PhD Research Progress Report (2018-2019)

TITLE: Genetic diversity of banana nematodes in Nigeria and a novel approach for rapid resistance screening

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Timeline of study: 2018-2022

University: Ghent University, Belgium

Research Objectives

A. To develop an efficient rapid screening method for nematode resistance and screening of improved Plantain hybrids (PITAs) and parents against *Radopholus similis*.
B. To characterize the plant parasitic nematode populations in the main plantain and banana growing areas in Nigeria.
C. To assess the root-knot, *Hoplolaimus* spp., *Helicotylenchus* spp. and root-lesion nematode diversity from plantain and banana in Nigeria.
D. To establish DNA barcoding, and phylogeography of *Pratylenchus* spp. from banana in East Africa/Nigeria.

Achievements

A. Rapid screening methods and screening of PITAs and parents against *Radopholus similis*.

- The culture of banana and plantain nematodes in pots and on carrot discs (*R. similis*) was established.
- We were able to observe nematode penetration in susceptible plantain cv. Agbagba using TC plantlets in Murashige and Skoog Media. Next, we will do a comparative study between two reference genotypes (susceptible and resistant genotype) with the objective of observing the nematode reproduction and penetration in order to characterize the responses to parasitism in both genotypes.
- Pot evaluation of available PITAs and parents against *Radopholus similis* in 2L nursery bag completed with experiment repeated once.

B. Diversity Plant-parasitic nematodes populations in the main plantain growing areas in Nigeria.

- Scanning of main banana and plantain growing areas in Nigeria to establish areas with nematode problems is 70% completed and evaluation of nematode diversity ongoing.

Introduction

The sustainable production of plantain and banana in Africa is threatened by pests and diseases especially plant parasitic nematodes (PPN) which causes snapping and toppling of mature plantain plants. In Nigeria, the plant-parasitic nematodes found on plantain and banana include *Pratylenchus coffeae*, *Radopholus similis*, *Helicotylenchus* spp., *Meloidogyne* spp. of which *P. coffeae*, *R. similis* are the most pathogenic species (Speijer *et al*., 1997, Rotimi *et al*., 2001). Management of banana nematodes relies mainly on the repeated use of chemical nematicides. Evidently, this should be limited because they are expensive, detrimental to the environment and the increasing concerns about groundwater contamination and toxicity. Integrated pest management (IPM) methods based on banana and plantain germplasm has proved to be promising, where resistant banana and plantain genotypes will be identified through resistance screening assays. Resistance screening is conventionally assayed in the field, pots, plastic bags, *in vitro* and single primary roots inoculation. A rapid screening model that needs lower nematode inoculum, fewer plants, less space, not influenced by root age or root growth rate is needed. Likewise, some genera of PPN on banana and plantain in Nigeria has not been carefully examined. The main objective of this research is to update the plant-parasitic nematodes (PPN) in the main banana and plantain growing areas in Nigeria and to develop an early rapid nematode resistance screening protocols for banana and plantain.

Summary of my research activities

The objective of my research is to identify natural sources of resistance to *R. similis* among the IITA improved plantain hybrids called PITAs and their parents, to develop efficient rapid screening methods for early and easy screening and to identify new sources of resistance. To achieve these aims and objectives, the nematode population from banana and plantain needed to be collected and cultured for the different optimization assays and for the screening of plantain and banana germplasm. The initial challenge occurred due to the difficulties to establish enough diversity of pure cultures of *Pratylenchus* spp and *R. similis*. This challenge was overcome after a few months, and *R. similis* cultures were established on carrot discs and also backed up in pot cultures in the screenhouse. *Helicotylenchus* spp, *Pratylenchus* spp and *Meloidogyne* spp. were also cultured in pots.

We are also looking to see if there is a rapid screening method we can design to evaluate resistance in banana and plantain. A method that will improve efficiency for space, time, nematode inoculum and which in turn will reduce variability. I tested a number of methodologies with limited success. We were able to confirm *R. similis* penetration in tissue culture (TC) plantain *cv* Agbagba by staining with acid fushin after 10-days. In 2018/2019, we couldn't do a comparative study to observe nematode penetration and nematode reproduction between reference resistant and susceptible genotypes due to the unavailability of susceptible and resistant reference genotypes.

In the second part, the available PITAs and parents were phenotyped in 2Litre nylon bags for resistance to *R. similis*. Macropropagated plantlets were used for the host-plant

response experiment. Three sword suckers of 20 different PITAs and 19 identified parents were collected and treated with hot water (52°C-55°C). Roots were removed and the leaf sheets were cut away exposing the buds. The entire corm was planted in the macropropagator. After 4-6 weeks, plantlets were extracted from the macropropagation chamber, and then transplanted in 2L nursery bags. Nine PITA hybrids and 8 parental lines were screened against *R. similis* in the first experiment, and eleven PITA hybrids and ten parental lines were screened in the second experiment for resistance against *R. similis* in Nigeria. False Horn cv. "Agbagba" was included as a susceptible control, Yangambi Km5 or/and Pisang Jari Buaya as the resistant controls. Macropropagated plantlets were inoculated with 1000 *R. similis* 4 - 8 weeks after transplanting into 2 litre pots filled with sterilized loamy soil. The plants were maintained in the screen house and harvested 8 weeks after inoculation and the reproduction factor, percentage of necrosis, the total number of nematodes (roots + soil), and other agronomic characteristics. The experiment was repeated once.

On the side of the collection of plant parasitic nematode specimens from different geographic locations in Nigeria. In 2019, we were able to collect PPN specimens from South Eastern Nigeria and a few specimens from Southwest, the survey will continue in 2020. The samples were analyzed, nematode were extracted and identified to the genus level, and out of all the samples, *Hoplolaimus* spp. was abundant from the soil and root samples collected from IITA plantain breeding plantation at Onne High Rainfall research station in River state Nigeria. The *Hoplolaimus* spp. was identified in all the samples, they were kept in DESS and fresh samples kept in water and DESS for further analysis.

Next step

January 2020, the *Hoplolaimus* spp. will be identified using morphological and molecular analysis. The nematode will be fixed and the morphological characteristics such as number of lines in the lateral field, vulva location in relation to body length, tail terminus, female and male body, lip annuli, shape of stylet knobs, stylet length will be recorded. Molecular identification to further confirm morphological identification will also be performed, the DNA will be extracted from single adult nematodes and the evaluation of ITS, 28S (D2/D3), 18S loci and a portion of the mitochondrial COI will be performed. PCR product will be sequenced, and the sequence will be deposited in GenBank. The sequence will be compared with sequences on NCBI's nucleotide database.

For rapid screening development, a comparative study between Yangambi Km 5 (resistant to *R. similis*) and Grand Naine (susceptible to *R. similis*) will be performed to observe nematode penetration and reproduction using TC plantlets in MS media. The TC plantlets will be obtained from the International Transit Centre (ITC) of Bioversity International at K.U.Leuven, Belgium. Each TC plantlets will be inoculated with 50 mixed stages of *Radopholus similis*. Two plantlets will be removed from the medium daily starting from the 1 dpi up to 15 dpi. To compare nematode penetration and reproduction, the roots will be cut into 1 – 2cm fragments and will be stained in acid fuchsin using the method described by Byrd *et al*. (1983). The stained nematodes will be counted with the aid of a microscope. Nematodes will be extracted from the medium by the modified Baermann technique (extraction tray method). The extracted nematodes

will be counted with the aid of a microscope. The mean at each time point will be calculated. Photographs will be taken using a high performance imaging system.

References

Bybd Jr, D.W., Kirkpatrick, T. and Barker, K., 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of nematology*, *15*(1), p.142.

Rotimi, M.O., De Waele, D. and Speijer, P.R., 2001. Plant parasitic nematodes associated with plantain (Musa spp., AAB-group) in southern Nigeria and their relative importance compared to other biotic constraints. *Nematology*, *3*(5), pp.423-436.

Speijer, P.R., De Waele, D. (1997). Screening of *Musa* germplasm for resistance and tolerance to nematodes. INIBAP Technical guidelines N°1. INIBAP, Montpellier, France.