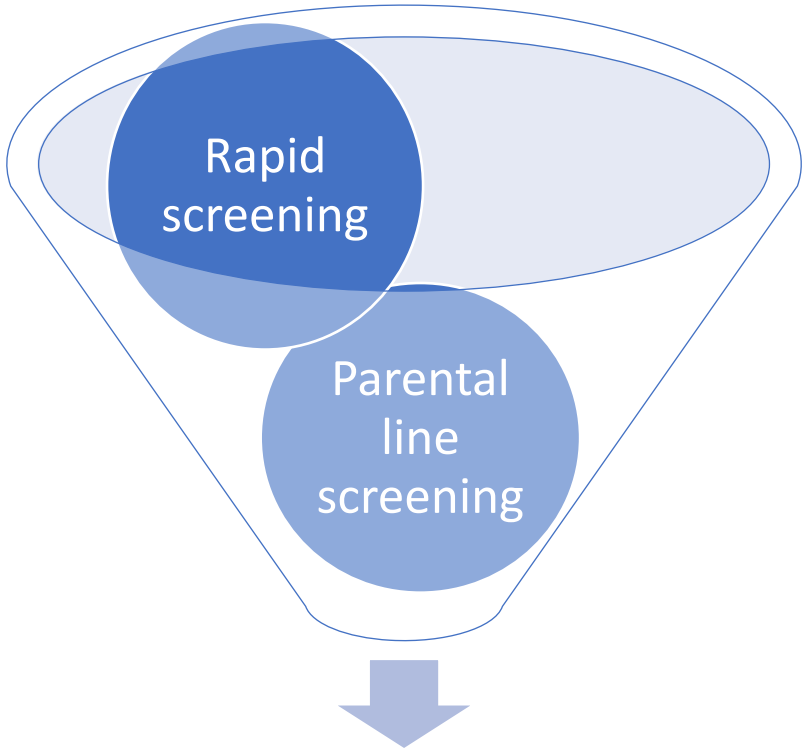
Nematode infection



Necrosis rating

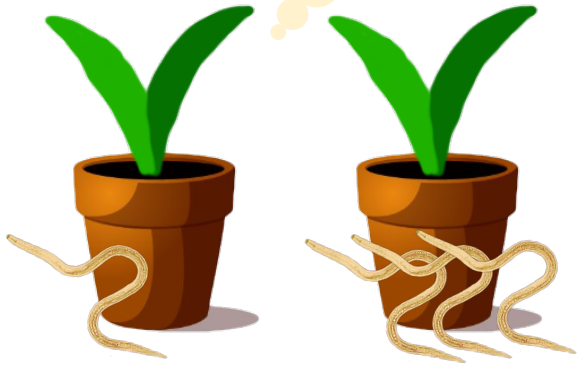
We are making progress in developing rapid screening method & screen for the nematode resistant parental lines.

Accurate and high-throughput screening system



Better banana breeding for nematode resistance

Which parental line to use for breeding?



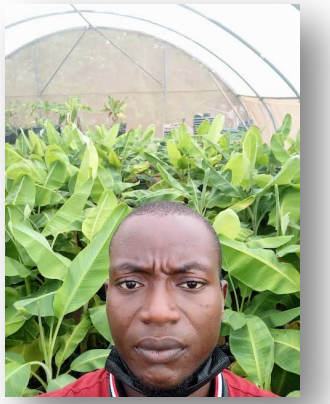
Thank you!

Kanan Saikai (Kenya)

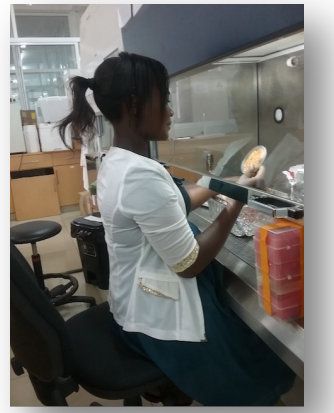
James Kisaakye (Kenya)

Joseph Kisitu (Uganda)

Emmanuel Olajide (Nigeria)



Happyness Justine (Tanzania)



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