Screening for resistance to the root burrowing nematode (*Radopholus similis*) in banana

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Introduction

Plant-parasitic nematodes (PPNs) such as *Pratylenchus* spp., *Helicotylenchus* spp., and *Radopholus* spp. amongst others are a major constraint to sustainable bananas (*Musa* spp.) production, (Stover and Simmonds 1987) and have been identified as a major factor contributing to declining production (Speijer *et al.*, 1999) especially among small holder farmers. the root burrowing nematode, *Radopholus similis*, has been reported as the most destructive nematode parasite of banana in the tropics (Gowen, 1993). To avert the yield reducing constraints due to nematode infestations, timely management and control of these bio-constraints is warranted. Nematodes can be controlled with chemicals, however, most of these chemicals have adverse effects to the environment and human health (Nyczepir and Thomas 2009). Furthermore, the chemical nematicides are too expensive for the small holder farmers (Savangikar 2004). Besides other nematode control and management strategies such as, development of nematode resistant banana genotypes through breeding is a promising strategy for the management of nematode infection in banana (Speijer and De Waele, 1997). This standard operating procedure focuses on screening banana breeding genotypes for resistance to *R. similis.*

Materials and methods

Plant materials

1) TC generated plant material including:
   a) Test genotypes (Parental genotypes and newly developed hybrids)
   b) Resistant controls:

i. SH3142
ii. Calcutta 4

c) Land race controls:
i. Mbwazirume
ii. Mchare
iii. TM-28 OBINO LEWAI

A few plants of Valery and Yangambi KM5 are included a side and not as part of the experimental treatments to test the efficacy of the nematode inoculum used

Other materials

1. Sterile forest soil
2. Sterile sand
3. 840-micron sieves
4. 1-liter plastic pots
5. Watering cans
6. Nematode cultures

Multiplication, weaning, and transplanting of tissue culture plantlets

- Banana plantlets multiplied in tissue culture laboratory are weaned and maintained in a humid chamber for 4 weeks before transplanting into 1-liter plastic pots containing a 1:1 mixture of sterile forest soil and sand sieved through 2mm sieve.
- The plantlets are left to acclimatize in the screen house for 4 weeks prior to inoculation.
- to effect quality control of the substrate, the pre-sterilized substrate is examined for nematode contamination by subjecting it to nematode extraction and identification using the modified Baermann technique as described in Coyne et al. (2007) prior to establishment of the experiment.

Experimental design

Parental genotypes

A partially replicated experimental design (P-Rep) is adopted with parental genotype occurring in duplicate and the checks in triplicate for the entire experimental set up. P-rep designs are useful when plant materials and space are limiting factors. In fact, P-rep designs allow for repeated trial evaluations at different locations. The experimental design as will be laid out in a screen house for the available 51 parental genotypes including checks is

shown in Table 1 below. The average efficiency for this design was 0.99 indicating that the design is optimal.

Table 1. P-rep lay out for banana nematode screening for 51 parental lines including controls

Block 1	29	6	42	**S5**	24	47	27	28	17	11
	51	45	1	10	39	**S4**	38	41	19	15
	34	44	14	**S6**	**S2**	37	26	50	32	18
	43	48	4	49	33	**S1**	9	36	22	**S3**
Block 2	39	**S4**	**S2**	28	45	23	19	24	35	**S6**
	7	20	44	3	16	48	1	2	26	31
	5	30	38	18	50	49	10	21	25	**S1**
	8	40	4	**S3**	46	12	47	**S5**	13	33
Block 3	27	41	43	**S3**	**S6**	35	13	36	51	11
	S1	46	31	2	29	14	42	21	16	40
	30	**S5**	6	20	**S4**	34	25	22	32	37
	15	8	12	17	**S2**	9	3	23	7	5

Note: *Numbers used in the blocks represent respective genotypes to be screened, and the alphanumerical characters represent controls in each block. The trial will comprise of 5 pseudo replicates per plot and 2 plots (hence 2 true replicates) in the entire trial for each test parental banana genotype.*

Hybrids

The augmented design resulting into an incomplete block design where standard checks are replicated in each block and test genotypes 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.

Inoculum preparation and Inoculation

- After isolation and multiplication of *R. similis* (annexes 1, 2, & 3), nematodes are washed from carrot discs and petri dish inner surfaces and suspended in water in a 500ml conical flask to the 500ml mark to form a stock suspension
- Determine the concentration of the stock solution by taking and quantifying (using a compound microscope) three 2ml aliquots of nematode suspension from the stock suspension.
- Basing on the concentration, constitute a working suspension from the stock suspension and transfer into another 500ml conical flask

- Prior to inoculation, calibrate a pipette to draw 250 nematodes per inoculation aliquot. An inoculation volume not less than 4ml suspension should be used
- Drill 4 holes (~5cm) at the plant base using a pencil and introduce 250 nematodes per hole using a pipette to make 1000 nematodes per plant, cover back the holes with soil after inoculation.
- Plants are drenched with soluble NPK following a bi-weekly interval at a rate of $2.5gL^{-1}$
- Uproot and observe the susceptible check plants (Valery) for nematode multiplication and necrosis to inform termination of the main experiment.

Nematode damage assessment

- Individual plants in respective pots are removed after softening the soil by watering
- Wash plant roots under running tap water to free the plants of any debris
- Harvest and weigh all roots from the corm
- Sort the roots into functional (OK) or dead (DE) and count the total number of roots in each group
- Select Ten (10) roots for root necrosis assessment
- Each of the 10 roots is chopped to a length of 5cm
- The 5cm root segments are dissected longitudinally to expose the cortical region
- For each root segment, one side of the exposed cortical regions is used to score for necrosis
- Each longitudinal root section is scored at scale of 20 for cortical necrosis and the score value expressed as a percentage
  Root necrosis data will be analyzed using the linear mixed models (random) under REML: *Genotype response = μ+ check effect + block effect + treatment effect + error*
- After scoring for necrosis, all roots from an individual plant are chopped into about 0.5cm pieces including the strands used in scoring for necrosis
- The chopped pieces are homogenized and 10g of the homogenate used in nematode extraction using the modified Baermann technique as described in Coyne et al. (2007) as described above
- After extraction, the nematode suspension is decanted to 25ml from which three 2ml aliquots are pipetted to compute for nematode density
- The nematode density of each plant is subjected to plant root weight adjustment to obtain to total nematode population for each plant root system

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Annexes

Annex 1: Isolation and multiplication of *R.similis*

- Banana roots infected with nematodes are obtained from the field, cleaned with tap water to free them of soil debris
- The roots are then subjected to nematode extraction by cutting them into ca. 1cm pieces, blended and subjected to nematode extraction using the modified Baermann technique as described in Coyne *et al*. (2007)

- The nematode suspension is then collected from the Modified Baermann tray set up into a baby jar
- After decanting the nematodes to 25ml volume, 2ml aliquots are pipetted into a nematode counting slide and identified under a stereo microscope
- Using morphological features, *R. similis* females and juveniles are picked from the suspension using a wire loop,
- The picked nematodes are then sterilized using streptomycin sulphate and transferred to readily prepared carrot discs in petri-dishes under a lamina flow hood
- The nematodes are then left to multiply in an incubator with temperature set at 28 ^{o}c

Annex 2: Sub-culturing of *R. similis* on carrot disks

- Select good discs containing nematodes
- Wash nematodes from the petri dish (2 ml suspension) into sterile test tube.
- Each petri dish should be washed into an individual test tube
- Prepare an ant-biotic solution by dissolving 0.06g of ant-biotic (streptomycin sulphate) in 10 ml of sterile and distilled water.
- Using a syringe, suck in the ant-biotic solution
- Fit the micro filter on the syringe (2 µm pore size)
- Release the solution through the micro filter into a sterile test tube
- Pipette 2.5 ml of the solution to each test tube
- Add 3 ml to each test tube. The final volume will be 7.5 ml
- Leave the samples to stand for about 2 hours
- Wash sample by Pipetting/reducing the sample volume to a negligible volume
- Add 5 ml of sterile and distilled water to each sample
- Leave sample to stand for 1 hour
- Again wash samples by pipetting/reducing sample volume to a negligible volume
- Add 3 ml of sterile and distilled water
- Leave the samples to stand for 30 s
- Add to the sample little volume of water (sterile and distilled) enough to inoculate the previously prepared carrot discs

Annex 3: Preparation of carrot discs for nematode culturing

- Select clean and sizeable carrots from market
- Wash the carrot with tape water and rinse with distilled water
- Dry the carrot by wiping them with a tissue paper
- Under the aseptic hood, hold the carrot with a pair of forceps
- Spray the carrot with 96 % ethanol and flame until it is dry
- Peel the carrot lightly with a potato peeler

- Again spray ethanol on the peeled carrot and flame until it is dry
- Peel the carrot and the cut it into sizeable disks that in your petri dishes
- Introduce the discs into petri-dishes
- Inoculate 2-3 micro drops (50-100 nematodes/micro drop) of previously prepared nematode suspension per disc
- Seal the petri-dishes with a parafilm
- Put the sealed petri-dishes into a container and place in an incubator at 28 °C