

# ANNEX 1

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## 1. Governance

### 1.1 Project Team Composition

#### Members of Steering Committee (SC):

1. Victor Manyong (Chair), IITA
2. Rony Swennen, IITA
3. Jerome Kubiriba, NARO
4. Altus Viljoen, SU
5. Brigitte Uwimana, IITA
6. Inge van Den Bergh, Bioversity International
7. Lucas Mueller, BTI
8. Jim Lorenzen, BMGF (non-voting member)
9. Danny Coyne (secretary), IITA

#### Members of Science Advisory Group (SAG):

1. Steve Rounsley (Chair), Crop Breeding and Molecular Markers, Dow Agrosiences, USA
2. Hale Ann Tufan, Bioinformatics, Project Manager NextGen Cassava, Cornell University, USA
3. Jane Gibbs, Agribusiness Development and Management (Crop Physiology and Breeding), The University of Western Australia
4. Eva Weltzien, Crop Breeding, previously ICRISAT, Mali, now independant, Germany
5. Richard Sikora, Plant and Soil Health, University of Bonn, Germany

#### IITA Management Team (MT):

1. Project Coordinator- Rony Swennen
2. Project Manager- Danny Coyne
3. Project Administrator- Scola Ponera

#### Work Package (WP) Leadership

- |      |   |
|------|---|
| WP1. | Jerome Kubiriba, NARO<br>Deputy: Robooni Tumuhimbise, NARO  |
| WP2. | Altus Viljoen, SU<br>Deputy: Diane Mostert, SU  |
| WP3. | Brigitte Uwimana, IITA<br>Deputy: Elizabeth Aitken, UQ  |
| WP4. | Inge van Den Bergh, Bioversity International<br>Deputy: Rhiannon Crichton, Bioversity International |
| WP5. | Lucas Mueller, BTI<br>Deputy: Guillaume Jean Bauchet, BTI   |
| WP6. | Danny Coyne, IITA<br>Deputy: Scola Ponera, IITA   |



## Improvement of banana for smallholder farmers in the Great Lakes Region of Africa

### 1.2 Minutes of the Project progress workshop

24-27<sup>th</sup> April 2017

#### Day 1 - 24<sup>th</sup> April

##### 9.00-9.05 Welcome from the Chair; by Wilberforce Tushemereirwe (Chair)

The Breeding Better Bananas project (BBB) has been on-going for two and a half years now and this is the 3<sup>rd</sup> annual meeting held with all participants of the project to assess progress on activities. The three main objectives of this annual meeting related to the project are:

- **To reflect** on achievements, reasons for underperformance.
- **Evaluate progress.**
- **Project** on tangible deliverables for this year- and propose adjustments on the work plan –if needed for the coming ones.

#### Further reasoning for this workshop is to:

- Welcome new and potential partners that have come on board recently (FAO, Palacky University -Czech Republic, and the University of Malaya - Malaysia).
- Strengthen the team.
- Discuss on students' support through the project.
- Foster interactions and feedback between the steering committee (SC), the scientific advisory group (SAG) and the work packages (WPs).

#### The management and overall coordination of the project of the BBB projects relays on the following IITA members:

- Team management/team Leader: **Rony Swennen**- based in Arusha (Tanzania) – at IITA offices at the Nelson Mandela African Institute of Science and Technology.
- Administrative tasks: **Scola Ponera**- based in Arusha (Tanzania) – at IITA offices at the Nelson Mandela African Institute of Science and Technology.
- Danny Coyne: Project Manager (technical, logistics and advisory tasks) - based in Nairobi (Kenya) – at the IITA offices of *icipe* campus.

Before the session continues, a minute of silence and reflection are requested for Mr Mgenzi Byabachwezi who deceased early in March 2017. Mgenzi was the Principal Agriculture Research Officer at ARI-Maruku and Leader of the Tanzanian National Banana Program. He was one of the five site coordinators for the regional testing. His knowledge of banana production systems and his unmatched skills to work with local banana-producing communities were crucial in the successful conduct of the baseline study. Full of energy and humour at work, Mgenzi made every task look easy and enjoyable, especially when working with smallholder farmers in rural areas.

##### 9.05-9.15 Remarks from Project Leader & Manager; by Rony Swennen and Danny Coyne.

#### Danny Coyne:

- The schedule for the meeting during the following days; a lot of emphasis is placed on the **need to be socially-interactive**, and to get WPs to interact among themselves as much as possible, and with the SC and the SAG as well, in order to get their advisory.

- The website is ready and functional, and is in the process of being continuously improved (<http://bananabreeding.iita.org/>). More comments and suggestions to be provided during communication session –day 3.
- Early reporting and accomplishment of deadlines has improved massively but still some groups need to deliver on time to ensure the project is not penalized for that (see more on this on session “Communication”- Danny Coyne- Day 3).
- A list with the names and the e-mail addresses of all attendees to the BBB workshop are available on the intranet.
-  See on the Intranet

 Participant List Banana Breeding Project Meeting 24-27th April,2017 Kampala- Uganda(001)

[Download](#)

### Rony Swennen:

He provides the technical summary of the projects' progress.

#### Technical highlights of WP1:

- Seed production has increased 3-fold as opposed to the 15-20% initial target.
- 48 hybrids for PIY.
- 31 Improved diploids received from EMPBRAPA and resistance to *Fusarium oxysporum* is confirmed here in Uganda.
- Seed set increased to 108%.

#### Technical highlights of WP2:

- A collection of 3 pests/diseases has been created already.
- The pest and disease manual is ready and printed; will be distributed here among all the participants.
- Pest and disease training workshop took place in 2016 with the participation of relevant stakeholders.

#### Technical highlights WP3:

- Diploid segregating populations being phenotyped/genotyped for QTL mapping.
- Genomic prediction: predictive model for 2 cycles.

#### Technical highlights WP4:

- Five testing sites have been established in Uganda/Tanzania.
- Base line study is being processed, and data will be released soon. This will help us to better understand traits for importance to end users.

#### Technical highlights WP5:

- MusaBase is ready and functional (<https://MusaBase.org/>);
- mutual linkages between MusaBase and the BBB's website have been created in both portals.
  -  See on the Intranet: Presentation 1-Welcome remarks Coyne-2017

**9.15-9.25**

**East Africa Operations; by IITA Director East Africa and SC chair (Dr. Victor Manyong)**

*Dr. Victor Manyong* is the East Africa Director Hub of IITA, based in Dar es Salaam (Tanzania). Dr. Manyong will be replacing Dr. Ylva Hilbur as the SC chair, who recently departed IITA. General acknowledgments are provided to the the National authorities and representatives of the Uganda government, the entire BBB team, and the newly arrived partners. Dr. Manyong emphasizes on the need of having breeding projects which are keen on ensuring that the research developed is reaching the populations that need it the most, and he states that the BBB project is firmly working towards that approach, with a strong participatory component that will translate into a better access of local communities of the improved banana varieties.

### 9.25-9.35 **BMGF Feedback;** by Jim Lorenzen

*Dr. Jim Lorenzen* is the Program Coordinator of the BBB project at the Bill and Melinda Gates Foundation (BMGF). He indicates that the banana crop came into the portfolio of the BMGF only 4 years ago, as it was listed as a key crop for providing food security in East Africa (EA) highlands. Jim says it is very positive to see the great south-south interaction as an outcome of this project, within Africa, but also between Africa and India, Brazil or Malaysia where other partners are. On this regard, Jim points out that “*progress happens where communities develop and cooperation is enhanced*”, and that the BBB project is a good example of that.

Related to the National Agriculture Research Organization (NARO) in Uganda, it is acknowledged that it is a key stakeholder in the region and in BBB project, who is conducting an enormous effort to invest on breeding and high quality research for banana in the region, and due to that NARO is becoming a point of reference for other National Research Organizations in EA related to banana breeding. As final remarks, Jim says that interaction among stakeholders is key to make breeding more efficient and improve genetic gain, and that the BMGF is key on knowing more on how this interaction will revert into tangible results on breeding better bananas for the region.

### 9.35-9.45 **Remarks Scientific Advisory Group and introductions of members;** by Steve Rounsley

*Dr. Steve Rounsley* is the chair of the SAG of the BBB project. He welcomes the national authorities of Uganda and the members of the BBB projects. He takes the opportunity to thank the management team of the project for having included him as a member of the SAG. His professional background is mainly on genomic work and he has worked in private seed companies, and now he is currently working on animal genetic improvement at Genus as a Senior Director, Applied Genomics since July 2016, based in Wisconsin, USA. He expects that his expertise will help the BBB project to achieve its main objectives and improve banana breeding in the long term. The other present members of the SAG present in the meeting are introduced:

- Jane Gibbs (member of the SAG) - Main expertise on plant physiology on banana- Australia
- Eva Weltzien-Rattunde (member of the SAG) - Main expertise on seed dissemination issues and gender- Germany.
- Richard Sikora – Main expertise on nematology, entomology, & soil ecology. Professor emeritus Bonn University- Germany.

### 9.45-10.00 **Welcome remarks and opening;** by Dr. Ambrose Agona, Director General, NARO

*Dr. Ambrose Agona* is the Director General of the National Agricultural Research Organisation (NARO) in Uganda. Dr. Agona presents it respects to the other members of the national Ugandan organizations, the BMGF, the BBB team and the press. He starts his speech stating that the BBB project is moving towards the right direction, by strengthening the aspects of the banana that increase production in the field. Uganda produces around 10 MT of banana and this crop is the number one source of carbohydrates consumed in the country, therefore research in this field is crucial to ensure food security in the country among the poorest households. Therefore, the BBB project should be able to respond to the increment of the local population, and the reciprocal increase on the demand of banana as a staple food, by improving the productivity of the local banana varieties within the region. Dr Agona indicates that the excellent achievements of the BBB project are visible already, as some of the objectives have been achieved and even overpassed. Still, he proceeds, yield gaps on farm persist and this is one of the main issues that the project should be able to address.

Additionally, the Director General urges researchers and partners to ensure that the new breeding lines developed within the BBB project are taken to the field and tested there to prove improved performance under the local agro-ecologic and phytosanitary conditions of the area. At the end of the day, he says, the project must translate into increased incomes of Uganda’s smallholders. The poverty index among households in Uganda has gone from 60% down to 19%, and this has been mainly achieved through contributions in agriculture, and banana has been one of the main crops that has helped on this. Therefore, the BBB project is particularly relevant and the new banana projects associated to it.

### 10.40-11.00 **Introducing the BMGF Banana Agronomy Project;** by Jerome Kubiriba

Jerome Kubiriba is banana breeder at the NARO in Uganda, and leader of WP1. In this session he will be presenting the Banana Agronomy Project (BAP), funded by the BMGF, where NARO will be the lead partner and IITA will be a partner collaborating in the implementation of it. There is an urgent need to intensify banana production, and as Dr. Agona has indicated previously, research to bridge the yield gap is a crucial factor to ensure that the new breed

lines from the BBB project, plus the non-improved local varieties, can outperform once they are in the field. Therefore, the project will be focussing on addressing the bio-physical stress suffered by the banana crop in the field, as this is one of the most limiting constraints that this crop faces. On the other side, the benefit of integrating management systems has been proved and should be promoted and expand the knowledge among farmers. To ensure success of this project, a wider stakeholder involvement on it is required.

☞ See on the Intranet: Presentation 2 Banana agronomy launch April 2017

### 11.00-13.00 Work Package update reports and general Discussion; *by all.*

A PDF document with all the presentations of the WPs for this session is available on the intranet<sup>1</sup>.

☞ See on the Intranet: Presentation 3 Session 1100-1300 h. Day 1-2017

#### WP1: Banana Breeding

##### Main highlights on achievements:

- 5,825 hybrids from 4x-2x crosses
- 3,461 hybrids from 2x-2x crosses
- 173 hybrids from 3x-2x crosses
- 185 hybrids from Mchare-2x crosses
- 48 hybrid selections from an EET of 930 plants (4x-3x)
- Over 1000 hybrid seedlings across ploidy levels to be planted
- Matooke and Mchare floral development and fertility studies-on going

For most of the outputs the WP has achieved 100% of the expected, and in many cases the target has exceeded 100%; only in very few outputs the team has not been able to achieve the expected targets but it is expected that these will be achieved by September.

##### Main challenges encountered have been:

- A microscope to conduct the study of the stigma development has not been acquired yet, so this year's deliverable is yet pending. To be procured soon.
- Pictorial data on catalogue of the banana flower: it has been achieved at 60%; remaining 40% to be achieved by October 2017.
- Challenges in the field trials have also been faced, such as drought and the construction of an electrical line that went across one of the fields where the banana plants were maintained.
- Finally, research funds coming late have been an additional challenge - but timely submission of technical reports should be able to help to get the installments disbursed on time.

**Question 1:** What have been the main progress related to flowers' fertility?

**Answer:** The team has been working on the use of sucrose solution to improve stigma's receptivity and banana fertility. Results are quite promising (see poster "*Effect of Glucose Solution on Stigma Receptivity and Subsequent Seed Set in EAHBs*", by Waniale A., A. Tugume, R. Tumuhimbise and R. Swennen).

**Question 2:** What is the current % of germination?

**Answer:** It is between 20-30%.

#### WP2: Pests and Diseases in Bananas.

##### Main highlights on achievements:

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<sup>1</sup> Individual PP presentations of this session of each WP are also available on the BBB website.

- A marker for *Fusarium oxysporum* f. sp. *cubense* (Foc) which is very accurate has been developed. And new markers will be available with the next 6 months. Characterization of 208 isolates has been completed up to VCG level; VCG groups or VCG complexes of Foc identified in the five screening sites (Kawanda, Mbarara, Arusha, Mbeya, Kagera) and varieties affected.
- Identification of plant parasitic nematodes (PPNs) is being conducted morphologically and for the banana weevils, the molecular markers are already validated. Banana weevils were captured and reared on detached banana corms. Eight banana weevil populations are currently being maintained at Kawanda.
- Mapping of the distribution of pests/diseases: GPS coordinates have been taken; the mapping of the distribution of nematodes using GPS coordinates is ongoing in Tanzania with good progress. A comprehensive map with all the GPS coordinates for all the pest and diseases has not been created yet, and will be done once the coordinates missing for certain sites and pests will be available.
- A training to enumerators on collecting Sigatoka data took place, using the harmonized disease screening protocols developed under this project (Viljoen, A., Mahuku, G., Massawe, C., Ssali, R.T., Kimunye, J., Mostert, G., Ndayihanzamaso, P. and Coyne, D.L. 2017. *Banana Diseases and Pests: Field Guide for Diagnostics and Data Collection*. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria)<sup>2</sup>.

Main challenges encountered have been:

- *P. fijiensis* isolated collected in Uganda originally failed to discharge spores. What we know now is that isolations have to be conducted from freshly collected samples, and this is the methodology applied now.
- Evaluations of Sigatoka at regional testing sites has been completed for Mbeya, Arusha and Kawanda. Samples of diseased leaves have been collected from evaluated plants and are being tested to confirm pathogen identity; there is a 50% variance related to the expected progress, and that is due to the fact that the regional trials were planted late and 2016/2017 was extremely dry. This affected plant establishment and symptom expression.
- Only the Tanzania team has been able to collect samples of nematodes to identify distribution and provide data on abundance on three sites nematode species of *Radopholus similis* and *Pratylenchus goodeyi*. NARO in Uganda will be collecting nematode populations for identification and pathogenity assessment.
- Research on the weevil component has been slowed down due to a delay on the transfer of funds to the team in Tanzania.

No questions from the audience.

### WP3: Molecular tools and development of genomic selection

Main highlights on achievements:

- The WP is progressing well, considering the initial time-line established for the project:
  - Year 1 and 2- Populations were developed for mapping (QTL analysis) and training (phase 1 for genomic selection).
  - Year 2, 3 and 4: Phenotyping of the mapping and training populations.
  - Year 4: Genotyping
  - Year 4 and 5: Genotyping + Phenotyping data (linkage maps and QTL mapping) and development of predictive models.
- Foc SR4:
  - The team is building well on previous research from the University of Queensland and University of Malaya;
  - UQ there has been a fine mapping of the resistance region (from 33 to 15 candidate genes in this region).
  - The UM population was lost. New crosses are now progressing. Availing genotypic and transcriptomic resources.
- Weevil: phenotyping of two banana populations is on-going in Kawanda (62%) and Sendusu (23%) and it is expected to be finished by March 2018.
- Nematodes: phenotyping of two banana populations is on-going in Sendusu and it is expected to be finished by November 2017.

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<sup>2</sup> Available on the BBB-Website.



- Phenotyping for flowering and harvest for 3 cycles is going on in 4 sites (Sendusu-low input; Sendusu-optimum input; Mbarara; Sendusu ETT lines).
- Progress in genotyping:
  - Use of 19 SSR markers at IEB to check for pollination mistakes
  - Complete for Kasaska x Borneo
  - Genotyping with 20 ISSR and 1 IRAP from UM (Monyet x Kokopo)
  - Dense SNP markers to be provided this year (Chip)
- Capacity building of 4 *PhD* students (SLU; UM; SU; IEB) and 3 *MSc* students (KU Leuven; Makerere University).

Main challenges encountered have been:

- On the phenotyping of Foc R1 in Arusha there are no indications of damage of the pests/diseases on the plants of the two populations that are being studied there –this needs to be discussed internally in the WP3 meeting.
- Mapping populations with pollination mistakes.
- Communication- a lot of different time zones, that makes communication among all packages members difficult over skype.

**WP4: Regional testing**

Main highlights on achievements:

- Analysis on the Baseline Survey is progressing well:
  - 5 target regions surveyed.
  - 1000 households (HH) visited- 1325 respondents (aprox. 50% men and 50% women).
  - 100 focal group discussions (FGDs) (separately for men and women, plus mixed groups).
  - Notes from FGD are being typed and translated into English. Coding of the FGD is on-going.
  - The programming R<sup>3</sup> code to generate descriptive statistics and plots, has been created.
  - Work on baselines data analysis and compilation of technical report, and papers, will be dominating activity of 2017
- Dr Pricilla Marimo (Post-Doctoral Fellow) has been recruited as gender specialist by Bioversity International to study and identify the gender-differentiated trait preferences across the banana value chain, in order to bring these into our breeding process in East Africa; her terms of reference (ToRs) have been shared within the all the project members.
- On the standardization of field protocols:
  - The data collection protocols have been standardized and compiled.
  - The crop ontology dictionary has been updated with a set of traits and variables and it is now available on-line.
- Related to participatory trials:
  - All fields planted in April and May 2016.
  - All field planted with QR codes and plants are labelled individually and registered with the codes (including alpha-numerical format as well).
  - Weather station installed in each site.

Main challenges encountered have been:

- Budget for trial maintenance insufficient to cover all costs for the necessary activities (i.e. mulching or manure application) was delayed; in addition, the money for data/internet connection raised by site managers as issue. Currently, Bioversity is topping up from own budget for most, but not all, items – this to be discussed with the SC and the project management.
- Battery life of tablets problematic, and connection to power banks not possible; New tablets and power banks bought; will be distributed to partners end of April / early May 2017.

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<sup>3</sup> R- Refers to the statistical free software for analyses.

**Question 1:** Is there any code of conduct/ethical policy in place related to the contentment provided by interviewees and the publication of the personal data gathered from HH-interviews for the baseline survey?

**Answer:** At the beginning of the questionnaire there is a question where interviewees are being asked if they “consent” to reply the questionnaire, and they are informed that the data will be used sole for research purposes.

**Further feedback from the audience:** This should be clearly indicated and backed up with in order to publish the results of the baseline survey.

## WP5: MusaBase Update

### Main highlights on achievements:

- **About 270 terms in ontologies have been inserted into MusaBase.** Still, the team/project has to focus a lot on uploading data (WP2-WP4) in the coming months.
- MusaBase now provides improved support for trial design and for plant-level phenotyping.
- Barcoding has improved, and now it includes 2-D barcodes, support for field book and database-direct phenotyping and easier printing.
- Database-direct Phenotyping: now we can use MusaBase website directly in the field from tablet
- The crossing manager currently supports different types of crosses that can be documented (multicross, polycross, reciprocal).

### Main challenges encountered have been:

- The main one is that, despite the joint efforts in 2016 to produce a single ontology list, WP1 and WP2 are using a different ontology than WP4, and many times the differences are difficult to reconcile (i.e. finger diameter, measured in cm or in mm?). This needs to be sorted as soon as possible.

## Plenary session for discussion:

Comment 1 from the audience related to the “Banana Agronomy Project”: Farmers are not using fertilizers now so the process of intensification should be done carefully to ensure that farmers are applying sustainable intensification practices. Cost-benefit analysis should be conducted. Breeding and agronomy projects should be connected to ensure that we do breeding for agronomic traits and that this translates into benefits once sustainable intensification is applied.

## Day 2: 25<sup>th</sup> April

**8.00 AM to 8.30 AM**

**What should be discussed during WP meetings, Briefing on results tracker & Matters arising;** by *Rony Swennen*

Presentation by Fazil Dusunceli- FAO representative (Plant Production and Protection Department): Introduction of **Efforts of FAO and the Global Programme on Prevention of Banana Fusarium Wilt Disease (Foc TR4).**

FAO is currently hosting the Secretariat for the World Banana Forum<sup>4</sup> that involves all players in banana value chain (from production to marketing), farmers’ organizations, researchers, agronomists, etc. Within this, FAO is leading a global platform aimed at joining efforts globally for the control of *Fusarium* Wilt Disease (Foc TR4) in other to strengthen resilience of the banana systems, reducing diseases risks and impacts. This project fits into FAO’s strategic objectives (SOs) SO2 and SO5 and on the EMPRES (Emergency Prevention Systems) group’s activities. The three main objectives of the program are:

<sup>4</sup> See: <http://www.fao.org/world-banana-forum/about-the-forum/en/>

1.- Interact at the global level, to enable a better environment:

- Collaboration facilitated among already existing projects.
- Development of policies and strategies at the regional level.
- Improve the capacities of national plant protection/agricultural programs.

2.- Improve the existing prevention methods (early warning systems).

3.- Improve integrated management of TR4, plus other *Fusarium* races and other pests/diseases (i.e. nematodes).

There needs to be global effort to manage and control Foc TR4 for Asia (already TR4 there), Africa (on alert) and Latin America (on a prevention mode). The implication of FAO was more than justified considering the widespread of this disease (see Figure 1). The platform has identified the need for breeding against TR4, and is IITA currently leading the breeding efforts of this FAO platform<sup>5</sup>.

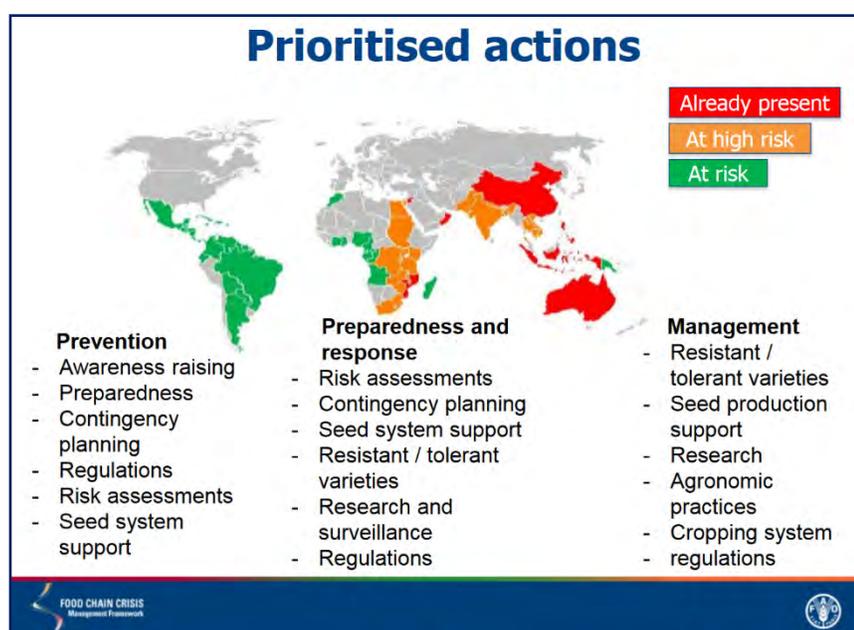


Figure 1<sup>6</sup>. Geographic location and priority interventions against FoC TR4 of areas affected (in red) and at high and low risk (orange and green, respectively).

The global programme on prevention of FOC TR4 has the following outcomes & outputs:

**Outcome 1.- Improved prevention for spread of Foc TR4 into banana-growing countries and regions.**

- Output 1: Policies and strategies improved and awareness level enhanced at all levels for improved prevention.
- Output 2: Surveillance, early detection and monitoring approaches and systems improved
- Output 3: Risks assessed and plant health-related legislation and phytosanitary practices enhanced.

<sup>5</sup> For more information, please visit: <http://www.fao.org/world-banana-forum/projects/fusarium-tr4/en/>

<sup>6</sup> Fazil Dusunceli, FAO (2016); available at: [http://www.fao.org/fileadmin/templates/banana/documents/Docs\\_Resources\\_2015/TR4/FAO\\_s\\_efforts\\_and\\_the\\_global\\_programme\\_on\\_prevention\\_of\\_banana\\_Fusarium\\_wilt.pdf](http://www.fao.org/fileadmin/templates/banana/documents/Docs_Resources_2015/TR4/FAO_s_efforts_and_the_global_programme_on_prevention_of_banana_Fusarium_wilt.pdf)

## Outcome 2.- Improved preparedness and the integrated management of Foc TR4 at field level.

- Output 4: Capacities strengthened to improve preparedness and prevention.
- Output 5: Integrated management strategies improved and implemented to reduce disease impact and pathogen spread.

## Outcome 3: Enhanced international synergy and collaboration.

Output 6.- Regional and international interaction, collaboration and information sharing enhanced.

☞ See on the Intranet: FAO TR4.Progr.Framework.xls

**8.30 AM to 8.50 AM**

**What should be discussed during WP meetings, briefing on results tracker & Matters arising; by Rony Swennen**

The project is ahead of time and focused; WPs are requested to look at their variance and identify why delivery is delayed and address the delays or draft an alternative strategy to overcome those areas which are behind on implementation. During today's sessions, what should be discussed? Some ideas for discussion are presented by Rony:

### WP1 should reflect on:

- Which Matooke delivered the NARITA?
- Which Matooke delivered the new 48 selected hybrids (this is not linked to amount of seeds and amount of hybrids)
  1. All PYT material from IITA and NARO should be evaluated in the same field
  2. Reduce breeding blocks because:
    - Enough seeds
    - Enough hybrids
  3. Is there a need to use genetic relationships as part of the crossing schemes?
  4. Should EMBRAPA and IEB/IITA use the same SSR?
  5. What can WP2-5 offer NOW to WP1-There is a need to open up information to the breeders.

### WP2 should reflect on:

- List of germplasm screened:
  - Different for each pest/disease
  - No link to breeding material
  - No link to segregation populations
- Mapping of pests/diseases:
  - Country
  - Testing sites

### WP3 should reflect on:

- QTL analysis for Breeding Selection (BS)? No molecular markers for Sigatoka are being done?
- Genomic prediction: Are we using all the criteria of the farmers for the genomic prediction models?
- Use average or individual info per genotype
- What needs to be improved when we redo the work for plantain?
- Are we using all criteria of the farmers?
- Plantain sequencing: what about EAHB?

### WP4 should reflect on:

- Start baby trials when mother trials are finished
- Will we process the new 48 hybrids in the same way as the current NARITAs?
- Any suggestions for the current breeding selection criteria?
- Linkage of the information from this packages with the breeders should take place as soon as possible.

#### WP5 should reflect on:

- There is mislabeling among accessions.
- No IITA ontology = there is breeder's ontology
- When can we actually start using this website?

**8.50 AM to 1 PM**

**Individual Work Package meetings in parallel: discussion of progress and collaboration WP leaders; by all**

During this session each group met and discussed internally and presented a power point presentation during session **3:30- 5:30 PM; Report back and update on progress and forward planning for Work Packages, informing on** the main topics discussed; all presentations are available on-line.

☞ See on the Intranet: Presentation 4-Session 1530-1730 h. Day 2-2017

Below in this section, the questions and comments that were raised from the other WPs and the team in the room are presented.

#### WP1- Discussion and questions

**Question 1:** Why do you want to have a leaf archive?

**Answer:** There are a good number of phenotypes that have been screened and can contribute to the training populations. It is a DNA archive.

**Question 2:** MusaBase is central. Are there resources enough to upload the information on MusaBase?

**Answer:** A training has been provided for all WPs and a central person with each WP should be the focal person to upload the information on the MusaBase. Also Margaret (new recruited staff from IITA based at ILRI Campus-under Trushar's supervision) will be assisting in this process and will go to the different stations to support these tasks.

**Question 3:** have we identified aneuploidy and how to use them?

**Answer:** These have been identified and we need to see how we can use them in the breeding program.

**Question 4:** Can WP1 provide tentative information on a potentially resistant genotype to WP2 and not the other way round?

**Answer:** There are few materials collected at the preliminary yield evaluation + diseases. Then a few genotypes are selected and submitted to pathologists to do in depth research on the resistance/tolerance.

#### WP2: Discussion and questions

**Question 1:** What is the rationale to screen for Sigatoka if there are already trials?

**Answer:** There seems to be a change in the virulence on the Sigatoka and there is a screening for virulence on it.

**Question 2:** What is the role of WP2 on field trials of WP4?

**Answer:** Yes, the team is monitoring the populations of pests and diseases in the trials in the fields- there is screening on the WP4 trials.

#### WP3: Discussion and questions

**Comment:** WP4 should be also involved in the field discussion on the farmers' traits and preferences together with WP1 and WP3.

**Question 1:** Genetic selection on diploids can be good enough to predict on polyploids?

**Answer:** there is at the moment a mixture of everything, so we are predicting on all the materials, but not individually.

#### WP4: Discussion and questions

**Question 1:** How many baby trials have you anticipated for each site?

**Answer:** 20 per site; although data collections will be done through mobile apps/IT and maybe there is no need to go and collect data on all sites. Needs to be discussed further among us.

**Question2:** The baby trials are going to be in randomized or in block designs?

**Answer:** They will be randomized. The program call Clim-up will help to analyze the data; Farmers will be able to rank from A to C all the banana varieties.

#### WP5: Discussion and questions

**Comment:** Related to synonyms, there is a checklist at ProMusa with around 7000 cultivars that can help to fine-tune names into the MusaBase.

**Feedback:** Related to the synonyms this will be looked up. But some of the names such as Machare of Mchare should be defined between the teams in Uganda and Tanzania.

**Ronny's comment:** Ensure what WP are going to be doing through a work plan in order for the teams to make sure that the deliverables for this year are clear and achievable. This will be checked during the next reporting period.

**Rodo's comment:** Today we could already know how much it would cost to do marker assisted breeding vs traditional selection, and to see the cost of it to direct the mode of action/utility of the same.

**Rony's comment:** A team from BMGF which is assessing all the breeding programs in the world "Breeding Program Assessment Tool" will look at all our lines and pipelines... and this will be able to give us information to see the way forward; this will be done end of the project as well.

#### 2 PM-3 PM

#### Poster Sessions

15 posters were presented (see details in Annex 2).

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- See on the Intranet: Presentation 5- Posters competition (all)-2017

Day 3: 26<sup>th</sup> April

8.15 AM to 2 PM Inter-WP meetings

Notes from the meeting of WP2 with the other WPs:

**Meeting with WP1:**

*What WP1 needs from WP2:*

- Disease survey information: Maps on Fusarium and Sigatoka distribution will be provided to WP1 by end of June (George responsible)
- Rapid screening methods:
  - Rony does not trust tissue culture testing, but WP2 is confident that such material has good value in rapid screening methodology
  - It is requested that germplasm is harmonized as representative set for controls
  - It is proposed that Sigatoka evaluation can be done in screen house with natural infection.
  - Screening must also involve virulence problems of pathogens.
  - First screening priority is Fusarium, then nematode/weevil small plant screening.
- Germplasm needs to be harmonized with a representative set for controls
- Evaluating males for resistance

*General comments:*

- Reports should be clearer – not always easy to understand
- Bukoba can work instead of Arusha for black Sigatoka field screenings
- WP1 and WP2 will work together as a team.
- WP1 will contact disease leaders directly for problems
- All material that needs to be screened is in the field already. So Sigatoka and other assessments can be done immediately.

*What WP2 needs from WP1:*

- WP1 need to identify the priority pests: Depends on region
- Continuous screening of pathogens and pests
- Logistics need to be in place not to have unrealistic expectations: time needed to multiply inoculum, greenhouse space, growth of organisms, time for symptoms to develop, etc.

*General comments:*

For breeders and others to consider: There is a reason why WP2 is involved in this project. If one WP could have done all the work in this project, then the others were not needed. There is certain expertise and knowledge required for executing the work, not when all goes well, but particularly when things go wrong. Knowledge that WP2 will bring to this group is:

- A proper knowledge of each of the respective pathogens and pests involved, from their biology, epidemiology, pathology and genetics.
- Understanding of life cycle and virulence of pathogens and pests
- Understanding the importance of inoculum load and environments for disease to occur
- Understand the effect of plant age, tissue affected and response of plants
- Understand the value of proper storage of organisms needed to maintain virulence
- Understand the importance of continuous characterization and evaluation of pathogen. Please do not go and isolate your own culture and not let us characterize it, because it can be a different species, biotype or VCG.

### Meeting with WP3

- WP3 requires screening protocol for Fusarium wilt and weevils: In Pest and Disease guide. Additional information will be provided to WP3 as required.
- Phenotypic data for field screenings will be provided to correlate with the marker-associated resistance to Foc race 1. Leaf samples from the phenotype screening will be provided to WP3 for marker testing.
- A question was asked about conflict regarding the resistance responses in field and greenhouse evaluations due to juvenile and mature plant resistance: This is the case, and this is why techniques are optimized to limit variation between greenhouse and field testing.
- WP3 will do the testing of their materials themselves. Kennedy, Tendo and Danny will assist WP3.
- Does ploidy play a role in resistance? It appears as that the nature of diploid and triploid resistance differs for Fusarium, but not for weevil/nematodes. This is why marker testing is being done.
- WP3 indicate that segregating populations will be screened against all pathogens and pests, and not only Fusarium.

### Meeting with WP4

- NARITA trials have been planted at five sites.
- When will data for baseline collections be available: Fusarium and Sigatoka at the end of June?
- Shooting happening right now – what about sample taking?
  - Kennedy and Janet will be collecting Sigatoka and Fusarium every 4 months.
  - Inge will let WP2 know when Fusarium starts developing.
- Inge will provide us with disease data within 1 month after ratings.

### Additional notes from the meeting of WP4 with WP2 (By Inge):

#### Disease data from WP2 survey done during baselines

- They will prepare a report for WP1, so they will share with us as well.
- They expect to have maps etc. generated by end of June.

#### Samples for black leaf streak

- They do not expect many changes over time, so they do not see the need to come in and take regular samples.

#### Samples for Fusarium wilt

- Fusarium wilt can come up very quickly and kill plants in 2-3 months' time.
- We need to tell them when we observe Fusarium wilt symptoms in the trials, and they will then come in to take samples.
- Action: Noel to keep WP2 people updated as soon as we suspect Fusarium wilt in one of the trials.

#### Nematode and weevil ratings and samples

- They expect low levels of infestation in first cycle.
- The suggestion is to send someone in to do a rapid assessment on the susceptible check.
- Nematode rating + extraction
- Weevil rating + trapping
- If levels are low – no need for further action.
- If levels are high – proceed with more rigorous sampling and rating.
- Mbwazirume is susceptible to both, so can be used for rapid assessment.
- We need to tell them when we expect Mbwazirume to be ready for harvest, at all five sites.
- Tendo/Cornel will then arrange for a technician to come and do the ratings/sampling.
- Action: Noel to keep WP2 people updated on when we expect shooting/harvest of Mbwazirume in all five trial sites.



### Disease data from five trials

- They would like to have access to the data as soon as possible after data collection.
- Action: Noel/Rhiannon to provide copy of data collection to WP2 people as soon as possible after data collection.

### Armillaria at TACRI

- Armillaria incidence is very high in the Moshi trial; it may also mask Fusarium wilt symptoms.
- We need to agree on a protocol specifically for that trial.
- Kennedy is very close there – so he can follow up.

### General communication and visits

- We need to inform WP2 people whenever we see something strange; we should not ignore anything we see.
- We can use WhatsApp or email to send our observations to WP2 people.
- WP2 people pay regular visits to the sites; we should see if we can make them coincide sometimes with Noel's visits.
- We can also exchange travel reports.
- WP2 people would also like to get access to the weather data.

### General comment

- Do not use any pesticides in the trials – we want to see symptoms.

*End of additional notes from WP4 & WP2 discussions*

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### Final General Comment from WP2

WP2 is not aware of all trials in the project, and the existence of trials is important to know. These trials can be of great value for resistance screening, and new trials then do not have to be established. If information about new trials is made available during the planning phase, the value of such trials can be maximized. WP5 indicated that all trial information is available on MusaBase.

### Notes from the meeting of WP3 with WP1:

#### How can WP3 serve WP1?

It was pointed out that many marker-trait association studies in different crops are never used in breeding work but remain on shelves after spending a lot of money on them. How can we make sure that our work will be beneficial to the breeding work (to WP1)? Here we revised our mapping populations and noted that at least one of the parents of the mapping populations being phenotyped for different traits is either currently used, or are in the pedigree of the diploids or tetraploids used in breeding. In the case where the parent used is the susceptible one, it was decided that hybrids in the mapping population carrying resistance and the right traits from the susceptible parent will be selected and used in breeding.

**2 P to 2:30 PM**

**Data platform update;** by *Trushar Shah, Lukas Mueller, Allan Brown*

**Allan Brown:** MusaBase is a very powerful tool, and should be used more often and populated by all of us. At this stage of the project, we should be using it to document our breeding activities. Input should be proactive to Lukas' team to make sure that all the information is there.

**Lukas Muller:** Tomorrow a session has been planned with each WP having one representative; s/he will be trained to become the focal point to upload data from each WP into MusaBase. These will be:

- WP1: Rabooni
- WP2: Kennedy
- WP3: Violet
- WP4: Noel

There have been some discussions on the issue of sharing data openly again! Scientist should not be afraid of publishing their results and the information on MusaBase. We need to start uploading data!

**Trushar:** Using the Banana Breeding Management Tool should help to make data available on MusaBase almost on real time. Repots can also be generated coming from the MusaBase website- which will be the primary source of information for all these data. Workflows have been developed in the system and are available in open data kits (ODK) for mobile phones and tablets. A demo will be provided tomorrow to the representatives of each WP team. Suitable hardware of peripherals to support this system is being evaluated at the moment. There will be a Data Platform week end of September in Uganda-dates yet to be defined.

☞ See on the Intranet: Presentation 6-Banana Data Platforms (WP5)-2017

**2:30 to 2:45 PM**

**Communication;** by *Danny Coyne*

- Technical reports from each WP are all available on the website. Annual report for 2016 (possibly 2015) presented and individual reports from partners will be put on the website.
- Reporting on time is key to ensure that the money is sent on time to each partner, to make sure that contracts can be renewed after approval of the reports. This applies to the six month reports. Discipline is crucial! **Both technical reports and financial reports** have to be ok and submitted on time.
- **For the final report, the delay of one single partner would delay all of us, as the annual report is submitted for all packages at the same time.**
- Use of logo with the BMGF should be approved by the communication team of BMGF and e-mail should be sent to them with copy to Jim Lorenzen; they will approve on the artwork and the technical content. The BMGF prefers to have all logos in the same size and all the funding sources present in an equal way, including theirs, and not some of them outstanding more than others.
- **For Publications:** For sub-contracted partners publications will have to acknowledge that the research outcomes are coming from this project (Breeding Better Bananas) under IITA; for IITA scientists we can directly acknowledge directly the BMGF.
- We need to plan on publications and the potential use of working-information sharing platforms such as Agshare and Scriptoria; Danny is exploring the possibility on having a training on how to best publish, how to make it faster, etc. for the students of the project in 2018 (this year finally the training could not materialize).
- This year we have tried new communication styles to reach more people – Such as Posters in my Pocket.
- Whatever we do, we need to communicate our efforts and activities!!!! So please report an all documents, brochures, booklets, etc... so this can be put on the website.
- Report on all training activities as much as we can with details of everything what we do: every report and info matters!
- Every IITA student needs to be registered under the Capacity development Unit at IITA, using an IITA Graduate Application form. For more info on this, please contact: Mrs. Omolara Olugbenga, (IITA) [O.Olugbenga@cgiar.org](mailto:O.Olugbenga@cgiar.org).
- **Open Access-** Please see communication from the BMGF on Annex 3.
- Minutes to be put in the website with the annual reports. The reports are only accessible to those with passwords –also the annual reports- but for the moment these documents are not open access.
- SAG- password protected.
- SC- password codes.
- All partners- password codes.

**Webmaster:** Each individual project member will be issued with a password for individual access.

**2:45 to 3 PM**

**Matters arising; by all**

**Rony: We will have** a checklist of the deliverables and achievements expected for this year from each WP. This will have to be sent to him and he will compare the expected deliverables vs. the actual ones.

**3:30 PM to 4:30 PM**

**Poster competition & Management feedback; by SAG, SC, PL and BMGF.**

#### **Poster competition (all):**

Members of the SAG, based on the guidelines provided for evaluating the posters, decided that the winning posters were:

- **Award WP1:** *Breeding 'Matooke': Do Men and Women's Needs and Preferences Matter?* Ssali T.R., Sanya, N. L., Namuddu, M. G., and Mayanja, S.
- **Award WP2:** *Molecular markers for the detection of Fusarium oxysporum f. sp. cubense in East and Central Africa;* Ndayihanzamaso, P., Karangwa, P., Mostert, D., Mahuku, G., and Viljoen, A.
- **Award WP3:** *Trait variation in a banana training population for genomic selection;* Nyine, M., Uwimana, B., Swennen, R., Batte, M., Brown, A., Christelová, P., Hřibová, E., Lorenzen, J., and Doležel, J.

#### **Remarks from the SAG (Steve Rounsley)**

**What is well done?** Results presented in this 3 –day workshops clearly indicate that the reject has accelerated since last year with is good progress, as well as communication was improved since last year as well. More science and more advancement from student projects for next year is expected. Related to each WP:

- WP1: Seed production numbers are outstanding and should move forward into producing more seed.
- WP2: Tremendous improvement in the last 12 months which is good. Screening is in great position to continue.
- WP3: Genomic selection – huge potential is in there; hope this can have a deep impact on the project. Implementation of genomic selection though, will be a lot of work.
- WP4: Great interaction among this and the other packages. Looking to have the baseline out to the breeders soon.
- WP5: MusaBase to be used as a powerful tool to change the banana breeding program.

#### **What to improve?**

- **Communication:** As inclusive, as persistent, and as often as possible!
- **Coordination:** More coordination among the packages- more efforts to be put in place to coordinate the activities.
- **Project as a pipeline:** This project should be considered as a breeding pipeline with different modules- that needs to be kept in mind for the next 12-24 months.
- **Focus:** Conscious effort to focus on the most important priorities of the project. This will reduce burden on communication and coordination. The SAG to play a crucial role on this one.
- “Breeding better bananas and less focus on producing better papers”

#### **Remarks from the SC (Victor Manyong).**

The Project is moving well. But major issues are related to communication and this needs to be addressed- all components of the project should contribute to the breeding program.

1.- What can we do to improve communication? Actions recommended:

- Among WP-leaders and the management SC and SAG– more meetings; therefore we will have our next on-line meetings to be scheduled on 12<sup>nd</sup> Thursday, 8<sup>th</sup> June, 12<sup>th</sup> October and 14<sup>th</sup> December.

- Within each WP: recommended one per month; before the SC meetings will take place, each WP will have its own meeting so they can inform on the progress to the following SC skype conference. To be organized by each WP-leader.

2.- Technical issues to be handled: we will explore possible changes in the subcontracts rejected on Uganda and Tanzania on the weevils and the nematode issues.

3.- Publication and open access (OA): guidelines for publications available? How many papers in each WP? All papers should be OA, Not in predatory journals, follow BMGF guidelines for Publication- on this particular point, please see Annex 3.

4.-Website: All technical reports to go there after approved and protected with password. How to increase the visibility of the website and someone being the focal point of the website- Laura Cortada (ITA) to be this person.

5.- Planting material for the baby trials- no International tissue culture material available on time (especially for Tanzania)- therefore a new tissue culture lab (TCL) will have to be identified. In Dar es Salaam there is a TC lab and the SC will contact them to find out more; if this does not function there will be a plantation in the field to multiply material.

8.- Post-harvest analysis of the NARITAs. The most urgent need there is to develop physico-chemical analyses of the fruits.

10.-Shall we explore a penalty for those who report late??? 10% budget cut for those reporting late and delaying the submission of reports could be explored.

→ **23<sup>rd</sup> April to 26<sup>th</sup> April 2018 our next annual BBB meeting.**

#### **Remarks from the Project Leader- (Rony Swennen)**

- “We are catching up”
- New partners on board, we need to take advantage on this as it can improve the work we do.
- Increased efficiency through a list of action points that will be circulated.
- SAG - We are grateful for the continuous support and feedback.

#### **Remarks from BMGF (Jim Lorenzen)**

Thanks to the SAG and the SC. This has been a year of good progress: things have changed and have gained progress by WP1 (more hybrids in the field, embryos, seeds) which is very good for the project; on the breeding program design, the targets should be even set higher to be a real breeding program. But how to focus and do less and faster? Can we work more with data from our preliminary yield trials (PYTs)? It would be good to get and save as much DNA as possible for the future years.

- WP2: Progress and phenotyping is being conducted in coordination with WP1 and WP3.
- WP3: Progress on the genetic side has been good- with the genotyping aspect of the project which is coming on the right time. Good results are expected for next year.
- WP4: Great to see all the trials in the field, the surveys have been completed and we are all eager to see the results of the baseline and the trials on the fields.
- WP5: Great responsiveness to the other WPs. We all need to use the MusaBase much more – and this has to happen BEFORE TRIALS ARE PLANTED! An increased use and uptake of MusaBase will help us to keep track on what we do; this will also reflect how our breeding program is becoming more accurate and precise.
- Low density markers and high density markers – what are we going to do in the next months for breeding selection? Hope to receive feedback in the next months.

**Synergies with other projects:** In addition, the Banana Agronomy project can help the BBB-project much: maps with agro-ecological data, socio-economic information, etc. All this new information can help to better identify which

are the most representative sites for our work... especially for the baby trials. This can also help us to be more efficient in our delivery: we should explore and aim at to have as many synergies as we can.

Quality and palatability is crucial in our varieties at the end of the day: more productive yes! But they need to be appealing to consumers. Organoleptic trades to breeders... Food science of quality of the bananas is key.

**Integration of packages:** better breeding by working well together - better communication and coordination are also a corner stone of our work.

**Reporting:** BMGF would like to see better what we do but not only as tables as appendixes - good varieties adopted by farmers will take many years, but meanwhile we should be able to pitch a **story** on how we are we making impact! Technical reports and stories that transmit a message on the impact.

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*End of Minutes 24<sup>th</sup> to 26<sup>th</sup> April 2017.*

## Minutes Annex 1: List of acronyms and abbreviations

*(in order of appearance in the text)*

Breeding Better Bananas project (BBB).  
FAO (Food and Agriculture Organization of the United Nations).  
Steering committee (SC).  
Scientific advisory group (SAG).  
Work packages (WPs).  
Bill and Melinda Gates Foundation (BMGF).  
International Institute of Tropical Agriculture (IITA).  
National Agriculture Research Laboratories (NARL), Uganda.  
National Agriculture Research Organization (NARO), Uganda.  
Banana Agronomy Project (BAP).  
Households (HH).  
Focal group discussions (FGDs).  
Terms of reference (ToRs).  
Open data kits (ODK).  
Tissue culture lab (TC).  
Preliminary yield trials (PYTs).

## Minutes Annex 2: Posters Presentations

| SN | Title  | Authors                              | WP  |
|----|--|--------------------------------------|-----|
| 1  | Use of sensory parameters as a tool in selecting Matooke hybrids   | Rabooni et al., 2017                 | WP1 |
| 2  | Breeding 'Matooke': Do Men and Women's Needs and Preferences Matter?   | Tendo, et al., 2017                  | WP1 |
| 3  | Effect of Glucose Solution on Stigma Receptivity and Subsequent Seed Set in EAHBs  | Waniale A et al., 2017               | WP1 |
| 4  | Suitability of existing <i>Musa</i> morphological descriptors to characterize East African highland 'Matooke' bananas  | Batte MICHAEL., A. et al., 2017      | WP1 |
| 5  | Chromosome doubling in diploid bananas for efficient breeding  | Oyesigye, N. et al., 2017            | WP1 |
| 6  | Molecular markers for the detection of <i>Fusarium oxysporum</i> f. sp. <i>ubense</i> in East and Central Africa   | Ndayihanzamaso PRIVATE. et al., 2017 | WP2 |
| 7  | A rapid screening method for response to banana weevils ( <i>Cosmopolites sordidus</i> )   | Kemigisa J. et al., 2017             | WP2 |
| 8  | The adaptation range for lack Sigatoka causal pathogen shifting towards higher altitudes in Uganda   | Janet Kimunye et al., 2017           | WP2 |
| 9  | Genetic diversity of banana ( <i>Musa</i> spp.) and its relation to plant parasitic nematodes in Tanzania  | Mgonja DOREEN.M. et al., 2017        | WP2 |
| 10 | Distribution of plant parasitic nematodes associated with banana crops in Tanzania   | NESSIE. Luambano, et al., 2017       | WP2 |
| 11 | Trait variation in a banana training population for genomic selection  | Nyine M., B. et al., 2017            | WP3 |
| 12 | Genetic dissection of FOC resistance using <i>Musa acuminata</i> ssp. <i>malaccensis</i>   | Chen ANDREW., et al., 2017           | WP3 |
| 13 | Genetic analysis of resistance against <i>Fusarium oxysporum</i> f. sp. <i>ubense</i> (Foc) in selected banana populations using molecular markers and linkage mapping | Arinaitwe K.I, et al., 2017          | WP3 |
| 14 | Phenotyping of a diploid population for resistance to <i>Radopholus similis</i>  | Batte MICHAEL., J et al., 2017       | WP3 |
| 15 | Building a breeding database for African banana programs: MusaBase   | Bauchet GUILLAUME., et al., 2017     | WP5 |

## Minutes Annex 3: Open access publication with BMGF projects- e-mail communication 16th May 2017

Open access publication with BMGF projects



Dear Banana Breeding Colleague,

Regarding our recent discussion on open access publication of articles arising from our project, please note that as soon you have an open access article accepted or resubmitted with modifications to a journal, please send the following email and ensure the OPP ID is included in the subject line as per below:

Dear Ashley (<[openaccess@gatesfoundation.org](mailto:openaccess@gatesfoundation.org)>)  
**Subject:** Open Access publication - OPP1093845

Dear Ashley

I am project member of

- **Grantee:** International Institute of Tropical Agriculture
- **Investment ID:** OPP1093845
- **Investment Title:** Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa
- **Investment Start Date:** October 01, 2014

Our paper has been accepted

The title is: XXXXXX

Authors are: YYYYYY

The article has been resubmitted on DATE and we expect it to be approved soon (see copy attached).

There will be costs involved for Open Access publication. As we know that the Foundation will cover these costs, please open for us an account.

Thanks

NAME OF PROJECT PARTNER

CC: Rony Swennen



**Improvement of banana for smallholder farmers  
in the Great Lakes Region of Africa**

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**Annual Project Planning Meeting**

**24-27<sup>th</sup> April 2017**

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**1.3 SAG and Steering Committee Meeting, 26th April 2017**

**Kabira Country Club, Kampala**

**Present**

SC: Victor, Danny, Jerome, Altus, Inge, Lukas, Rony, Brigitte, Jim

SAG: Steve, Jane, Richard, Eva

**Duration**

Started: 16.55

Finished: ~18.00

**Agenda**

Steve provided some feedback from the SAG meeting and suggested this a useful strategy, as used by other similar projects.

*Communication*

Is lack of communication the problem or a symptom of a larger problem of team not engaging due to disinterest/disconnectedness? SAG was initially concerned that different groups/individuals were not thinking as team members. However, following discussions on the last day of the meeting, the SAG saw lots of evidence of teamwork and willingness to operate for the benefit of other parts of the project. The discussions on Wednesday also provided more vision of a pipeline that links the project together.

There was concern expressed about this in the SAG meeting but now there is more of a feeling of much more cohesion. There remains a need for vigilance on communication however, and that the ideas proposed and put forward need to be put into action and made concrete. Some time needs to be invested in describing explicitly which products or type of products we are trying to produce – in order to help foster teamwork and for the project team and partners to come behind a vision.

Also, the breeding team needs to build into their work the partners of the other teams.

The SC needs to take responsibility to communicate this vision and guide the team, keeping them inspired and in support of the project vision. Suggested that the SC discuss what the vision is and mechanisms on how to inspire the team to come behind it.

*SC meetings and vision of the project*

Discussions / feedback is more tactical as opposed to strategic discussion. Short term tactical as opposed to longer term strategy.

The group was asked if time is spent discussing the longer term vision. This is necessary - in order that the SC believes in the vision themselves and in order to relate the vision to the team.

Rony: The vision needs to be flexible. One vision with multiple products. And consequently what are and how many products will be produced?

Jerome: Thinking about 5 year timeframe and therefore what can be achieved in those 5 years. But perhaps we need to think about the longer term considerations, beyond the 5 years. e.g. how many people are being pulled out of poverty?

Victor: the vision is detailed in the proposal, and we need to remind people and partners what it is.

### *Science*

Richard: Would like to see science presentations in the next meeting. Science and data arising from the project should be presented. Should also have an internal review on science outputs and data produced.

The inclusion of a poster session this year was praised and provided a very useful addition. The value of the evaluation and announcing winners however, was at first thought unnecessary. However, upon seeing the reaction of the students to the announcement of the winners and receiving prizes it was realized that this meant a great deal and so the value of this was process was much better appreciated.

There is value in getting more detailed data and presentation – e.g. genomic detail.

### *Additional SAG member*

SAG proposes the addition of a SAG member from the breeding community. Ideally a breeder from the private sector to provide a different style of thinking, especially from an efficiency perspective. Along these lines a similar project, NextGen cassava with HQ in Cornell, and activities in Namulonge has several breeders involved, who could help the banana project on how they have approached a breeding project like this. They also use a SAG system towards leveraging the experience of the SAG to look for opportunities.

### *Reporting*

There is perhaps a need to think a little bit more on how people will benefit from the project and what they will get out of it when reporting.

There is a large contrast between NextGen and BBB. However, they are double the scale and started at a higher level. But BBB still needs to assess reducing the burden of reporting.

Lukas: two reports a year is burdensome and heavy. A little too much and once annually may be better. Altus in agreement with once annual reporting, although the twice annual report helps identify problems and thus address them.

Reporting is somehow imposed by IITA and effectively it is not too much of a requirement. So perhaps we should ignore the institutional system and think about relieving the 6-monthly reporting. Look at what helps and what works best for the project and keep momentum.

SAG is assuming that there are reports to each other on progress and underachievement during the SC meetings. Also too many deliverables with an overwhelming numerical amount. As a general theme look to find opportunities to focus people's time and energy on the most important deliverables.

Rony likes to see deliverables, against promises made.

There is also a sense that some activities are overloading and people spread too thin.

### *Jim*

Wished to emphasize a few terms that had cropped up regularly during the meeting.

Team – which requires teamwork and coming together to create teams.

Pipeline – which is a system that has been / is being built to generate increasing number of products – hybrids. The project is building a system that is providing better varieties and the impact pathway is clear. Need to think about the system for the longer term however and beyond the project, and its sustainability, which will come from demonstrating something that is valuable and in demand.

Do not be afraid of showing public goods, such as superior parents that will be valuable for dessert bananas and plantains as well as cooking banana.

Need to think about population improvement and faster turnover of breeding populations - not about exploiting long past parents.

*Rony*

The project looks forward for the visit by the Breeding Performance Assessment Team (BPAT) in May 2017. That will provide useful info.

The SAG enjoyed the meeting and have learnt much about banana.

#### **Action points:**

1. Update vision
2. More strategic discussion
3. Define:
  - a. products we are trying to produce: what phenotype?
  - b. Pipeline
  - c. Impact on farmers
  - d. Relate to proposal
4. Addition of a SAG member from the breeding community
5. Foster more teamwork by bringing more people into the breeding work
6. Next meeting:
  - a. science presentations by project partners
  - b. posters as before
7. Reporting: once a year???

## 1.4 Breeding Better Bananas website update

During this year, the website for the Breeding Better Bananas (BBB) project was finalised for launch/release and has consolidated its contents to act as a resource platform both for the general public as well for the project members (intranet). A password-protected area enables project members to access more project specific data and documents for internal project use only. The web site can be accessed at both <http://breedingbetterbananas.org> as well as <http://bananabreeding.iita.org/> which redirects to the new domain. Once the main contents and details of the project and partners was established, the site was launched. However, it is being continuously added to as information, data and documents are made available, as well as being updated and refreshed. In order to improve visibility and facilitate sharing of the contents from the BBB web site, the page now includes in each work package section, as well as on the main page, **icons for easy sharing on the social networks** (i.e. Facebook, Twitter, LinkedIn, Pinterest), as well as an easy-sharing icon to send website's contents directly through e-mail; also a friendly printing icon has been included in order to facilitate the printing of the website materials for the end users. In addition, since July 2017, the website presents a **“contact us” link** that can be used to directly contact the project through a standardized form, that once is filled it sends the information request and/or any other comments into a single centralized email address that has been created for this purpose ([Breedingbetterbananas@cgiar.org](mailto:Breedingbetterbananas@cgiar.org)); this email is checked daily and key members of the project (project manager, project coordinator and project administrator) have access to it. An **engine to search content within the website** (Figure 1) has been also been added during 2017, which allows end users to quickly search for any contents within the projects' website.

The project members have also been provided with individual registration passwords which allow personal access to the intranet in order to report, share and consult information.

Related to the information that appears on the website, this has been timely updated as new events evolved during 2017, including new partners, press news, links to other banana-related websites and projects, and technical publications from the work packages.

The website statistics indicate that this year 57.3% of our visitors were new comers; in the last quarter of the year (From June to September) the site had 762 page views, and 57.43% of new sessions; more information on the website's metrics are available in Figure 1.

In order to help safeguard the project logo and name, its registration as a trademark is currently being undertaken in Nigeria.

## All web site data audience overview for June –September 2017.

Banana Breeding  
All Web Site Data

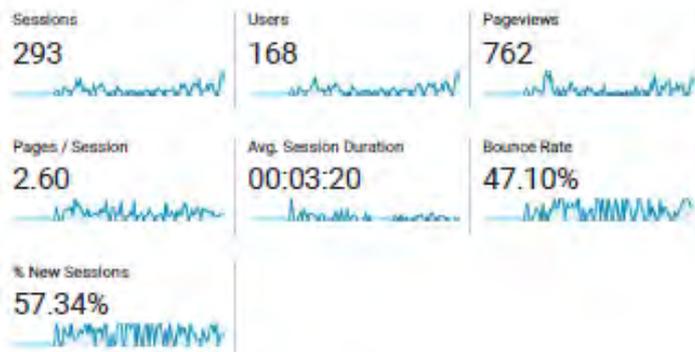
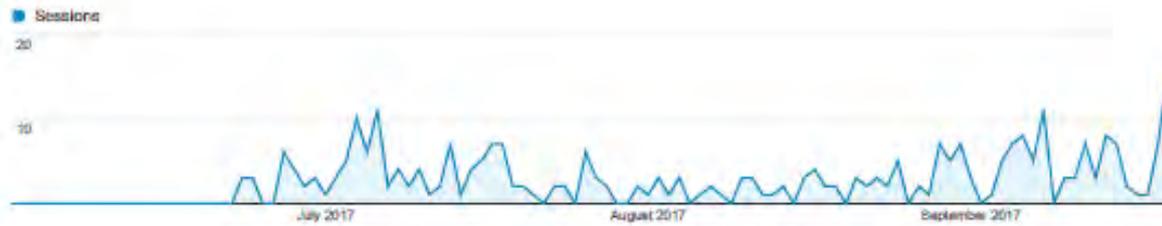
[GO TO REPORT](#)

### Audience Overview

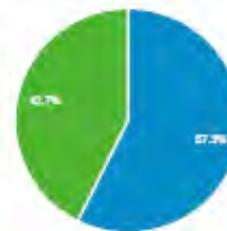
All Users  
100.00% Sessions

Jun 1, 2017 - Sep 20, 2017

#### Overview



■ New Visitor ■ Returning Visitor



| Country           | Sessions | % Sessions |
|-------------------|----------|------------|
| 1. Kenya          | 59       | 20.14%     |
| 2. India          | 27       | 9.22%      |
| 3. Nigeria        | 25       | 8.53%      |
| 4. United States  | 24       | 8.19%      |
| 5. Tanzania       | 23       | 7.85%      |
| 6. Uganda         | 17       | 5.80%      |
| 7. Belgium        | 14       | 4.78%      |
| 8. United Kingdom | 11       | 3.75%      |
| 9. China          | 6        | 2.05%      |
| 10. Spain         | 6        | 2.05%      |

## 1.5 Publications and Communication Outputs

Supported and produced through or relevant to the BMGF Improvement of banana for smallholder farmers in the Great Lakes Region of Africa

### PUBLICATIONS

#### Books / Book Chapters

1. Viljoen, A., Mahuku, G., Massawe, C., Ssali, R.T., Kimunye, J., Mostert, G., Ndayihanzamaso, P. and Coyne, D.L. 2017. *Banana Pests and Diseases: Field Guide for Disease Diagnostics and Data Collection*. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Pp. 73.

#### Peer Reviewed Journal Articles

1. Batte, M., Mukiibi, A., Swennen, R., Uwimana, B., Pocasangre, L., Hovmalm, H.P., Geleta, M., and Ortiz, R. 2017. Suitability of existing *Musa* morphological descriptors to characterize East African highland 'matooke' bananas. *Genetic Resources and Crop Evolution*. 10.1007/s10722-017-0562-9. <https://doi.org/10.1007/s10722-017-0562-9>
2. Christelova, P., De Langhe, E., Hribova, E., Cizkova, J., Sardos, J., Husakova, M., Van den houwe, I., Sutanto, A., Kay Kepler, A., Swennen, R., Roux, N., and Dolezel, J. 2017. Molecular and Cytological Characterization of the Global *Musa* Germplasm Collection Provides Insights into the Treasure of Banana Diversity. *Biodiversity and Conservation*, 26(4), 801-824. DOI 10.1007/s10531-016-1273-9. <http://dx.doi.org/10.1007/s10531-016-1273-9>
3. Janssens, S.B., Vandeloek, F., De Langhe, E., Verstraete, B., Smets, E., Van den houwe, I., and Swennen, R. 2016. Evolutionary dynamics and biogeography of Musaceae reveal a correlation between the diversification of the banana family and the geological and climatic history of Southeast Asia. *New Phytologist*, 210(4), 1453-1465. 10.1111/nph.13856. <http://dx.doi.org/10.1111/nph.13856>
4. Kissel, E., Vanhove, A.-C., Garcia, S., Panis, B., Rouard, M., Cenci, A., Roux, N., Zorrilla, C., Swennen, R., and Carpentier, S. 2016. Abiotic stress research in crops using -omics approaches: drought stress and banana in the spotlight. *Acta Horticulturae*, 1114, 81-90. DOI 10.17660/ActaHortic.2016.1114.11. <http://dx.doi.org/10.17660/ActaHortic.2016.1114.11>
1. Mostert, D., Molina, A.B., Daniells, J., Fourie, G., Hermanto, C., Chao, C.-P., Fabregar, E., Sinohin, V.G., Masdek, N., Thangavelu, R., Li, C. Yi, G., Wei, Y., Mostert, L. and Viljoen, A. 2017. The distribution and host range of *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups in Asia. *Plos One* 12 (7) e0181630. <https://doi.org/10.1371/journal.pone.0181630>.
5. Nyine, M., Uwimana, B., Swennen, R., Batte, M., Brown, A., Christelova, P., Hribova, E., Lorenzen, J., and Dolezel, J. 2017. Trait variation and genetic diversity in a banana genomic selection training population. *PLoS ONE*, 12(6), e0178734. <https://doi.org/10.1371/journal.pone.0178734>
6. Nyine, M., Uwimana, B., Blavet, N., Hřibová, E., Vanrespaille, H., Batte, M., Akech, V., Brown, A., Lorenzen, J., Swennen, R., Dolezel, J. Genomic Prediction in a Polyploid Crop: Genotype by Environment Interaction and Allelic Dosage Effects on Predictive Ability in Banana, (submitted) *The Plant*
7. Zorrilla, J., Rouard, M., Cenci, A., Kissel, E., Do, H., Dubois, E., Nidelet, S., Roux, N., Swennen, R., and Carpentier, S. 2016. Differential root transcriptomics in a polyploid non-model crop: the importance of respiration during osmotic stress. *Scientific Reports*, 6. 22583. DOI 10.1038/srep22583. <http://www.nature.com/articles/srep22583>

#### Peer Reviewed Articles under process

1. Coyne, D.L., Dubois, T. and Daneel, M. Integrated Pest Management in Bananas. In: *Integrated Pest Management in the Tropics*. CAB International, UK. (in press)
2. Coyne, D. and Kidane, S. Nematode Pathogens. In: Jones, D. (ed) *Diseases of Banana, Abacá and Enset*. 2<sup>nd</sup> Edn. CAB International, Wallingford, UK, pp. (in press)
3. Karangwa, P., Blomme, G., Beed, F. and Viljoen, A. 2017. Genetic diversity of *Fusarium oxysporum* f. sp. *cabense* in East and Central Africa. *Plant Disease* (In Press).
4. Hung, T.N., Mostert, D., Viljoen, A. Chao, C.P. and Molina, A.B. 2017. First report of *Fusarium* wilt of Cavendish bananas, caused by *Fusarium oxysporum* f. sp. *cabense* tropical race 4 (VCG 01213/16), in Vietnam. *Plant Disease* (In Press).
5. Ndayihanzamaso, P., Karangwa, P., Mostert, G., Blomme, G., Beed, F., Mahuku, G. and Viljoen, A. 2016. Multiplex PCR assay for the detection of Lineage VI of *Fusarium oxysporum* f. sp. *cabense*. In preparation
6. Sikora, R.A., Coyne, D.L., Hallman, J. and Timper, P. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (third edition)*. CAB International, UK. (in press)
7. Sikora, R.A., Coyne, D.L. and Quénéhervé, P. Nematode Parasites of Bananas and Plantains in: Sikora, R.A., Coyne, D.L., Hallman, J. and Timper, P. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (third edition)*. CAB International, UK. (in press).

### Technical Briefs/ Protocols/

1. De Buck, S., and Swennen, R. 2016. Bananas, the green gold of the South: VIB, pp 55.

## CONFERENCE OUTPUTS

### Oral Presentation

1. Adheka, J.G., Dhed'a Djailo, B., Blomme, G., Karamura, D., Swennen, R., and De Langhe, E. 2016. Actual plantain diversity status in the Democratic Republic of Congo and future prospects. III All Africa Horticultural Congress. Ibadan, Nigeria. 7-12 August 2016. Oral abstract.
2. Adheka, J.G., Komoy, J., Sivirihauma, C., Karamura, D., De Langhe, E., Swennen, R., Dhed'a Djailo, B., and Blomme, G. 2016. Banana and plantain diversity and uses in Oriental province, Democratic Republic of Congo. X International Symposium on Banana: ISHS-ProMusa Symposium on Agroecological Approaches to Promote Innovative Banana Production Systems. Montpellier, France. 10-14 October 2016.
3. Brown, A., Massawe, V.F., Mduma, H., Zinga, M.K., Uwimana, B., and Swennen, R. 2017. Pollen Viability and Genetic Diversity of East African Diploid Bananas and Their Impact on International Musa Breeding. 2017 ASHS Annual Conference. Hawaii. 19-22 September 2017. <https://ashs.confex.com/ashs/2017/meetingapp.cgi/Paper/26502>.
4. Carpentier, S., Panis, B., Van den Bergh, I., Vandenhoeve, I., and Swennen, R. 2017. The quest for climate smart varieties: phenotyping the banana biodiversity. The third general meeting of COST action FA1306 "Field phenotyping technologies from woody perennials to annual crops". Oeiras, Portugal. 27-29 March 2017. [http://www.plant-phenotyping.org/home\\_of\\_3rd\\_cost\\_meeting](http://www.plant-phenotyping.org/home_of_3rd_cost_meeting).

5. Dusunceli, F., Van den Bergh, I., Swennen, R., and Liu, P. 2017. Awareness, prevention and rapid response is key for managing banana *Fusarium* wilt TR4 globally: efforts of FAO and its partners to minimize impact on food security and livelihoods. 7th International Banana Congress CORBANA 2017. Miami, USA. 26-29 September 2017. <https://congresointernacionaldebanano.com/?lang=en>.
6. Mostert, W. O'Neill, S. Perry, L. Mostert and A. Viljoen. 2017. The banana wilt fungus *Fusarium oxysporum* f.sp. *cubense* more diverse than previously anticipated. Australasian Plant Pathology biennial conference, Brisbane, Australia. 26-28 September. Abstract.
7. Njukwe, E., Ekesa, B., Ocimati, W., Blomme, G., Kamira, M., Amah, D., Swennen, R., Okafor, C., and Ndayisaba, P.C. 2016. Intensification and diversification of banana production systems: Key drivers for increased income and food and nutritional security in the Great Lakes region. Humidtropics/FARA - Marketplace event on 'Systems research in agri-food systems'. Ibadan, Nigeria. 15-17 November 2016. Abstract.
8. Van Wesemael, J., Hueber, Y., Kissel, E., Campos, N.A., Swennen, R., and Carpentier, S. 2017. Quantification and identification of allele specific proteins for polyploid non-model crops: Proof of principle for 3 banana genotypes/phenotypes. The third general meeting of COST action FA1306 "Field phenotyping technologies from woody perennials to annual crops". Oeiras, Portugal. 27-29 March 2017. [http://www.plant-phenotyping.org/home\\_of\\_3rd\\_cost\\_meeting](http://www.plant-phenotyping.org/home_of_3rd_cost_meeting).
9. Zorrilla, J., Kissel, E., van Wesemael, J., Swennen, R., and Carpentier, S. 2017. From an in vitro to a greenhouse model: the role of glycolysis and fermentation during drought stress in a polyploid crop. The third general meeting of COST action FA1306 "Field phenotyping technologies from woody perennials to annual crops". Oeiras, Portugal. 27-29 March 2017. [http://www.plant-phenotyping.org/home\\_of\\_3rd\\_cost\\_meeting](http://www.plant-phenotyping.org/home_of_3rd_cost_meeting).
10. Zorrilla, J., Kissel, E., van Wesemael, J., Swennen, R., and Carpentier, S. 2017. The role of glycolysis and fermentation during drought stress in a polyploid crop. Confirmation of a lab model in a greenhouse model. The third general meeting of COST action FA1306 "Field phenotyping technologies from woody perennials to annual crops". Oeiras, Portugal. 27-29 March 2017. [http://www.plant-phenotyping.org/home\\_of\\_3rd\\_cost\\_meeting](http://www.plant-phenotyping.org/home_of_3rd_cost_meeting).
11. Zorrilla, J., Rouard, M., Cenci, A., Kissel, E., Roux, N., Swennen, R., and Carpentier, S. 2016. How do roots respond to osmotic stress? a transcriptomic approach to address this question in a non-model crop. COST FA1306 meeting 'The quest for tolerant varieties - Phenotyping at plant and cellular level' Copenhagen, Denmark. 18-20 April 2016. Oral abstract.
12. zum Felde, A., and Swennen, R. 2016. Effects of planting density and irrigation on *Musa* AAB cv. 'Agbagba' under sub-optimal agroecological conditions. X International Symposium on Banana: ISHS-ProMusa Symposium on Agroecological Approaches to Promote Innovative Banana Production Systems. Montpellier, France. 10-14 October 2016. Oral abstract.

### Poster Presentation

1. Adheka, J.G., Tutu, S., Dhed'a Djailo, B., and Swennen, R. 2016. A contribution to sustainable plantain cropping in the Democratic Republic of Congo (DR Congo). III All Africa Horticultural Congress. Ibadan, Nigeria. 7-12 August 2016. Poster abstract.
2. Carpentier, S., Kissel, E., Janiak, M., Rouard, M., Zorrilla, J., and Swennen, R. 2016. The quest for tolerant varieties: integration of -omics techniques to understand stress in non-model crops. International Plant & Animal Genome XXIV. San Diego, USA. 9-13 January 2016. Poster abstract. <https://pag.confex.com/pag/xxiv/webprogram/Paper21736.html>.
3. Cenci, A., Sardos, J., Hueber, Y., Zorrilla-Fontanesi, J., Van Wesemael, J., Swennen, R., Roux, N., Carpentier, S., and Rouard, M. 2017. A genomic view of the banana (*Musa* spp.) diversification: the case of triploid ABB genome group. Plant genome evolution. A current opinion conference. Sitges,



- Spain. 1-3 October 2017. Poster abstract. <https://www.elsevier.com/events/conferences/plant-genome-evolution>.
4. Christelova, P., Hribova, E., Bartos, J., Swennen, R., Amah, D., and Dolezel, J. 2017. Behind the missing bud - genetic and epigenetic variation of African plantains. Plant genome evolution. A current opinion conference. Sitges, Spain. 1-3 October 2017. Poster abstract. <https://www.elsevier.com/events/conferences/plant-genome-evolution>.
  5. Drapal, M., Carvalho, E., Roux, N., Rouard, M., Amah, D., Swennen, R., and Fraser, P.D. 2016. A metabolomics approach to the assessment of banana diversity and quality traits. International Plant & Animal Genome XXIV. San Diego, USA. 9-13 January 2016. Poster abstract. <https://pag.confex.com/pag/xxiv/webprogram/Paper22048.html>.
  6. Drapal, M., Carvalho, E., Van den houwe, I., Rouard, M., Sardos, J., Amah, D., Swennen, R., Roux, N., and Fraser, P.D. 2016. A metabolomics approach to the assessment of banana diversity and traits. International Plant & Animal Genome XXIV. San Diego, USA. 9-13 January 2016. Poster abstract. <https://pag.confex.com/pag/xxiv/webprogram/Paper22107.html>.
  7. Nyine, M., Uwimana, B., Swennen, R., Batte, A., Hribova, E., and Dolezel, J. 2016. Genomic breeding approaches for East African bananas. International Plant & Animal Genome XXIV. San Diego, USA. 9-13 January 2016. Poster abstract. <https://pag.confex.com/pag/xxiv/webprogram/Paper19935.html>.
  8. Ndayihanzamaso, P., Karangwa, P., Mostert, G., Mahuku, G. and Viljoen, A. 2017. Molecular markers for the detection of *Fusarium oxysporum* f.sp. *cubense* in East and Central Africa. 50<sup>th</sup> Congress of the Southern African Society for Plant Pathology, Champagne Castle Resort, South Africa. 15-18 January. Poster abstract.
  9. Sabura, S., Swennen, R., Deckers, J., Aerts, R., Weldeyes, F., Abebe, G., Hailemichael, A., Weldesenbet, F., Blomme, G., and Vancampenhout, K. 2016. Agro-Ecological Niche of Bacterial Wilt (*Xanthomonas Campestris* pv. *musacearum*) of Enset (*Ensete Ventricosum* (Welw.) Cheessman) in Gamo Highlands of Ethiopia. Tropentag "Solidarity in a competing world - fair use of resources". Book of abstracts. Vienna, Austria. 18-21 September 2016. 116. Poster abstract. <http://www.tropentag.de/abstract.php?code=xy8ojvi4>.
  10. Van den Bergh, I., Swennen, R., Crichton, R., Madalla, N., Marimo, P., Kubiriba, J., Tumuhimbise, R., Okurut, W.A., Massawe, C., Kindimba, G., Mbongi, D., Byabachwesi, M. 2017. NARITA hybrids for East Africa. Poster presented at the RTB World Café on "Scaling RTB technologies", 10 March 2017, Dar es Salaam, Tanzania. Poster abstract.
  11. Zorrilla, J., Rouard, M., Cenci, A., Kissel, E., Do, H., Dubois, E., Nidelet, S., Roux, N., Swennen, R., and Carpentier, S. 2016. Transcriptomic profiling in *Musa*: a look into processes affected by mild osmotic stress in the root tip. International Plant & Animal Genome XXIV. San Diego, USA. 9-13 January 2016. Poster abstract. <https://pag.confex.com/pag/xxiv/webprogram/Paper19933.html>.
  12. Zorrilla, J., Rouard, M., Cenci, A., Kissel, E., Do, H., Dubois, E., Nidelet, S., Roux, N., Swennen, R., and Carpentier, S. 2016. Transcriptomic profiling in *Musa*: a look into processes affected by mild osmotic stress in the root tip. COST FA1306 meeting 'The quest for tolerant varieties-Phenotyping at the cell level'. Versailles, France. 1-2 February 2016. Poster abstract.
  13. Zorrilla, J., Rouard, M., Cenci, A., Kissel, E., Roux, N., Swennen, R., and Carpentier, S. 2016. How do roots respond to osmotic stress? a transcriptomic approach to address this question in a non-model crop. Plant Biology Europe. EPSO/FESPB 2016. Prague, Czech Republic 26-30 June 2016. Poster abstract. <http://www.europlantbiology2016.org/>

1. Rabooni, et al., 2017. Use of sensory parameters as a tool in selecting Matooke hybrids.
2. Tendo, et al., 2017. Breeding 'Matooke': Do Men and Women's Needs and Preferences Matter?
3. Waniale, A. et al., 2017. Effect of Glucose Solution on Stigma Receptivity and Subsequent Seed Set in EAHBs.
4. Batte M., A. et al., 2017. Suitability of existing *Musa* morphological descriptors to characterize East African highland 'Matooke' bananas.
5. Oyesigye, N. et al., 2017. Chromosome doubling in diploid bananas for efficient breeding
6. Ndayihanzamaso, P. et al., 2017. Molecular markers for the detection of *Fusarium oxysporum* f. sp. *cubense* in East and Central Africa.
7. Kemigisa, J. et al., 2017. A rapid screening method for response to banana weevils (*Cosmopolites sordidus*)
8. Kimunye, J. et al., 2017. The adaptation range for lack Sigatoka causal pathogen shifting towards higher altitudes in Uganda.
9. Mgonja, D.M. et al., 2017 Genetic diversity of banana (*Musa* spp.) and its relation to plant parasitic nematodes in Tanzania.
10. Luambano, N. et al., 2017. Distribution of plant parasitic nematodes associated with banana crops in Tanzania.
11. Nyine, M., B. et al., 2017. Trait variation in a banana training population for genomic selection.
12. Chen, A., et al., 2017. Genetic dissection of FOC resistance using *Musa acuminata* ssp. *Malaccensis*.
13. Arinaitwe K.I., et al., 2017. Genetic analysis of resistance against *Fusarium oxysporum* f. sp. *cubense* (Foc) in selected banana populations using molecular markers and linkage mapping.
14. Batte M., et al., 2017. Phenotyping of a diploid population for resistance to *Radopholus similis*.
15. Bauchet G. et al., 2017. Building a breeding database for African banana programs: MusaBase.

### Miscellaneous

1. De Buck, S., and Swennen, R. 2016. Bananas, the green gold of the South: VIB, pp 55.
2. Nyine, M., and Blavet, N. 2016. Alleledosage R function.  
[http://olomouc.ueb.cas.cz/system/files/users/public/scripts/AlleleDosage\\_R\\_function.docx](http://olomouc.ueb.cas.cz/system/files/users/public/scripts/AlleleDosage_R_function.docx)

### TRAINING WORKSHOPS

1. R-script used for allelic dosage in SNP markers can be accessed from the link:  
[http://olomouc.ueb.cas.cz/system/files/users/public/scripts/AlleleDosage\\_R\\_function.docx](http://olomouc.ueb.cas.cz/system/files/users/public/scripts/AlleleDosage_R_function.docx)
2. Musabase workshop, Kampala, Uganda. 21-22<sup>nd</sup> April 2017.

## MEDIA

### Press Releases

1. Daily Monitor story: <http://www.monitor.co.ug/Magazines/Farming/689860-3915228-su82gb/index.html>
2. 1500 banana farmers to benefit from Sh18b project, New Vision, Friday 28<sup>th</sup> April, 2017
3. Banana high yields seeds in the offing, The Daily News, 8<sup>th</sup> May, 2017.
4. Ray of hope for pest, disease-free bananas, The East African, 27<sup>th</sup> May, 2017.
5. Research body develops new disease resistant banana variety- The Guardian, 29<sup>th</sup> May, 2017.
6. 48 new banana hybrids available, The Citizen, 29<sup>th</sup> May, 2017.
7. Uganda gets 1.7b banana labs: Besides bacterial wilt the experts are testing another variety of bananas that is resistant to nematodes and weevil borers. New Vision, Uganda, 25<sup>th</sup> September 2017.

### Web-based Outputs / Announcements / Videos

1. Global programme seeks to contain serious threat to the world's bananas, FAO, Rome <http://www.fao.org/news/story/en/item/1044761/icode/>
2. Banana Fusarium Wilt Disease. Interview with Prof. Altus Viljoen, FAO, Rome, July 26 2016. <https://www.youtube.com/watch?v=AXm9KRiYe7I>

### TV / Broadcast

1. UBC TV (National broadcaster) <https://www.youtube.com/watch?v=oNvaJW6t31w>
2. NBS TV (Private) <https://www.youtube.com/watch?v=9XXHZspdZFY>
3. IITA, April 27, [International experts on banana improvement meet in Uganda to strengthen breeding efforts in the region](#)
4. CGIAR, [International experts on banana improvement meet in Uganda to strengthen breeding efforts in the region](#),

### Radio Broadcast

1. [NARO: New Banana Breed Are Disease Resistant](#), YouTube, 2<sup>nd</sup> May, 2017.

## 2. Work Package 1

### 2.1 Banana Genotypes Selected From Early Evaluation Trial Established in 2015

| N  | Genotyp  | Cross   | HT | G  | N  | Y  | HT  | N  | B | H | F | F | N  | N  | D  | Rem   |
|----|----------|---------|----|----|----|----|-----|----|---|---|---|---|----|----|----|-------|
| 1  | 10/569-  | 365K-   | 24 | 39 | 7  | 5  | 14  | 8  | 9 | 7 | 9 | 6 | 98 | 0  |    | *     |
| 2  | 10/569-  | 365K-   | 19 | 38 | 10 | 9  | 16  | 6  | 8 | 6 | 1 | 1 | 86 | 0  | 18 | ****  |
| 3  | 10/574-3 | 199K-   | 21 | 41 | 8  | 7  | 12  | 4  | 6 | 6 | 8 | 7 | 86 | 0  | 14 | ****  |
| 4  | 10/579-1 | 1411K-  | 19 | 36 | 9  | 9  | 15  | 4  | 1 | 7 | 1 | 9 | 96 | 4  | 19 | ***** |
| 5  | 10/579-2 | 1411K-  | 19 | 36 | 12 | 1  | 16  | 6  | 1 | 7 | 1 | 1 | 86 | 3  | 19 | ****  |
| 6  | 10/579-3 | 1411K-  | 20 | 36 | 13 | 1  | 19  | 4  | 1 | 6 | 1 | 1 | 90 | 2  | 19 | ****  |
| 7  | 10/579-4 | 1411K-  | 22 | 40 | 10 | 8  | 14  | 4  | 1 | 7 | 1 | 1 | 98 | 4  | 63 | ***** |
| 8  | 10/585-6 | 401K-   | 24 | 45 | 11 | 8  | 14  | 3  | 2 | 8 | 1 | 1 | 12 | 2  | 16 | ****  |
| 9  | 10/585-7 | 401K-   | 24 | 40 | 8  | 6  | 15  | 2  | 8 | 8 | 1 | 1 | 10 | 0  | 15 | *     |
| 10 | 10/585-8 | 401K-   | 22 | 36 | 9  | 6  | 10  | 3  | 9 | 8 | 1 | 1 | 10 | 0  | 14 | ***   |
| 11 | 10/595-1 | 199K-   | 23 | 32 | 9  | 7  | 19  | 2  | 8 | 6 | 1 | 1 | 98 | 0  | 13 | ****  |
| 12 | 10/601-2 | 1154K-  | 21 | 35 | 12 | 9  | 30  | 1  | 1 | 9 | 1 | 1 | 12 | 3  | 13 | ****  |
| 13 | 10/601-5 | 1154K-  | 20 | 36 | 11 | 9  | -   | 0  | 1 | 6 | 1 | 1 | 89 | 2  | -  | ****  |
| 14 | 10/601-7 | 1154K-  | 21 | 49 | 8  | 7  | 15  | 7  | 1 | 6 | 1 | 1 | 98 | 0  | 17 | ****  |
| 15 | 10/669-  | 1411K-  | 20 | 40 | 12 | 1  | 17  | 8  | 5 | 4 | 1 | 1 | 48 | 0  | 17 | ***** |
| 16 | 10/669-  | 1411K-  | 21 | 39 | 14 | 8  | 16  | 5  | 1 | 8 | 1 | 1 | 88 | 2  | 16 | ****  |
| 17 | 10/669-5 | 1411K-  | 21 | 43 | 15 | 1  | 16  | 9  | 1 | 7 | 1 | 1 | 10 | 1  | 17 | *     |
| 18 | 10/669-  | 1411K-  | 25 | 44 | 9  | 7  | 15  | 8  | 7 | 7 | 1 | 1 | 98 | 0  | 17 | **    |
| 19 | 10/669-  | 1411K-  | 21 | 39 | 9  | 5  | 14  | 7  | 6 | 7 | 1 | 1 | 89 | 2  | 16 | **    |
| 20 | 10/669-  | 1411K-  | 19 | 38 | 9  | 5  | 16  | 7  | 8 | 8 | 1 | 1 | 88 | 1  | 17 | **    |
| 21 | 10/671-5 | 376Kx40 | 21 | 33 | 8  | 5  | 14  | 4  | 8 | 6 | 1 | 1 | 66 | 0  | 18 | **    |
| 22 | 10/672-1 | 401Kx40 | 21 | 30 | 6  | 5  | 18  | 6  | 7 | 7 | 1 | 1 | 97 | 0  | 14 | ***   |
| 23 | 10/672-2 | 401Kx40 | -  | -  | -  | -  | -   | 0  | 1 | 6 | 1 | 1 | 87 | 3  | -  | ****  |
| 24 | 10/672-4 | 401Kx40 | 15 | 31 | 10 | 9  | 13  | 6  | 6 | 5 | 1 | 1 | 55 | 0  | 17 | ***** |
| 25 | 10/672-5 | 401Kx40 | 18 | 34 | 12 | 1  | 10  | 5  | 7 | 7 | 1 | 1 | 49 | 0  | 18 | ***   |
| 26 | 10/672-8 | 401Kx40 | 21 | 37 | 11 | 9  | 16  | 6  | 7 | 6 | 1 | 1 | 87 | 1  | 16 | ****  |
| 27 | 10/686-  | 365Kx40 | 20 | 29 | 9  | 9  | 14  | 6  | 5 | 6 | 1 | 9 | 76 | 0  | 16 | ****  |
| 28 | 10/687-5 | 660K-   | 22 | 30 | 8  | 6  | 18  | 4  | 5 | 5 | 1 | 1 | 10 | 0  | 14 | ***   |
| 29 | 10/689-5 | 660K-   | 17 | 40 | 15 | 1  | 13  | 3  | 7 | 4 | 1 | 1 | 54 | 1  | 16 | ***   |
| 30 | 10/689-7 | 660K-   | 19 | 39 | 9  | 9  | 15  | 6  | 6 | 4 | 1 | 1 | 51 | 1  | 19 | ***   |
| 31 | 10/700-  | 660K-   | 25 | 40 | 11 | 9  | 17  | 3  | 1 | 6 | 1 | 1 | 10 | 1  | 16 | ***   |
| 32 | 10/700-  | 660K-   | 15 | 30 | 11 | 1  | 10  | 1  | 6 | 5 | 1 | 1 | 60 | 0  | 14 | ***   |
| 33 | 10/700-  | 660K-   | 26 | 35 | 7  | 6  | 17  | 6  | 1 | 7 | 1 | 1 | 94 | 0  | 16 | ****  |
| 34 | 10/700-  | 660K-   | 15 | 38 | 13 | 9  | 13  | 4  | 6 | 5 | 1 | 1 | 54 | 1  | 14 | ***   |
| 35 | 10/700-  | 660K-   | 19 | 38 | 9  | 7  | 16  | 7  | 6 | 5 | 1 | 1 | 51 | 0  | 16 | **    |
| 36 | 10/700-  | 660K-   | -  | -  | -  | -  | -   | 0  | 8 | 6 | 1 | 1 | 87 | 0  | -  | ****  |
| 37 | 10/700-8 | 660K-   | 24 | 40 | 11 | 9  | 20  | 6  | 8 | 6 | 1 | 1 | 10 | 0  | 15 | ***** |
| 38 | 10/702-2 | 199K-   | 20 | 39 | 12 | 1  | 15  | 6  | 1 | 6 | 1 | 1 | 91 | 0  | 18 | *     |
| 39 | 10/669-  | 1411K-  | 17 | 32 | 7  | 5  | 14  | 4  | 8 | 7 | 1 | 1 | 10 | 0  | 18 | ***   |
| 40 | 10/601-1 | 1154K-  | 17 | 36 | 10 | 1  | 11  | 3  | 1 | 7 | 1 | 1 | 10 | 2  | 17 | ****  |
| 41 | 10/601-3 | 1154K-  | 16 | 37 | 11 | 9  | 11  | 4  | 1 | 9 | 1 | 1 | 11 | 3  | 16 | ****  |
| 42 | Mbwazir  | N/A     | 21 | 46 | 8. | 6. | 14  | 2. | 7 | 5 | 1 | 1 | 85 | 3. | 97 | **    |
| M  |          |         | 20 | 37 | 10 | 8. | 14  | 4. | 8 | 6 | 1 | 1 | 87 | 1. | 16 |       |
| S. |          |         | 27 | 4. | 2. | 2. | 37. | 2. | 3 | 1 | 1 | 1 | 20 | 1. | 26 |       |
| C  |          |         | 13 | 12 | 21 | 2  | 26. | 5. | 3 | 1 | 1 | 1 | 23 | 12 | 16 |       |

HT=Plant height (cm), GTH= plant girth (cm), NSL= number of green standing leaves, YLS= youngest leaf spotted at flowering, HTTS= height of tallest sucker at flowering, NoS= number of suckers, BWT= bunch weight (kg), HDS= number of hands, FL= fruit length, FC= fruit circumference, NF= number of finger/bunch, NLH= number of leaves at flowering, and DTM=days to bunch maturity. **Remark<sup>3</sup>**= Defines the level of acceptance of the test genotypes in terms of pulp colour and sap content compared to Mbwazirume (a local check).

\*\*\*\*\* (5 stars) = No sap and deep yellow pulp colour same as that of Mbwazirume;

\*\*\*\* (4 stars) = No sap and yellow pulp colour almost same as that of Mbwazirume

\*\*\* (3 stars) = No sap and yellow colour slightly lighter than that of Mbwazirume

\*\* (2 stars) = With little sap and colour slightly lighter than that of Mbwazirume

\* (1 star) = With a lot of sap and white colour

## 2.2 Results of 75 Hybrids Selected From Two Evaluation Trials (Low-Input Management and High-Input Management Fields) of a Training Population in Sendusu

Mean performance of genotypes compared to local check, Enzirabahima using the least significant value at P=0.05: Category a (marked in red) indicates genotypes with mean significantly lower than the mean of Enzirabahima; b (white) is for genotypes equal to Enzirabahima; c (green) is for genotypes with a higher mean than for Enzirabahima.

| sn | Genotype   | Yield  | Maturity | Black Sigatoka | Suckering | Stature |        |         | Bunch orientation |
|----|------------|--------|----------|----------------|-----------|---------|--------|---------|-------------------|
|    |            | BWT    | DFM      | INSLF          | TS        | PGF     | PHF    | PGF/PHF | RP (mode)         |
| 1  | 25623S-11  | 23.44c | 160c     | 76.28c         | 5.27b     | 60.51c  | 315.4b | 0.190c  | 1                 |
| 2  | 26337S-11  | 22.14c | 147c     | 77.50c         | 4.79b     | 59.56c  | 327.2b | 0.182c  | 1                 |
| 3  | 25583S-2   | 18.44c | 137c     | 78.56c         | 4.36b     | 59.83c  | 316.2b | 0.184c  | 1                 |
| 4  | 26337S-34  | 17.20c | 142c     | 72.00c         | 2.34b     | 55.84b  | 347.9b | 0.167c  | 1                 |
| 5  | 26666S-1   | 17.04c | 151c     | 81.49c         | 6.31b     | 60.94c  | 316.9b | 0.192c  | 1                 |
| 6  | 28776S-2   | 15.62c | 146c     | 65.18b         | 4.38b     | 51.50b  | 270.0a | 0.193c  | 1                 |
| 7  | 24948S-10  | 15.53c | 144c     | 71.46c         | 3.74b     | 51.24b  | 295.3b | 0.174c  | 1                 |
| 8  | 26337S-43  | 13.75c | 139c     | 77.11c         | 3.89b     | 53.43b  | 299.4b | 0.179c  | 1                 |
| 9  | 27579S-3   | 13.61c | 132c     | 73.45c         | 7.94c     | 47.44b  | 287.8b | 0.164b  | 1                 |
| 10 | 24948S-9   | 13.57c | 148c     | 80.89c         | 4.26b     | 48.39b  | 283.6a | 0.172c  | 1                 |
| 11 | 28452S-11  | 13.42c | 135c     | 76.73c         | 5.68b     | 44.95b  | 281.6a | 0.160b  | 2                 |
| 12 | 27770S-4   | 12.89c | 128c     | 75.69c         | 4.72b     | 44.53b  | 261.4a | 0.172c  | 1                 |
| 13 | 28033S-23  | 12.87c | 159c     | 72.90c         | 3.37b     | 51.26b  | 265.6a | 0.193c  | 1                 |
| 14 | 28476S-8   | 12.84c | 127c     | 71.58c         | 6.75b     | 48.75b  | 283.7a | 0.172c  | 1                 |
| 15 | 27914S-1   | 12.79c | 140c     | 75.40c         | 5.79b     | 45.95b  | 250.7a | 0.184c  | 1                 |
| 16 | 26337S-22A | 12.69c | 137c     | 77.82c         | 8.56c     | 52.78b  | 355.0b | 0.148b  | 1                 |
| 17 | 25356S-1   | 12.43c | 144c     | 67.90b         | 3.36b     | 42.85a  | 235.4a | 0.182c  | 1                 |
| 18 | 27262S-3   | 12.21c | 139c     | 77.41c         | 4.70b     | 45.07b  | 247.5a | 0.181c  | 1                 |
| 19 | 26316S-7   | 12.15c | 125c     | 71.10c         | 3.67b     | 44.99b  | 276.2a | 0.165b  | 1                 |
| 20 | 28492S-1   | 12.06c | 154c     | 70.48c         | 4.33b     | 43.28b  | 250.0a | 0.174c  | 1                 |
| 21 | 27873S-26  | 12.00c | 164c     | 79.33c         | 4.73b     | 48.14b  | 282.0a | 0.177c  | 1                 |
| 22 | 27914S-24  | 11.88c | 141c     | 74.49c         | 4.71b     | 41.61a  | 294.7b | 0.144b  | 1                 |
| 23 | 25974S-11  | 11.74c | 122c     | 68.87c         | 4.75b     | 47.06b  | 267.0a | 0.175c  | 1                 |
| 24 | 25031S-7   | 11.63c | 190c     | 75.61c         | 4.05b     | 41.15a  | 223.7a | 0.187c  | 1                 |

| sn | Genotype  | Yield  | Maturity | Black Sigatoka | Suckering | Stature |        |         | Bunch orientation |
|----|-----------|--------|----------|----------------|-----------|---------|--------|---------|-------------------|
|    |           | BWT    | DFM      | INSLF          | TS        | PGF     | PHF    | PGF/PHF | RP (mode)         |
| 25 | 26840S-10 | 11.62c | 149c     | 76.16c         | 4.07b     | 48.21b  | 266.8a | 0.182c  | 1                 |
| 26 | 28246S-7  | 11.48c | 122c     | 75.26c         | 7.36c     | 41.75a  | 252.4a | 0.165b  | 1                 |
| 27 | 29285s-20 | 11.46c | 147c     | 71.01c         | 4.62b     | 47.47b  | 268.1a | 0.178c  | 1                 |
| 28 | 25117S-1  | 11.39c | 166c     | 69.44c         | 5.49b     | 48.99b  | 302.2b | 0.162b  | 1                 |
| 29 | 27914S-3  | 11.35c | 130c     | 74.21c         | 4.09b     | 48.20b  | 285.0a | 0.169c  | 1                 |
| 30 | 27914S-26 | 11.00c | 139c     | 78.20c         | 7.21c     | 40.48a  | 259.6a | 0.156b  | 1                 |
| 31 | 26260S-3  | 10.91c | 170c     | 73.21c         | 3.63b     | 41.74a  | 245.7a | 0.169c  | 1                 |
| 32 | 27346S-4  | 10.90c | 172c     | 75.58c         | 5.52b     | 52.65b  | 282.2a | 0.182c  | 1                 |
| 33 | 26337S-37 | 10.79c | 143c     | 86.31c         | 6.52b     | 52.69b  | 323.4b | 0.163b  | 1                 |
| 34 | 26315S-1  | 10.78c | 140c     | 64.89b         | 2.94b     | 48.97b  | 286.8b | 0.171c  | 1                 |
| 35 | 26815S-3  | 10.77c | 146c     | 69.82c         | 3.05b     | 46.77b  | 280.7a | 0.168c  | 1                 |
| 36 | 25974S-17 | 10.46c | 161c     | 84.31c         | 4.71b     | 51.04b  | 263.3a | 0.177c  | 2                 |
| 37 | 25737S-1  | 10.43c | 124c     | 71.63c         | 3.78b     | 43.18b  | 239.6a | 0.182c  | 1                 |
| 38 | 27184S-4  | 10.37b | 146c     | 82.7c          | 5.79b     | 43.13b  | 270.5a | 0.161b  | 1                 |
| 39 | 29586S-4  | 10.19b | 146c     | 70.29c         | 5.94b     | 46.28b  | 250.0a | 0.191c  | 2                 |
| 40 | 26337S-22 | 10.07b | 146c     | 77.17c         | 5.71b     | 45.29b  | 336.4b | 0.135a  | 1                 |
| 41 | 26975S-2  | 10.05b | 136c     | 73.06c         | 4.15b     | 51.70b  | 304.9b | 0.169c  | 1                 |
| 42 | 28434S-9  | 9.93b  | 141c     | 70.65c         | 4.00b     | 46.30b  | 269.3a | 0.172c  | 2                 |
| 43 | 24948S-13 | 9.84b  | 141c     | 69.12c         | 3.96b     | 46.06b  | 263.9a | 0.176c  | 1                 |
| 44 | 25435S-4  | 9.65b  | 155c     | 73.96c         | 2.94b     | 41.13a  | 257.1a | 0.162b  | 1                 |
| 45 | 28030S-6  | 9.59b  | 151c     | 76.82c         | 2.50b     | 52.19b  | 311.9b | 0.167c  | 2                 |
| 46 | 29114S-24 | 9.42b  | 166c     | 70.89c         | 4.67b     | 45.77b  | 273.2a | 0.171c  | 1                 |
| 47 | 28060S-8  | 9.06b  | 161c     | 76.87c         | 3.71b     | 43.24b  | 279.4a | 0.155b  | 1                 |
| 48 | 27262S-1  | 9.04b  | 132c     | 77.85c         | 6.44b     | 48.98b  | 306.1b | 0.162b  | 1                 |
| 49 | 28200S-3  | 8.96b  | 121c     | 85.38c         | 3.93b     | 44.89b  | 295.7b | 0.152b  | 1                 |
| 50 | 28260S-2  | 8.94b  | 134c     | 71.47c         | 3.83b     | 52.28b  | 302.2b | 0.174c  | 1                 |
| 51 | 28030S-2  | 8.78b  | 139c     | 79.88c         | 3.83b     | 46.06b  | 298.6b | 0.154b  | 2                 |
| 52 | 28068S-9  | 8.70b  | 115c     | 75.17c         | 3.64b     | 42.72a  | 242.6a | 0.172c  | 1                 |
| 53 | 27935S-1  | 8.62b  | 135c     | 70.79c         | 7.75c     | 39.31a  | 244.7a | 0.162b  | 1                 |

| sn | Genotype     | Yield       | Maturity   | Black Sigatoka | Suckering   | Stature      |             |              | Bunch orientation |
|----|--------------|-------------|------------|----------------|-------------|--------------|-------------|--------------|-------------------|
|    |              | BWT         | DFM        | INSLF          | TS          | PGF          | PHF         | PGF/PHF      | RP (mode)         |
| 54 | 25499S-7     | 8.61b       | 143c       | 63.81b         | 5.56b       | 41.83a       | 266.1a      | 0.157b       | 2                 |
| 55 | 28164S-3     | 8.39b       | 147c       | 84.6c          | 5.86b       | 44.24b       | 293.5b      | 0.150b       | 1                 |
| 56 | 25974S-30    | 8.28b       | 178c       | 80.45c         | 3.96b       | 45.51b       | 264.2a      | 0.175c       | 1                 |
| 57 | 25909S-3     | 8.20b       | 161c       | 69.44c         | 3.65b       | 47.67b       | 255.4a      | 0.186c       | 1                 |
| 58 | 26337S-22B   | 8.15b       | 125c       | 72.74c         | 7.45c       | 47.07b       | 322.2b      | 0.145b       | 1                 |
| 59 | 27401S-1     | 7.92b       | 153c       | 71.56c         | 4.56b       | 45.71b       | 244.1a      | 0.189c       | 2                 |
| 60 | 28465S-21    | 7.87b       | 142c       | 81.55c         | 4.83b       | 41.22a       | 294.9b      | 0.140a       | 2                 |
| 61 | 28257S-1     | 7.59b       | 153c       | 78.80c         | 5.20b       | 43.51b       | 276.8a      | 0.156b       | 2                 |
| 62 | 27914S-7     | 7.49b       | 140c       | 70.29c         | 7.81c       | 36.77a       | 222.5a      | 0.166b       | 2                 |
| 63 | 28164S-15    | 7.38b       | 123c       | 75.31c         | 5.75b       | 45.69b       | 294.9b      | 0.156b       | 2                 |
| 64 | 27914S-18    | 7.36b       | 143c       | 76.82c         | 5.16b       | 40.95a       | 256.0a      | 0.160b       | 2                 |
| 65 | 27579S-1     | 7.30b       | 147c       | 83.21c         | 3.19b       | 43.39b       | 264.4a      | 0.162b       | 2                 |
| 66 | 27346S-2     | 7.16b       | 158c       | 70.74c         | 2.99b       | 45.77b       | 277.2a      | 0.166b       | 2                 |
| 67 | 25974S-31    | 7.07b       | 160c       | 68.47c         | 4.17b       | 44.00b       | 218.6a      | 0.204c       | 2                 |
| 68 | 27885S-9     | 6.99b       | 131c       | 73.19c         | 7.11c       | 41.39a       | 286.4b      | 0.144b       | 2                 |
| 69 | 28256S-1     | 6.68b       | 126c       | 72.14c         | 7.14c       | 38.65a       | 240.1a      | 0.162b       | 2                 |
| 70 | 27524S-22    | 6.22b       | 148c       | 73.31c         | 4.27b       | 43.59b       | 273.8a      | 0.159b       | 2                 |
| 71 | 25328S-3     | 6.22b       | 170c       | 75.36c         | 3.25b       | 39.07a       | 213.9a      | 0.174c       | 2                 |
| 73 | 28506S-1     | 5.84b       | 148c       | 71.63c         | 5.31b       | 40.08a       | 286.4b      | 0.140a       | 2                 |
| 74 | 28165S-1     | 5.27b       | 153c       | 84.28c         | 5.06b       | 53.34b       | 297.6b      | 0.182c       | 2                 |
| 75 | 26990S-4     | 3.60b       | 182c       | 70.96c         | 3.75b       | 39.32a       | 229.9a      | 0.170c       | 2                 |
| 76 | 25031S-17    | 3.29b       | 156c       | 78.24c         | 3.29b       | 44.39b       | 240.8a      | 0.190c       | 2                 |
| 72 | Enzirabahima | 6.16        | 104        | 63.16          | 4.29        | 50.21        | 326.1       | 0.153        | 2                 |
|    | <b>LSD</b>   | <b>4.28</b> | <b>9.8</b> | <b>5.14</b>    | <b>2.48</b> | <b>7.132</b> | <b>40.4</b> | <b>0.013</b> |                   |

BWT = Bunch weight (kg), DFM = Days from flowering to harvest, INSLF = Index of non-spotted leaf at flowering, TS = Total number of suckers, PHF = Plant height at flowering, PGF = Plant girth at flowering, PGF/PHF = plant height by plant girth ratio, RP = rachis position (1: vertical, 2: at an angle)

Estimated yield considering bunch weight, planting density with a spacing of 2m x 3m and survival rate; and recommendation for advancement to AYT

| sn | Genotype   | BWT    | INSL   | Survival (%) | Yield (t/ha) | Yield (t/ha/year) | Recommendation to AYT |
|----|------------|--------|--------|--------------|--------------|-------------------|-----------------------|
| 1  | 25583S-2   | 18.44c | 78.56c | 83           | 30.7         | 25.6              | Yes - Group 1         |
| 2  | 24948S-9   | 13.57c | 80.89c | 100          | 22.6         | 22.6              | Yes - Group 1         |
| 3  | 27770S-4   | 12.89c | 75.69c | 100          | 21.5         | 21.5              | Yes - Group 1         |
| 4  | 27914S-1   | 12.79c | 75.40c | 100          | 21.3         | 21.3              | Yes - Group 1         |
| 5  | 27262S-3   | 12.21c | 77.41c | 100          | 20.4         | 20.4              | Yes - Group 1         |
| 6  | 28492S-1   | 12.06c | 70.48c | 100          | 20.1         | 20.1              | Yes - Group 1         |
| 7  | 25974S-11  | 11.74c | 68.87c | 100          | 19.6         | 19.6              | Yes - Group 1         |
| 8  | 28246S-7   | 11.48c | 75.26c | 100          | 19.1         | 19.1              | Yes - Group 1         |
| 9  | 26337S-43  | 13.75c | 77.11c | 83           | 22.9         | 19.1              | Yes - Group 1         |
| 10 | 27914S-3   | 11.35c | 74.21c | 100          | 18.9         | 18.9              | Yes - Group 1         |
| 11 | 27914S-26  | 11.00c | 78.20c | 100          | 18.3         | 18.3              | Yes - Group 1         |
| 12 | 26260S-3   | 10.91c | 73.21c | 100          | 18.2         | 18.2              | Yes - Group 1         |
| 13 | 26337S-37  | 10.79c | 86.31c | 100          | 18.0         | 18.0              | Yes - Group 1         |
| 14 | 28476S-8   | 12.84c | 71.58c | 83           | 21.4         | 17.8              | Yes - Group 1         |
| 15 | 25737S-1   | 10.43c | 71.63c | 100          | 17.4         | 17.4              | Yes - Group 1         |
| 16 | 26316S-7   | 12.15c | 71.10c | 83           | 20.3         | 16.9              | Yes - Group 1         |
| 17 | 27914S-24  | 11.88c | 74.49c | 83           | 19.8         | 16.5              | Yes - Group 1         |
| 18 | 25031S-7   | 11.63c | 75.61c | 83           | 19.4         | 16.2              | Yes - Group 1         |
| 19 | 26975S-2   | 10.05b | 73.06c | 100          | 16.8         | 16.8              | Yes - Group 2         |
| 20 | 29586S-4   | 10.19b | 70.29c | 100          | 17.0         | 17.0              | Yes - Group 2         |
| 21 | 27184S-4   | 10.37b | 82.7c  | 100          | 17.3         | 17.3              | Yes - Group 2         |
| 22 | 28506S-1   | 5.84b  | 71.63c | 100          | 9.7          | 9.7               | Yes - Group 2         |
| 23 | 27885S-9   | 6.99b  | 73.19c | 83           | 11.7         | 9.7               | Yes - Group 2         |
| 24 | 25974S-31  | 7.07b  | 68.47c | 100          | 11.8         | 11.8              | Yes - Group 2         |
| 25 | 27346S-2   | 7.16b  | 70.74c | 100          | 11.9         | 11.9              | Yes - Group 2         |
| 26 | 27914S-18  | 7.36b  | 76.82c | 100          | 12.3         | 12.3              | Yes - Group 2         |
| 27 | 27914S-7   | 7.49b  | 70.29c | 83           | 12.5         | 10.4              | Yes - Group 2         |
| 28 | 28257S-1   | 7.59b  | 78.80c | 100          | 12.7         | 12.7              | Yes - Group 2         |
| 29 | 28465S-21  | 7.87b  | 81.55c | 83           | 13.1         | 10.9              | Yes - Group 2         |
| 30 | 27401S-1   | 7.92b  | 71.56c | 100          | 13.2         | 13.2              | Yes - Group 2         |
| 31 | 25909S-3   | 8.20b  | 69.44c | 100          | 13.7         | 13.7              | Yes - Group 2         |
| 32 | 25974S-30  | 8.28b  | 80.45c | 83           | 13.8         | 11.5              | Yes - Group 2         |
| 33 | 28164S-3   | 8.39b  | 84.6c  | 100          | 14.0         | 14.0              | Yes - Group 2         |
| 34 | 27935S-1   | 8.62b  | 70.79c | 100          | 14.4         | 14.4              | Yes - Group 2         |
| 35 | 28068S-9   | 8.70b  | 75.17c | 83           | 14.5         | 12.1              | Yes - Group 2         |
| 36 | 28030S-2   | 8.78b  | 79.88c | 83           | 14.6         | 12.2              | Yes - Group 2         |
| 37 | 27262S-1   | 9.04b  | 77.85c | 100          | 15.1         | 15.1              | Yes - Group 2         |
| 38 | 24948S-13  | 9.84b  | 69.12c | 100          | 16.4         | 16.4              | Yes - Group 2         |
| 39 | 28434S-9   | 9.93b  | 70.65c | 83           | 16.6         | 13.8              | Yes - Group 2         |
| 40 | 28776S-2   | 15.62c | 65.18b | 83           | 26.0         | 21.7              | Yes - Group 3         |
| 41 | 25356S-1   | 12.43c | 67.90b | 83           | 20.7         | 17.3              | Yes - Group 3         |
| 42 | 26315S-1   | 10.78c | 64.89b | 83           | 18.0         | 15.0              | Yes - Group 3         |
| 43 | 25623S-11  | 23.44c | 76.28c | 67           | 39.1         | 26.0              | Yes - Group 4         |
| 44 | 26666S-1   | 17.04c | 81.49c | 67           | 28.4         | 18.9              | Yes - Group 4         |
| 45 | 24948S-10  | 15.53c | 71.46c | 67           | 25.9         | 17.3              | Yes - Group 4         |
| 46 | 27579S-3   | 13.61c | 73.45c | 67           | 22.7         | 15.1              | Yes - Group 4         |
| 47 | 26337S-34  | 17.20c | 72.00c | 50           | 28.7         | 14.3              | Yes - Group 4         |
| 48 | 28033S-23  | 12.87c | 72.90c | 67           | 21.5         | 14.3              | Yes - Group 4         |
| 49 | 26337S-22A | 12.69c | 77.82c | 67           | 21.2         | 14.1              | Yes - Group 4         |



| sn | Genotype     | BWT    | INSL   | Survival (%) | Yield (t/ha) | Yield (t/ha/year) | Recommendation to AYT |
|----|--------------|--------|--------|--------------|--------------|-------------------|-----------------------|
| 50 | 27873S-26    | 12.00c | 79.33c | 67           | 20.0         | 13.3              | Yes - Group 4         |
| 51 | 26337S-11    | 22.14c | 77.50c | 33           | 36.9         | 12.3              | Yes - Group 4         |
| 52 | 27346S-4     | 10.90c | 75.58c | 67           | 18.2         | 12.1              | Yes - Group 4         |
| 53 | 28452S-11    | 13.42c | 76.73c | 50           | 22.4         | 11.2              | Yes - Group 4         |
| 54 | 26840S-10    | 11.62c | 76.16c | 50           | 19.4         | 9.7               | Yes - Group 4         |
| 55 | 29285s-20    | 11.46c | 71.01c | 50           | 19.1         | 9.6               | Yes - Group 4         |
| 56 | 25117S-1     | 11.39c | 69.44c | 50           | 19.0         | 9.5               | Yes - Group 4         |
| 57 | 25974S-17    | 10.46c | 84.31c | 50           | 17.4         | 8.7               | Yes - Group 4         |
| 58 | 26815S-3     | 10.77c | 69.82c | 33           | 18.0         | 6.0               | Yes - Group 4         |
| 59 | 25499S-7     | 8.61b  | 63.81b | 100          | 14.4         | 14.4              | Yes - Group 5         |
| 60 | 25435S-4     | 9.65b  | 73.96c | 67           | 16.1         | 10.7              | Yes - Group 6         |
| 61 | 28030S-6     | 9.59b  | 76.82c | 67           | 16.0         | 10.7              | Yes - Group 6         |
| 62 | 29114S-24    | 9.42b  | 70.89c | 67           | 15.7         | 10.5              | Yes - Group 6         |
| 63 | 28060S-8     | 9.06b  | 76.87c | 67           | 15.1         | 10.1              | Yes - Group 6         |
| 64 | 28260S-2     | 8.94b  | 71.47c | 67           | 14.9         | 9.9               | Yes - Group 6         |
| 65 | 26337S-22B   | 8.15b  | 72.74c | 67           | 13.6         | 9.1               | No                    |
| 66 | 27524S-22    | 6.22b  | 73.31c | 83           | 10.4         | 8.6               | No                    |
| 67 | 25328S-3     | 6.22b  | 75.36c | 83           | 10.4         | 8.6               | No                    |
| 68 | 28164S-15    | 7.38b  | 75.31c | 67           | 12.3         | 8.2               | No                    |
| 69 | 27579S-1     | 7.30b  | 83.21c | 67           | 12.2         | 8.1               | No                    |
| 70 | 28200S-3     | 8.96b  | 85.38c | 50           | 14.9         | 7.5               | No                    |
| 71 | 28256S-1     | 6.68b  | 72.14c | 67           | 11.1         | 7.4               | No                    |
| 72 | 28165S-1     | 5.27b  | 84.28c | 83           | 8.8          | 7.3               | No                    |
| 73 | 26990S-4     | 3.60b  | 70.96c | 67           | 6.0          | 4.0               | No                    |
| 74 | 25031S-17    | 3.29b  | 78.24c | 67           | 5.5          | 3.7               | No                    |
| 75 | 26337S-22    | 10.07b | 77.17c | 0            | 16.8         | 0.0               | No                    |
| 76 | Enzirabahima | 6.16   | 63.16  | 83           | 10.3         | 8.6               |                       |

BWT = Bunch weight (kg), INSLF = Index of non-spotted leaf at flowering, +: yield per ha regardless of survival rate, ++: yield per ha per year considering survival rate

### 2.3 NARITA end user response: tentative

| Product          | Single purpose                | Dual purpose |    |
|------------------|-------------------------------|--------------|----|
| Steamed Food     | 2,4,7,8,12,14,15,18,23 and 24 | 17           | 32 |
| Katogo           | 6, 10 and 16                  | 17           |    |
| Juice            | 21                            | 13           |    |
| Dessert          | 31 and 33                     | 13           |    |
| Boiling plantain |                               |              | 32 |

31: Pisang Ceylon,

32: Cachaco

33: Gros Michel

## 2.4 Preliminary data on pollen quantity and bunch yield of some diploids in a trial at NARL

Pollen quantity was assessed on a scale of 0-4 where: 0 = no pollen, 1 = very little pollen, 2 = little pollen, 3 = moderate pollen, 4 = abundant pollen.

**Table 1:** Pollen quantity and bunch yield of some diploids in a trial at NARL

| Genotype       | Pollen Quantity  | Bunch weight (kg) |
|----------------|------------------|-------------------|
| 02145/1320     | 1.0 ± 0.5        | 3.0 ± 1.2         |
| 1019           | 1.0 ± 0.3        | 2.0 ± 0.8         |
| 1119           | 2.7 ± 0.3        | 1.6 ± 0.5         |
| 1603           | 2.4 ± 0.2        | 1.4 ± 0.4         |
| 1702           | 2.6 ± 0.2        | 2.4 ± 0.4         |
| 201087-3       | 3.0 ± 0.8        | 2.0 ± 1.7         |
| 201087-4       | 2.0 ± 0.8        | 5.0 ± 1.7         |
| 2215           | 4.0 ± 0.5        | 2.5 ± 1.2         |
| 2216           | 2.8 ± 0.3        | 2.5 ± 0.7         |
| 2710           | 2.5 ± 0.3        | 3.3 ± 0.7         |
| 5265-1         | 1.4 ± 0.2        | 2.7 ± 0.5         |
| 7197-2         | 2.8 ± 0.3        | 6.1 ± 0.6         |
| TMB2x8075-7    | 3.6 ± 0.2        | 3.8 ± 0.4         |
| 81k            | 3.1 ± 0.2        | 2.7 ± 0.5         |
| 919            | 2.0 ± 0.8        | 3.0 ± 1.7         |
| F1C4N          | 2.6 ± 0.3        | 1.7 ± 0.6         |
| UZAKAN         | 1.0 ± 0.5        | 9.5 ± 1.2         |
| Kasaska        | 2.0 ± 0.8        | 1.0 ± 1.7         |
| Khaithounguang | 2.5 ± 0.4        | 2.8 ± 0.8         |
| Makyungwe      | 1.0 ± 0.4        | 7.8 ± 0.8         |
| SH3142         | 2.0 ± 0.5        | 7.0 ± 1.2         |
| TMB2x5105-1    | 2.2 ± 0.3        | 3.4 ± 0.8         |
| TMB2x6142      | 2.0 ± 0.4        | 2.5 ± 0.8         |
| TMB2x9172      | 2.0 ± 0.4        | 2.2 ± 0.8         |
| TUU GIA        | 1.2 ± 0.3        | 4.8 ± 0.8         |
| Yalim          | 1.0 ± 0.5        | 5.0 ± 1.2         |
| Zebrina GF     | 1.4 ± 0.3        | 2.2 ± 0.8         |
| Calcutta 4     | 3.9 ± 0.3        | 2.4 ± 0.6         |
| Milembo        | 1.0 ± 0.5        | 9.0 ± 1.2         |
| Mean           | <b>2.6 ± 0.5</b> | <b>3.6 ± 1.4</b>  |

## 2.5 Studies on pollen quantity and quality for some of the commonly used males at IITA-Arusha

### POLLEN VIABILITY VARIABILITY AMONG EAST AFRICAN DIPLOIDS

#### 1. Quantity of pollen grains per cultivar

|               | Cultivar        | Quantity |         |         |         | SD     |
|---------------|-----------------|----------|---------|---------|---------|--------|
|               |                 | slide 1  | slide 2 | slide 3 | average |        |
| <b>Males</b>  | Calcutta 4      | 34250    | 33907   | 32494   | 33550   | 930.5  |
|               | Pisang Pahang   | 32124    | 31107   | 32190   | 31807   | 607.1  |
|               | CV rose         | 29367    | 28907   | 29345   | 29206   | 259.5  |
|               | Truncanta       | 25781    | 25721   | 24980   | 25494   | 446.1  |
|               | Zebrina GF      | 8930     | 8720    | 8447    | 8699    | 242.2  |
|               | Borneo          | 25784    | 36123   | 36430   | 32779   | 6059.8 |
|               |                 |          |         |         |         |        |
| <b>Mchare</b> | Huti white      | 5920     | 5790    | 5810    | 5840    | 70     |
|               | Huti green      | 4324     | 3912    | 4398    | 4211    | 261.9  |
|               | Mshare laini    | 5265     | 4876    | 4997    | 5046    | 199.2  |
|               | Mchale mlelembo | 2757     | 2858    | 2412    | 2675    | 233.9  |
|               | Akondro mainty  | 3260     | 3178    | 2645    | 3028    | 333.9  |
|               | Makyugu I       | 0        | 0       | 0       | 0       |        |
|               | Ijihu inkundu   | 0        | 0       | 0       | 0       |        |

#### 2. Viability percentage

|               | Cultivar        | Viability (%) |      |         |      |         |      | SD    |         |
|---------------|-----------------|---------------|------|---------|------|---------|------|-------|---------|
|               |                 | slide 1       |      | slide 2 |      | slide 3 |      |       | Average |
| <b>Males</b>  | Calcutta 4      | 76.4          | 80.1 | 84.3    | 80.4 | 78.6    | 76.6 | 79.4  | 4.6     |
|               | Pisang Pahang   | 67.3          | 80.1 | 80.9    | 74.3 | 78.6    | 70.6 | 75.3  | 5.      |
|               | CV rose         | 56.7          | 56.4 | 50.1    | 52.6 | 52      | 46.7 | 52.4  | 3.8     |
|               | Truncanta       | 68.9          | 67   | 82.6    | 64.6 | 72      | 74.3 | 71.5  | 5.8     |
|               | Zebrina GF      | 31.6          | 18.8 | 23.6    | 20   | 27      | 22.6 | 23.9  | 4.3     |
|               | Borneo          | 83            | 68.9 | 78.6    | 76.4 | 81.6    | 84.8 | 78.9  | 5.2     |
|               |                 |               |      |         |      |         |      |       |         |
| <b>Mchare</b> | Huti white      | 68.3          | 56.7 | 65.8    | 67.9 | 70.7    | 76.4 | 67.6  | 5.9     |
|               | Huti green      | 28.6          | 25.3 | 38.5    | 30.2 | 43      | 46.3 | 35.35 | 7.8     |
|               | Mshare laini    | 53            | 48.3 | 46.7    | 48.4 | 66      | 62.4 | 54.13 | 7.4     |
|               | Mchale mlelembo | 46.7          | 32.8 | 36.4    | 35.6 | 41.6    | 44.6 | 39.6  | 5       |
|               | Akondro mainty  | 12.6          | 18.7 | 31.3    | 34.3 | 28.9    | 27.3 | 25.5  | 9.5     |
|               | Makyugu I       |               | 0    | 0       | 0    | 0       | 0    | 0     | 0       |
|               | Ijihu inkundu   |               | 0    | 0       | 0    | 0       | 0    | 0     | 0       |

## 2.6 Pollen viability and seasonal variation in diploids and Mchare



**POLLEN VIABILITY AND SEASONAL  
VARIATION IN SELECTED WILD MUSA (AA)  
DIPLOIDS AND MCHARE CULTIVARS**

**Veronica Massawe, Hassan Mduma, Rony Swennen and Allan Brown**

**International Institute of Tropical Agriculture @NM-AIST, Arusha  
Tanzania**

# IITA Banana Breeding in Africa

| Arusha (Tanzania) | Sendusu/Namulonge (Uganda) | Ibadan (Nigeria) |
|-------------------|----------------------------|------------------|
| Mchare: AA        | Matooke: AAA               | Plantains: AAB   |



Dessert banana

Cavendish

AAA

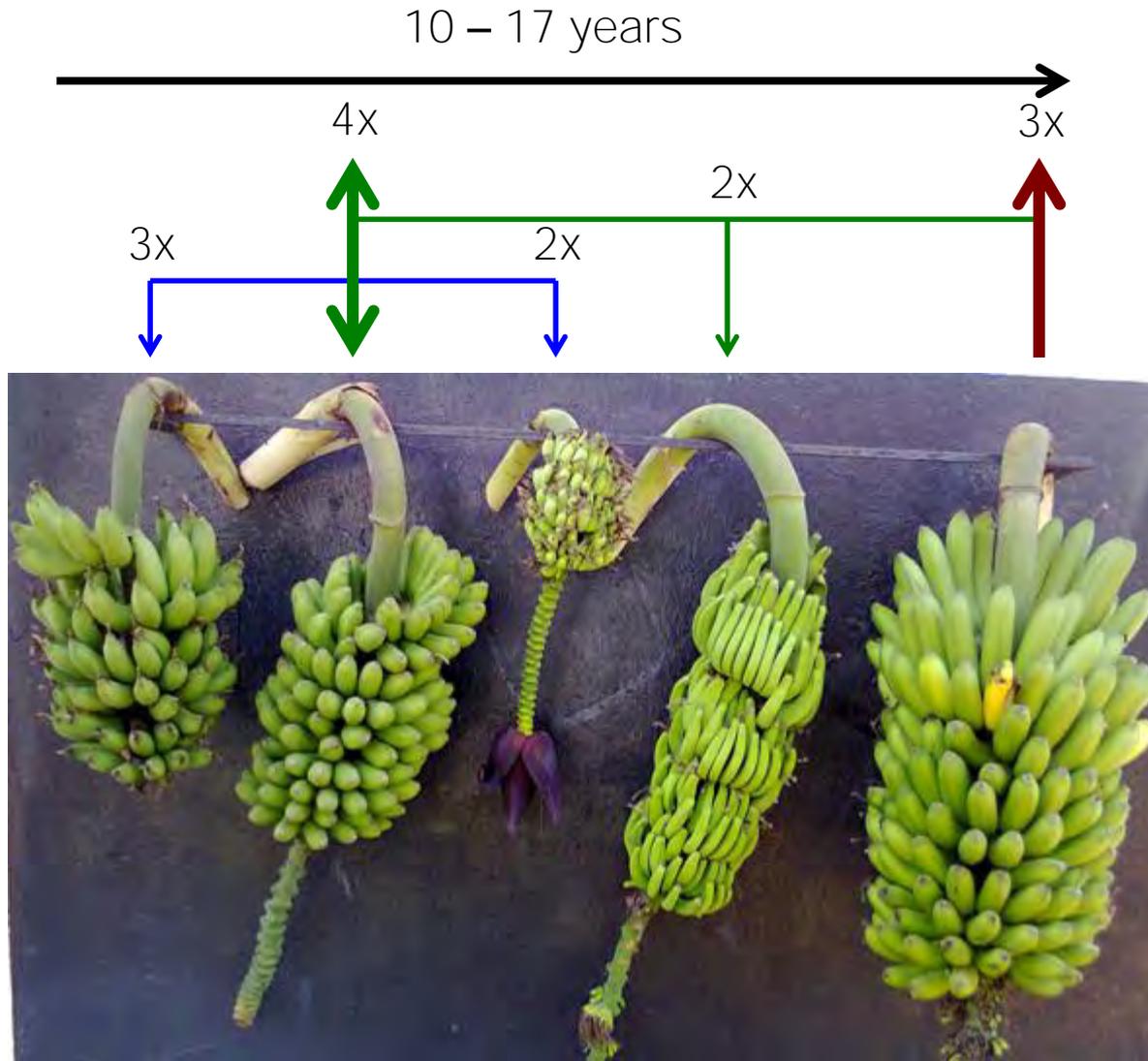


- Why triploid?
  - Seedless
  - Appropriate combination of large bunch and vigorous plant growth
- However...
  - Triploids take longer to breed
  - Take up more space
- Mchare are the exception to the rule





# Breeding strategy simpler than for triploids:



# Arusha Banana Breeding (Mchare)



Makhyugu I



Makhyugu I



Huti-white



Huti-white



Mchare Laini



Kahuti



Kahuti



Huti-green



Huti-green



Mchare Laini

Numerous biotic and abiotic pressures:

## Disease and insect pressure

- Black sigatoka
- Fusarium TR 1 and 4
- Nematodes
- Banana weevil
- Viruses



## Abiotic stress

- drought

## Post Harvest

# Mlalis, Mshale, Muraru: the « 3M group »



**Tanzania Musa Expedition 2001**

July 27<sup>th</sup> to July 30<sup>th</sup> 2001  
Dr. de Luyck, Dr. Tereza and A. Mwahele



The Exotic  
Produce



**LZARDI**  
LZARDI  
LZARDI

**TANZANIA MUSA EXPEDITION 2005**

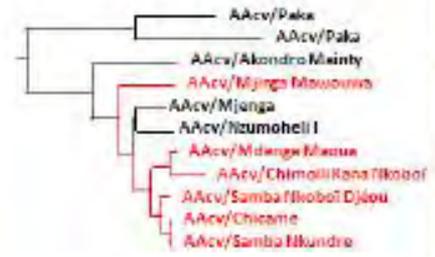
MOROGORO-REBETA REGION  
DECEMBER 2005

**KENYA MUSA EXPEDITION 2006**

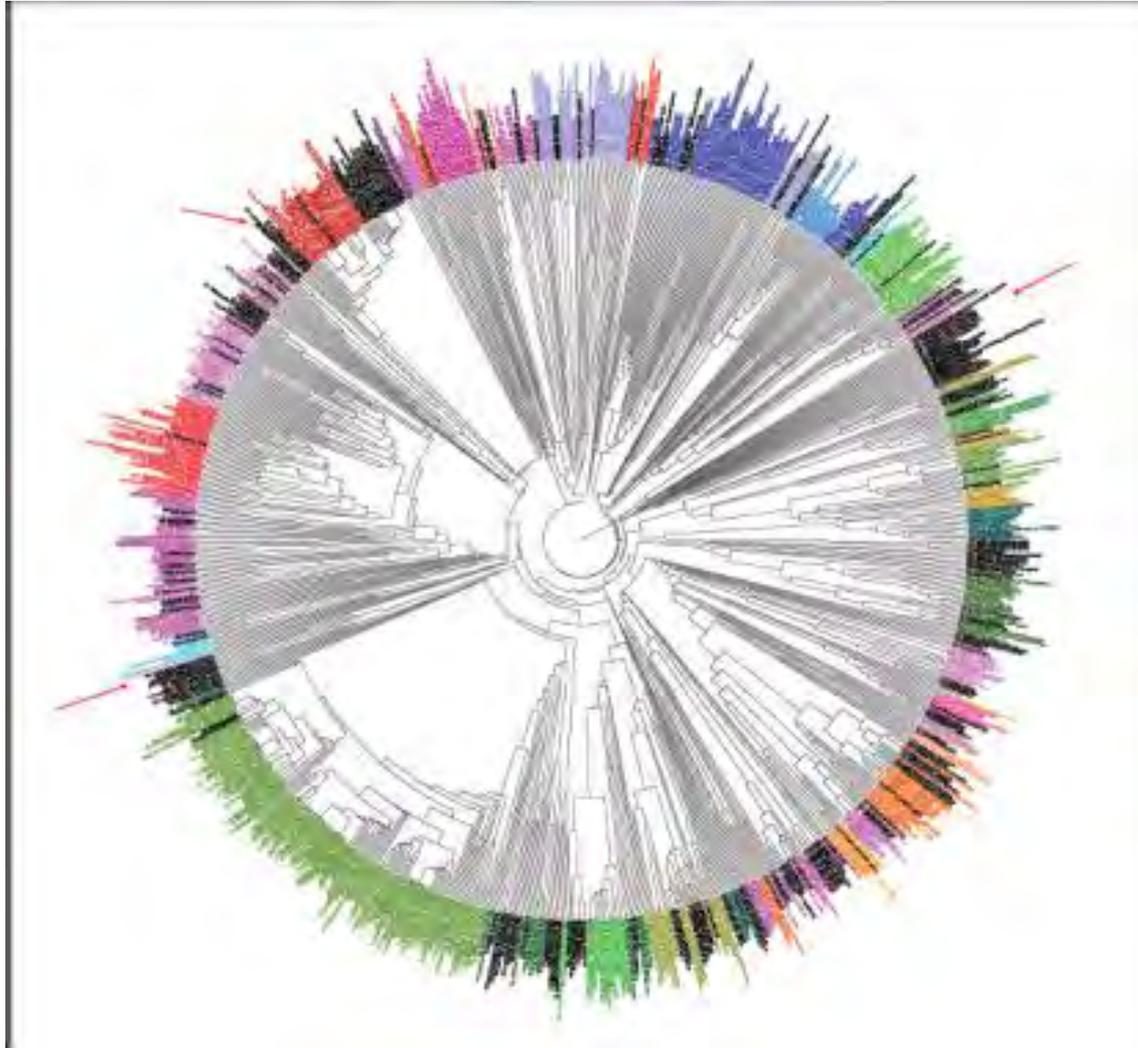
*Karamura, G., Njuguna, J. and Nyamongo, G.*







International Workshop on Pre-Breeding – February 2015







Huti White



Calcutta 4

Determine the genetic and morphological variation among East African Diploids (Mchare, Mlali, Muraru)

Identify fertile Mchare parent (done)

Develop Mchare hybrids with multiple sources of resistance

Attempt backcrosses with fertile Mchare

Intercross resistant Mchare to pyramid resistance

Produce Chromosome doubled plants



What needs to be done?



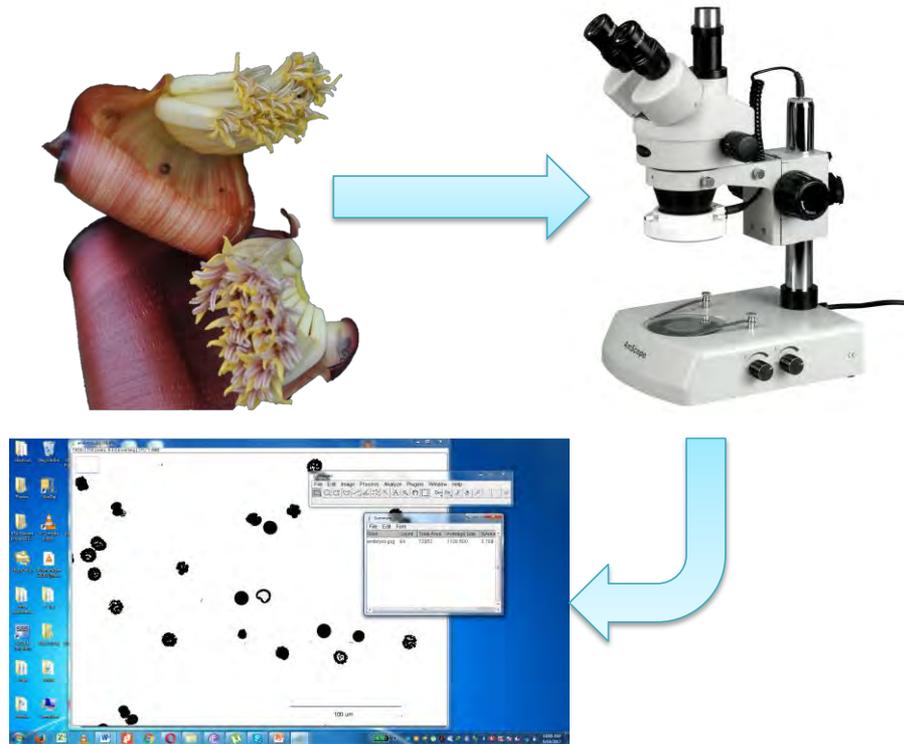
- To assess concentration and viability of pollen among wild diploids and Mchare.
- To identify the most fertile male Mchare cultivars that can be utilized in breeding schemes.



- 15 genotypes (7 wild accessions and 8 Mchare cultivars) for 12 months in Arusha, Tanzania.
- Pollen are collected at the same time ([8am](#)), once a month .

| SN | Genotypes       | Sub species/Group |
|----|-----------------|-------------------|
| 1  | Calcuta 4       | ssp burmannica    |
| 2  | Borneo          | ssp microcarpa    |
| 3  | CV rose         | ssp malaccensis   |
| 4  | P.Pahang        | ssp malaccensis   |
| 5  | P.Lilin         | ssp malaccensis   |
| 6  | Trucanta        | ssp truncata      |
| 7  | Zebrina GF      | ssp zebrina       |
| 8  | Huti white      | Mchare            |
| 9  | Huti green      | Mchare            |
| 10 | Mchare laini    | Mchare            |
| 11 | Mchare mlelembo | Mchare            |
| 12 | Makyugu II      | Mchare            |
| 13 | Akondro mainty  | Mchare            |
| 14 | Makyugu I       | Mchare            |
| 15 | Ijihu Inkundu   | Mchare            |

- Quantification is done by analyzing digital images taken by stereo microscope in Image J software.



Pollen counts taken once a month, 3 anthers per genotype at 8 am

- Staining by Triphenyl Tetrazolium chloride (TTC) method is used in assessing pollen viability.
- This procedure is adopted from (Soarez et al. 2016).
- A drop of TTC stain is added to a slide containing pollen grains.
- After incubation at room temperature for 2 hours, viable pollen grains take up the stain, while unviable pollen remains transparent
- Percentage pollen viability is calculated from obtained results



# Total pollen production

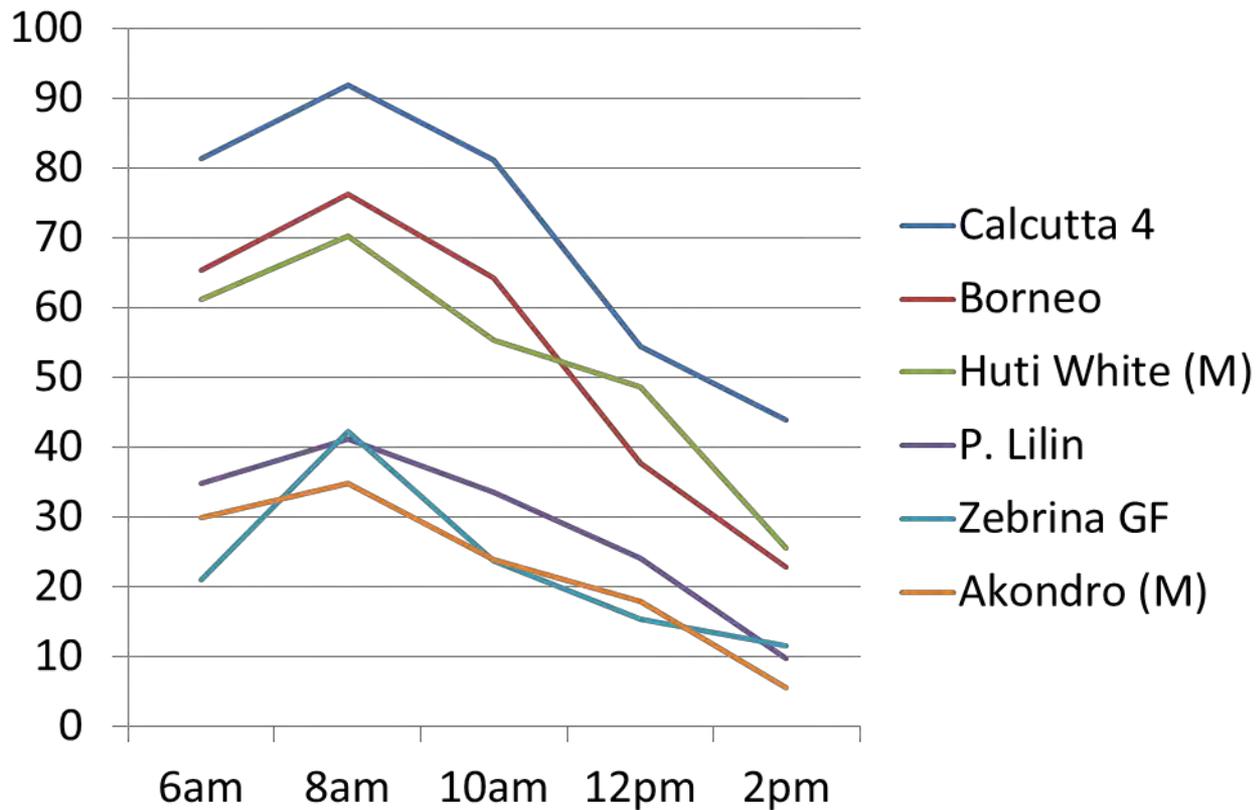
| Genotype       | Sub species     | Total Pollen | Significance * |
|----------------|-----------------|--------------|----------------|
| Calcutta 4     | ssp burmannica  | 31,863       | A              |
| Borneo         | ssp microcarpa  | 31,806       | A              |
| CV Rose        | ssp malaccensis | 28,847       | B              |
| Pisang lilin   | ssp malaccensis | 27,728       | B C            |
| Pisang Pahang  | ssp malaccensis | 26,652       | C              |
| Truncata       | ssp truncata    | 22,259       | D              |
| Zebrina GF     | ssp zebrina     | 11,835       | E              |
| M. Laini       | Mchare          | 7,875        | F              |
| Huti White     | Mchare          | 7,259        | F G            |
| Huti Green     | Mchare          | 5,702        | G H            |
| Makhyugu II    | Mchare          | 4,858        | H I            |
| M. Mlelmebo    | Mchare          | 4,570        | H I            |
| Akondro mainty | Mlali/Mchare    | 3,170        | I              |
| Makhyugu I     | ?               | 215          | J              |
| Ijihu Inkundu  | Mchare          | 155          | J              |

Average pollen production of wild diploids used in breeding and Mchare over 7 months, 21 anthers

# Results and discussion

| Genotype       | Sub species     | Total pollen | % Viable pollen | Significance <sup>1</sup> | Total viable pollen |
|----------------|-----------------|--------------|-----------------|---------------------------|---------------------|
| Calcutta 4     | ssp burmannica  | 31,863       | 74.2            | A                         | 23,642              |
| Borneo         | ssp microcarpa  | 31,806       | 74.2            | A                         | 23,600              |
| CV Rose        | ssp malaccensis | 28,847       | 64.6            | B                         | 18,635              |
| Huti White     | Mchare          | 7,259        | 59.3            | C                         | 4,305               |
| Pisang Pahang  | ssp malaccensis | 26,652       | 57.2            | C                         | 15,244              |
| Truncata       | ssp trucanta    | 22,259       | 50.0            | D                         | 11,130              |
| M. Laini       | Mchare          | 7,875        | 48.5            | D                         | 3,819               |
| Makhyugu II    | Mchare          | 4,858        | 46.7            | D E                       | 2,269               |
| Huti Green     | Mchare          | 5,702        | 43.3            | E F                       | 2,469               |
| Pisang Lilin   | ssp malaccensis | 27,728       | 42.9            | F                         | 11,894              |
| M. Mlelmebo    | Mchare          | 4,570        | 36.8            | G                         | 1,682               |
| Zebrina GF     | ssp zebrina     | 11,835       | 33.8            | G                         | 3,906               |
| Akondro mainty | Mlali/Mchare    | 3,170        | 26.1            | H                         | 827                 |
| Makhyugu I     | ?               | 215          | 9.7             | I                         | 21                  |
| Ijihu Inkundu  | Mchare          | 155          | 7.2             | I                         | 11                  |

Average percent viable pollen over 7 months (21 anthers)



Percentage viable pollen of select wild diploids and mchare (M) cultivars in Arusha, Tanzania March 2017

| Month    | Observations | Average Pollen quantity | Significance |
|----------|--------------|-------------------------|--------------|
| February |              | 14,026                  | A B          |
| March    |              | 14,581                  | A B          |
| April    |              | 15,420                  | A            |
| May      |              | 13,576                  | B            |
| June     |              | 14,902                  | A B          |
| July     |              | 13,506                  | B            |
| August   |              | 13,744                  | B            |

**Average pollen production in 7 months**

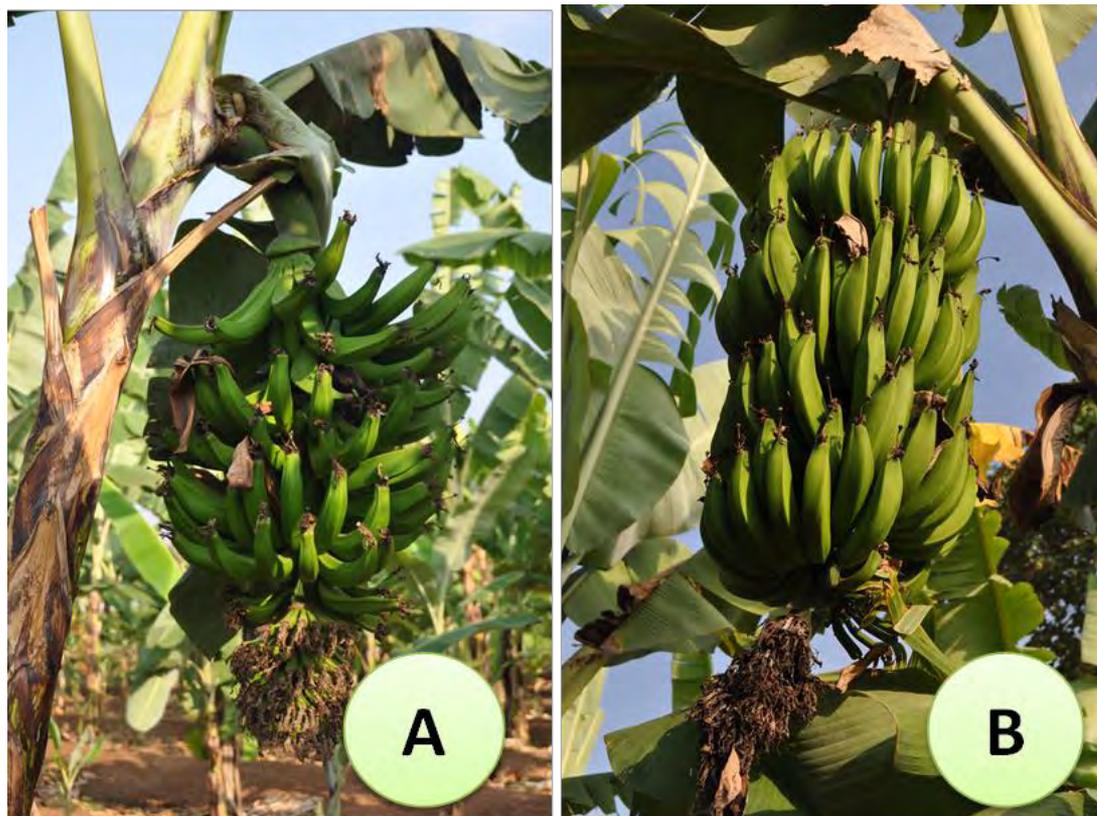


| Month    | Observations | Pollen viability |
|----------|--------------|------------------|
| February |              | 47.4 A B         |
| March    |              | 45.8 A           |
| April    |              | 48.9 B           |
| May      |              | 49.2 B           |
| June     |              | 42.9 C           |
| July     |              | 38.9 D           |
| August   |              | 41.7 C           |

**Average pollen viability in 7 months**

# Conclusion

- Significant pollen production and viability of pollen has been observed among Mchare.
- Fortunately, the two most fertile cultivars, also contain the most important quality traits.



**A:Mchare Laini B:Huti white**

- After successful introgression of resistant traits, Huti white and Mchare laini could be used in improving other bananas like EAHB and Cavendish.



- The study will continue for the next 5 months to provide further documentation for seasonal effects in pollen production

| Cross                   | Bunches | Seeds |
|-------------------------|---------|-------|
| Huti-white X Borneo     | 43      | 83    |
| Huti-white X CV Rose    | 33      | 45    |
| Huti-white X Calcutta 4 | 51      | 136   |
| Huti-Green X Borneo     | 22      | 3     |
| Huti-Green X Calcutta 4 | 22      | 0     |
| Huti-Green X CV Rose    | 16      | 0     |

**Number of Mchare hybrid seeds produced in 2 months period**





# BREEDING BETTER BANANAS



## 2.7 Mchare crosses in Arusha (to 2<sup>nd</sup> October 2017)

| Cross (Parents)              | Number of crosses | Number of seeds produced |
|------------------------------|-------------------|--------------------------|
| Huti-white X Borneo          | 68                | 127                      |
| Huti-white X CV Rose         | 36                | 45                       |
| Huti-white X Calcutta 4      | 69                | 144                      |
| Kisukari Mchare X Borneo     | 17                | 0                        |
| Kisukari Mchare X Calcutta 4 | 20                | 0                        |
| Kisukari Mchare X Zebrina GF | 1                 | 0                        |
| Huti-Green X Borneo          | 42                | 3                        |
| Huti-Green X Calcutta 4      | 45                | 0                        |
| Huti-Green X CV Rose         | 24                | 0                        |
| Ilayi X Borneo               | 1                 | 0                        |
| Ilayi X Calcutta 4           | 1                 | 0                        |
| Ilayi red X Calcutta 4       | 1                 | 0                        |
| Mchare Laini X Borneo        | 24                | 0                        |
| Mchare Laini X Calcutta 4    | 34                | 0                        |
| Mchare Laini X CV Rose       | 6                 | 0                        |
| Mchare Laini X Guyod         | 3                 | 0                        |
| Makhyugu I X Calcutta 4      | 8                 | 0                        |
| Makhyugu I X Borneo          | 5                 | 0                        |
| Makhyugu II X Borneo         | 4                 | 0                        |
| Makhyugu II X CV Rose        | 2                 | 0                        |
| Makhyugu II X Calcutta 4     | 7                 | 0                        |
| Ijihu inkundu X CV Rose      | 8                 | 0                        |
| Ijihu inkundu X Borneo       | 24                | 0                        |
| Ijihu inkundu X Calcutta 4   | 24                | 3                        |
| Ijihu inkundu X Truncata     | 4                 | 0                        |
| Akondro mainty X Borneo      | 15                | 0                        |
| Akondro mainty X Calcutta 4  | 23                | 3                        |
| Akondro mainty X CV Rose     | 13                | 0                        |
| Nshonowa X Borneo            | 22                | 29                       |



| Cross (Parents)                   | Number of crosses | Number of seeds produced |
|-----------------------------------|-------------------|--------------------------|
| <b>Nshonowa X Calcutta 4</b>      | 51                | 12                       |
| <b>Kahuti X Borneo</b>            | 2                 | 0                        |
| <b>Kahuti X Calcutta 4</b>        | 2                 | 0                        |
| <b>Kitarasa X Borneo</b>          | 4                 | 6                        |
| <b>Kitarasa X Calcutta 4</b>      | 9                 | 0                        |
| <b>Mchare Mlelembo X Borneo</b>   | 22                | 0                        |
| <b>Mchare Mlelembo Calcutta 4</b> | 32                | 0                        |
| <b>Mchare Mlelembo X CV Rose</b>  | 3                 | 0                        |
| <b>Mchare Mlelembo X Guyod</b>    | 1                 |                          |
| <b>Ndishu X Calcutta 4</b>        | 16                | 0                        |
| <b>Ndishu X Borneo</b>            | 19                | 1                        |
| <b>Ntindii I X Calcutta 4</b>     | 3                 | 0                        |
| <b>Ntindii I X Borneo</b>         | 1                 | 0                        |
| <b>Ntindii II X Borneo</b>        | 1                 | 0                        |
| <b>Mraru (mlalu) X Borneo</b>     | 1                 |                          |
| <b>Muraru red X Borneo</b>        | 1                 |                          |
| <b>Muraru red X Calcutta 4</b>    | 2                 |                          |
| <b>Muraru White X Borneo</b>      | 1                 |                          |
| <b>Muraru White X Calcutta 4</b>  | 1                 |                          |

## 2.8 Digital data capture in Banana: A system for tracking seed, monitoring progress and reporting results in Banana breeding programs

BTracT : Banana Tracking Tool

Trushar Shah, Margaret Karanja and Allan Brown: design and development of the tool

Rony Swennen: domain expertise on the Banana varieties.

Guillaume Bauchet, Nick Morales and Lukas Mueller: integration with Musabase.

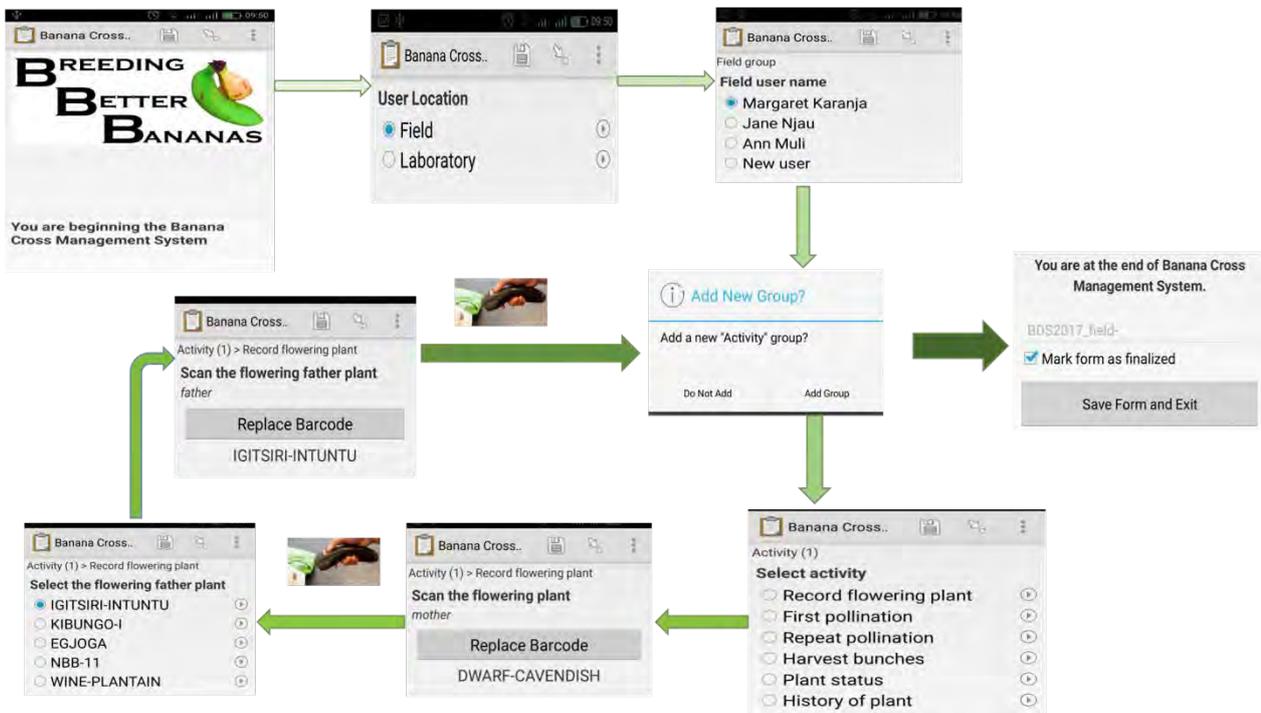
### Introduction

Banana breeding programs face a number of technical challenges such as ploidy and sterility of banana cultivars, slow propagation, space requirements and the time required for breeding. To overcome some of the logistical and management constraints in this long-winded process, we have come up with a data management system that is complementary and fully integrated with Musabase. This system allows accurate, timely and efficient data collection, management, analysis and interpretation that are crucial at all stages of the crop improvement cycle in Banana. Such information is not only important in monitoring progress but also identifying bottlenecks, providing biological insight and in providing alerts for situations where immediate intervention is required eg: plant death or disease outbreaks.

The salient features of the system were envisioned by the Banana breeding team, whose aim was to use an on-line data management system that will see reduced to zero data collection errors in the field, laboratory and screenhouses while providing instant access to information at any given time and place. The design and development of the system involved gathering of user requirements, mapping all the activities from the field to the laboratory and back to the field. The Open Data Kit (ODK) framework was used to develop the handheld-device based tools that help to manage and integrate mobile data collection activities remotely.

### Activity mapping

Banana pipeline is a large and complex process that uses an advanced form designed to capture all information regarding a banana plant in our field trials. The general idea in each step is to capture the plant/bunch/plantlet ID and the date of action. Once a cross is captured, it is followed throughout its life from pollination, harvesting, seed extraction, tissue culture and back to the open field as a plantlet. Figure 1 below illustrates the activities captured under the field based activities as displayed on the mobile application.



**Figure 1.** Screenshot of mobile application and workflow for handheld application

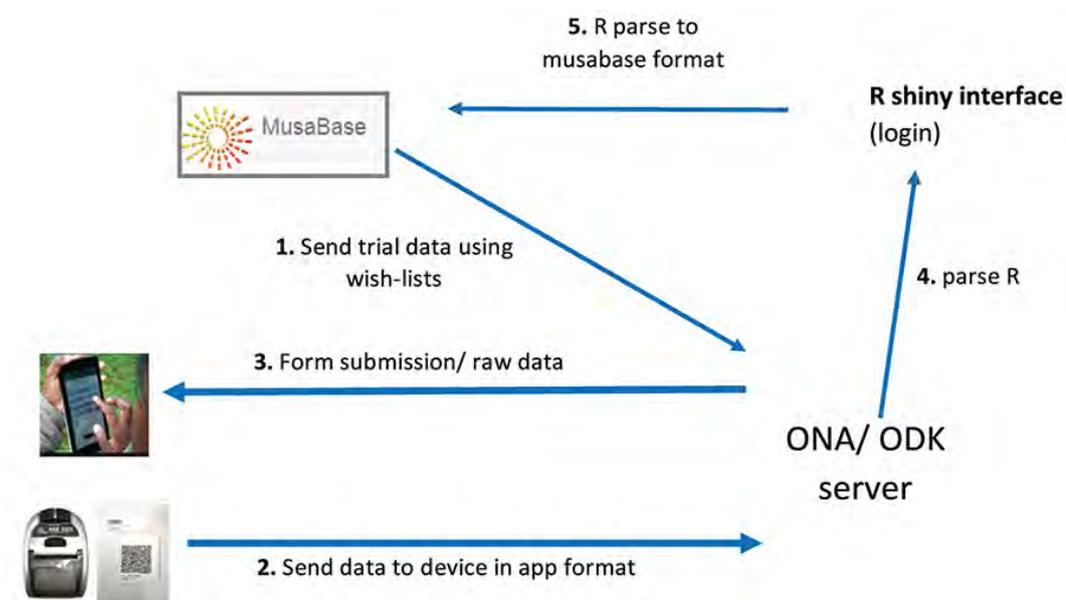
### Technical Methods Overview

The digital data capture system is built on a case management process that is integrated with Musabase, a server platform (Ona) that simultaneously aggregates data from the various users of the system and R (a statistical package used for data manipulation). This is illustrated in Figure 2.

If a breeder is interested in making crosses, he/she starts by creating a cross wish list to be used in pollination, from MusaBase where he selects the female and their respective male parents. Once this list is generated, it is immediately sent to Ona platform (mobile aggregation platform) and to the specific form as a media file after which the information is availed to the users. The field layout information is also provided from Musabase to the mobile application.

When the data is collected through the mobile phone, it is submitted to Ona platform and aggregated with its time stamps and geo-points. Using a daily scheduler, the dataset is pulled from Ona to R using Ona.R package. In the R environment, these data are structured and organized into the required formats and then pushed back to Ona and to the specific form as well as back to Musabase. This whole process ensures efficient tracking where an 'identifier' will proceed to the next step only if it has passed the previous one. ODK functionality such as relevance, constraints if any, and pull-data functions have made this process easy in ensuring data quality control.

Reports are generated in R as email alerts and also available through an R shiny dashboard view (Figure 3). These reports are accessible to authenticated users at any time and place. From the dashboard one can filter the reports to know the number of crosses made at any given day, bunches in the ripening shed, how many are at a particular stage and so on. Data sets can be filtered and downloaded for their intended use.

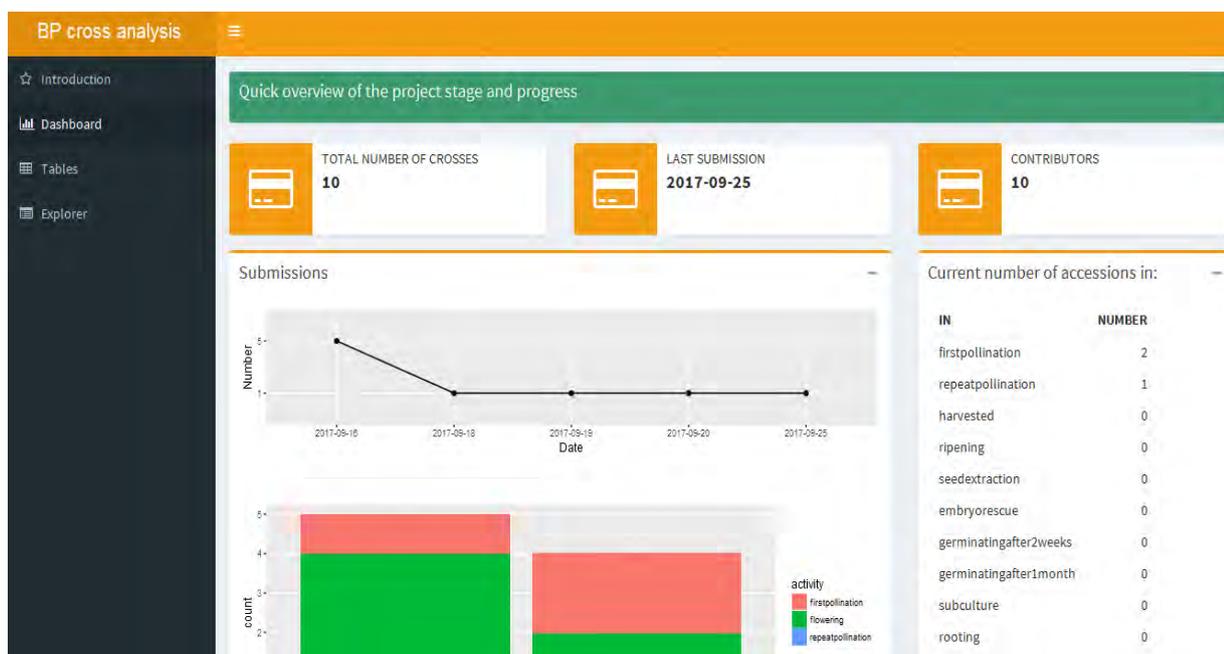


**Figure 2:** Technical overview of the system and data flow

During the project we have investigated different IT equipment and peripherals that are required. We have already identified recommendations for android handheld devices, mobile printers and barcode scanner.

The integration for Musabase has been done for obtaining data from Musabase (crossing wishlist and field layout) but is in progress for posting back to Musabase after the crossing and tissue culture workflows.

This system is now tested at the IITA banana breeding program in Arusha in October 2017 and we plan to have it operational by January 2018. Thereafter it will be transferred to the banana breeding activities of NARO and IITA, Uganda by March 2018.



**Figure 3.** R-Shiny dashboard for reports and real-time visualization

As the data in the Musabase has been accumulated from different sources mainly in Excel spreadsheets we have also tried to use fuzzy searches to identify duplications, misspelling and mislabeling of varieties in the

field. We are streamlining the naming of varieties to that existing in the ProMusa database as well as records from the International Transit Centre (ITC). This has been a very involving data curation exercise, but is essential to bring harmonization and standardization across breeding locations.

### Future improvements

In future additional features such as alerts, 'travelling salesman' algorithm for efficient pollinations in the field and improved a customized reports for users will be made available.

### EMBRAPA improved diploids



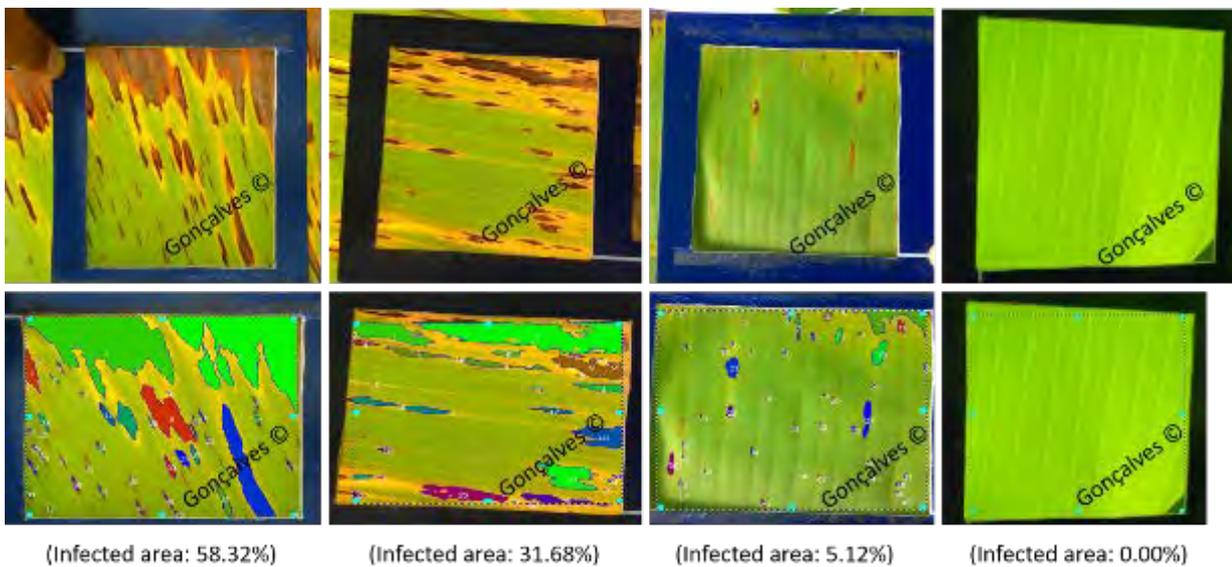
**Figure.** Experimental area artificially infested with *Fusarium oxysporum* f. sp. *cubense* for evaluating resistance of new improved diploids. Embrapa, September 2017.



**Figure.** Improved diploids without symptoms of Panama disease. Embrapa, September 2017.



**Figure.** Improved diploids with (A and C) and without (B and D) symptoms of Black Sigatoka. Embrapa, September 2017.



**Figure.** Image analysis associated with *M. fijiensis* infection in improved diploids. Embrapa, September 2017.

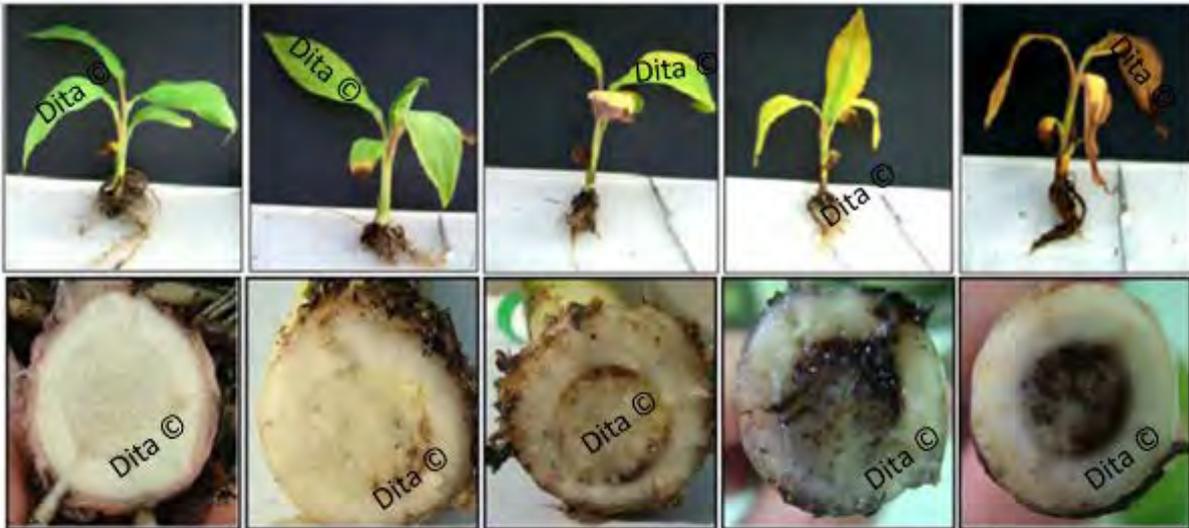


Figure. Scale of notes for evaluation of resistance to Fusarium wilt. Embrapa, September 2017.



Figure. New improved diploids. Embrapa, September 2017.



## 2.10 Recommendations of IITA/NARO Banana breeding in response to recent visit of the BPAT breeding team to Uganda and Tanzania

Representatives present: Allan Brown, Brigitte Uwimana (IITA), Robooni Tumuhimbise, Jerome Kubiriba (NARO)

Date: July 2017

Each Station (Arusha, Sendusu and Kwanda) made reports based on their interactions with the BPAT team (minutes attached)

Common themes were noted from the minutes.

1. **Specialization.** Following on the close collaboration that has developed between NARO and IITA in banana breeding, the opportunity is present to reduce redundancies in the production and evaluation systems. This will increase the overall efficiency of the program while allowing each team to specialize in one or more aspect of hybrid production. Arusha is the natural candidate for diploid improvement as the target phenotype of its breeding program is itself a diploid banana (Mchare). We need to define what would be an ideal diploid for use in triploid improvement (Matoke) and evaluate diploids not just for our own use but those that would have value in Uganda. Sendusu and Kwanda need to agree on ways to reduce overlap and a number of suggestions were made including allowing one station (Sendusu) to focus on tetraploid (3x X 2x crosses) production while the other focuses on secondary triploids (4x X 2x crosses) (Ka wanda). Another option would be for one station to broaden its genetic base by introducing lesser used germplasm while the other station continued to make and evaluate hybrids utilizing existing germplasm.
2. **Enlarging the genetic base of our breeding material.** The programs should focus less on the quantitative (number of seeds and hybrids produced) and more on the qualitative (number of superior hybrids produced). Robooni and Brigitte need to evaluate the crosses that have been made in the past and eliminate less useful plant material while focusing on the best performing parents both in terms of yield as consumer acceptance. We also need to evaluate material that has not been utilized previously either by introducing material from other partner programs (EMBRAPA and others) or by developing our own elite accessions.
3. **Need to develop product profiles.** Arusha (Mchare) and Sendusu and Kwanda (Matoke) need to clearly define what our short term and long term objectives are and document this. It will allow us to be more focused and avoid disruption if current team is no longer on board.
4. **Examine ways to increase the evaluation efficiency.** Can we reduce the number of cycle's, years, reps used in our evaluation pipeline. Using historical data, would the same material have been selected or advanced to the next cycle of evaluation if we reduced any of the current parameters?
5. **Need to develop a seed system in Uganda and Tanzania.** Need to develop a clear pathway for releasing new varieties and delivering true to type, virus indexed plant material to the growers. Need to identify all partners required to make this happen.

## 2.11 Progress report on Banana Chip construction

Summary: Work on developing a Illumina custom array has progressed steadily this year and is expected to be completed within the next 3 months. Delays have occurred as a result of the complexity of the project and the need to finalize multiple contracts with external vendors.

DNA from twenty accessions (table 1) has been extracted by KU Leuven, passed quality control standards and as of 16/10/17 been successfully sequenced by Genomics Core Research Facility, Cornell Medical School, NYC, USA.

In total, 302,766 Million base pairs of sequence have been generated. The custom array designed by Illumina will accommodate 10,000 SNPs and allows for the genotyping of 1504 accessions of our choosing.

**Table** Twenty accessions sequenced for SNP calling and subsequent base pairs obtained

| Cultivar              | ITC number | ploidy | sub-species/group     | Shepherd(1) | Mbp(2)         |
|-----------------------|------------|--------|-----------------------|-------------|----------------|
| CV Rose               | ITC0712    | AA     | ssp. malaccensis      | ST          | 17,419         |
| Borneo                | ITC0253    | AA     | ssp. microcarpa       | ST          | 18,014         |
| Kasaska               | ITC0591    | AA     | ssp. microcarpa       | ST          | 22,163         |
| Paliama               | ITC0766    | AA     | ssp. banksii          | ST          | 22,747         |
| Tomolo                | ITC1187    | AA     | ssp. banksii          | ST          | 15,421         |
| Pisang Lilin          | ITC1121    | AA     | ISEA 1                | NM          | 18,216         |
| Paka                  | ITC1254    | AA     | ?                     | NM          | 20,135         |
| Calcutta 4            | ITC0249    | AA     | ssp. burmannicoides   | N1&2        |                |
| Zebrina GF            | ITC0966    | AA     | ssp. zebrina          | J           | 23,545         |
| Kahuti                | ITC1468    | AA     | Mchare                |             | 16,997         |
| Akondro mainty        | ITC0281    | AA     | Mchare                |             | 19,016         |
| Nshonowa              | ITC 1466   | AA     | Mchare                |             | 17,294         |
| Pisang mas            | ITC1493    | AA     | sucrier               | ?           | 18,362         |
| Guyod                 | ITC0299    | AA     | IndonTriPh            | ?           | 18,875         |
| SH3142                | ITC0425    | AA     | FHIA hybrid           | ?           | 19,625         |
| TMB2x 9128-3          | ITC1437    | AA     | IITA hybrid           | ?           |                |
| Enzirabahima          | ITC1354    | AAA    | matoke                |             | 16,749         |
| 1 Plantain French     | ITC0109    | AAB    | plantain (french)     |             |                |
| 1 Plantain False horn | ITC0111    | AAB    | plantain (false horn) |             |                |
| Petite-Naine          | ITC0654    | AAA    | Cavendish             |             | 18,188         |
| <b>total Mbp</b>      |            |        |                       |             | <b>302,766</b> |

1 translocation group as described by Shepherd 1999. These refer to the 7 seven translocation groups inferred by Shep. ST=standard (same as Pahang, reference sequence), NM=Northern Malayan, N1 and N2 Northern 1 and 2, J=javanese

2 Millions of base pairs

Sequence data has been downloaded from vendor website and is currently housed at University of North Carolina at Charlotte's (UNCC) Biocomputing facility at Kannapolis, North Carolina. Dr. Robert Reid (UNCC) will process the data and align to 'Pahang' reference sequence. He will utilize software recommended by Illumina for SNP calling using their default parameters.

### Next Steps

1. SNPs meeting the quality control standards will be aligned to reference sequence and batch blasted against NCBI dataset to identify putative function/location of each SNP. SNPs will be chosen on basis

of location (providing complete coverage of all 11 chromosomes) and function (relating to breeding objectives of the project). Low frequency SNPs will be discarded.

2. Input has been received by some partners as to which genes and pathways should be targeted but we will continue to solicit input. The involvement of all partners needs to be emphasized on this as it will greatly impact not only the current project but the future utility of the resource.
3. Populations for genotyping need to be selected and quality DNA needs to be obtained.
4. Illumina will construct custom array after verifying that SNPs meet parameters.
5. Chips will be shipped to the David H Murdock Research Institute (DHMRI) for processing and data will be provided to Robert Reid (UNCC) for scoring.
6. Data will be utilized by Work package 3 for creating linkage maps and conducting QTL analysis.

The custom array will be available to the general public through Illumina, 6 months after we have successfully utilized it. Genomic sequence will be made available through public databases after we have utilized it for publication.

## 2.12 Global Program TR4 invitation letter from FAO to IITA



26 May 2017

Dear Mr Sanginga,

I have the pleasure of writing to you in connection with our efforts to develop a global programme on prevention and management of the Fusarium Wilt disease of banana.

As you know Fusarium wilt is an important disease of banana worldwide. Following the recent spread of its Tropical race 4 into Africa, the Food and Agriculture Organization of the United Nations (FAO) has been making efforts to develop a global programme. So far, discussions have taken place at the technical level.

As a next step, I wish to inform you that we are in the process of developing a global Banana Fusarium Wilt Programme and that we would like to see Bioversity International, International Institute of Tropical Agriculture (IITA) and the World Banana Forum as our main partners among the many other potential international and national partners and collaborators.

We highly value this partnership, which we believe would help the countries to more effectively prevent further spread of the fungus and manage it in the places where it already occurs. I believe that joining our specific and complementary strengths in our domains would be the key advantage of this initiative.

As per the attached draft executive summary of the programme, in addition to the components on international collaboration, policy development, capacity building, surveillance, risk assessment, containment and integrated management, we foresee a component on developing resistant varieties. We believe that this could be best achieved through a collaborative approach with relevant institutions and based on already ongoing breeding activities, and that IITA is best suited to coordinate this task.

In this regard I hope IITA, through its leading scientists, can elevate its current regional breeding efforts to a global level by establishing an international consortium for the development of banana varieties resistant to Fusarium wilt fungus with focus on Tropical Race 4. IITA taking leadership over this international consortium for the development of banana varieties, will not exclude IITA in being involved in the other programme components.

/..

Mr Nteranya Sanginga  
Director General  
International Institute of  
Tropical Agriculture (IITA)  
Ibadan  
Nigeria

cc: Mr Rony Swennen  
Banana Breeder, IITA  
Arusha  
Tanzania

We envisage that this consortium will work not only on sweet bananas, but also on cooking bananas and plantains. We believe that it can also function as an international platform towards breeding for resistance to other pests and diseases.

Unfortunately, we are currently not in a position to allocate any financial resources for this work but we will make all efforts to raise funds and we hope we can achieve this in collaboration with you and other partners.

Regarding the development of the programme itself, your technical officers may liaise with Mr Fazil Dusunceli (Fazil.Dusunceli@fao.org) who is following the process from our side. Please note that they may make any necessary contacts with relevant experts and institutions to establish an international consortium to develop bananas resistant to Fusarium wilt disease with focus on Tropical race 4 in the context of this programme.

We are planning a workshop in Autumn to define the work plans, activities and responsibilities and I would appreciate it if you could assign relevant staff to collaborate with us in designing and running the workshop, and in general for development and implementation of the programme. We will be communicating with you as the programme evolves, but in the meantime please do not hesitate to contact me on any issue that might need our attention.

We look forward to working with IITA, along with other partners, to develop and implement this global programme for improving productivity of bananas and its resilience to this deadly threat in Africa and at global level.

Yours sincerely,



Hans Dreyer  
Director

Plant Production and Protection Division

# Global Programme on Prevention of Fusarium wilt (Foc) Disease of Banana

## EXECUTIVE SUMMARY

1. Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cabense* (Foc) has been a major constraint to banana production for more than 100 years. The disease first gained prominence when it caused significant losses to Gros Michel bananas grown for export to the USA and Europe during the first half of the 20<sup>th</sup> century. To prevent the international export industry from complete collapse, Gros Michel was replaced with Cavendish bananas. However, in the last two decades Cavendish varieties succumbed to the disease, first in the subtropics and recently in the tropics. The reason for the outbreaks in the tropics was the discovery of a new variant of the Fusarium wilt fungus, called internationally Tropical Race 4 (Foc TR4), in Southeast Asia. Until early 2000s, Foc TR4 had been restricted to some Cavendish-producing countries of Asia Pacific, but it was recently discovered also outside this region as far as in the Middle East and Mozambique bringing the total number of infested countries to 19. This led to international concerns that the disease could threaten bananas worldwide, endangering food security and livelihoods of smallholders and damaging the banana trade.

2. Fusarium wilt of banana is particularly difficult to control. The responsible fungus is soil-borne and can survive for decades in the absence of bananas. Once susceptible bananas are planted in infested fields, the fungus infects the plants through the roots to cause a lethal wilt. Suckers taken from diseased areas spread the Fusarium wilt fungus over long distances, while it can be disseminated within and between fields with soil attached to shoes and plantation tools, vehicles and in drainage and irrigation water. Control of the disease by fungicides is impossible and the only means to protect bananas is to prevent the fungus from being introduced into disease-free fields through preventive measures, or by planting resistant varieties. Because of the wide host-range of Foc TR4, bananas grown as food crop and for local markets, as well as those grown for international trade, are all potential targets. Proper awareness and appropriate legislation is thus needed to secure the future of bananas worldwide.

3. This programme is designed in view of the magnitude of the risks posed by Foc TR4, as well as other races, to banana production, and considering the global mandate and strategic objectives of FAO. It aims to enhance international synergy and collaboration among the existing initiatives, in order to provide the necessary technical assistance to countries affected by and at risk of this devastating threat, through mobilising resources and catalysing efforts of organisations and institutions of the public and private sectors.

4. The programme also aims to fill in knowledge gaps by supporting development and exchange of the science based evidences, methodologies, experiences and tools in key priority areas such as biology and epidemiology of the causal fungus, risk assessments, detection and surveillance, soil health and beneficial organisms and development and adoption of resistant cultivars. These tools will help adoption of integrated disease management practices that assist prevention of disease spread and suppression of the fungus to ensure more resilient cropping systems in the long term.

6. The programme activities have been designed around eight major thematic areas as follows:

1. Enhancement of awareness, policies and strategies at national, regional and international levels for improved prevention;
2. Surveillance, early detection and monitoring approaches and systems;
3. Risk and impact assessments and improvement of phytosanitary measures and regulations;
4. Improved preparedness and containment techniques;
5. Strengthening of institutional and farmer capacities for improved management, containment, preparedness and prevention;
6. Collaborative development and deployment of germplasm and varieties resistant to Foc TR4;
7. Integrated disease management strategies to reduce disease impact and spread;
8. Regional and international interaction, coordination and information sharing for improved governance.

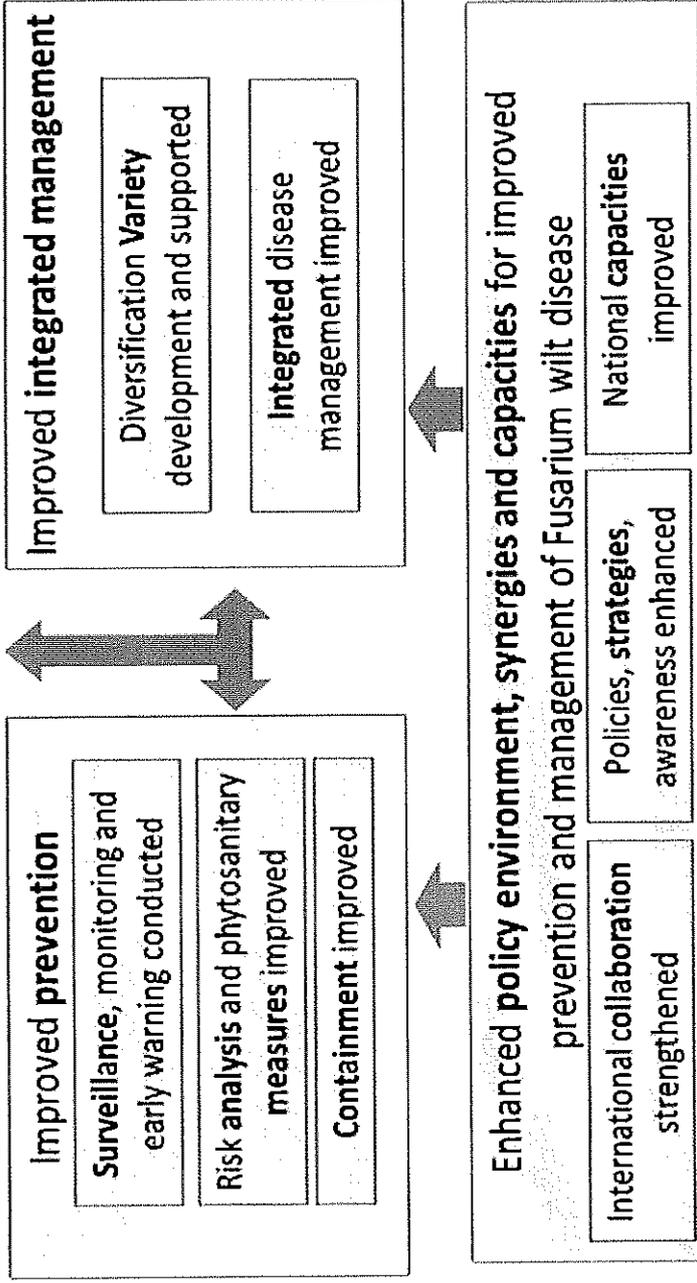
7. The programme will be hosted at FAO headquarters and implemented in close collaboration with the participating partners Bioversity International, World Banana Forum, IITA as well as others from various sectors and regions. Specific priorities and work plans will be established at regional levels, close to the field, around regional platforms in Asia, Near-East, Africa and Latin America and the Caribbean involving FAO regional offices, regional networks of Bioversity International and regional Plant Protection Organizations (RPPDs) linked with the IPPC (RPPO's) as well as other relevant international and national institutions. A programme steering committee consisting of representatives from FAO, Bioversity International, World Banana Forum, African Consortium on Foc TR4, Asia, The Near East and the Latin America and Caribbean will oversee the implementation and progress. This will be supported by a technical advisory committee which will consist of representatives of the collaborating institutions.

8. The programme activities will contribute greatly to sustainability of global banana production and livelihoods of around 400.000 million producers and workers engaged in the sector through enhancing international collaboration and strengthening capacities of countries to manage and prevent spread of the disease.



Objectives and expected outcomes of the Programme

**More resilient banana systems with reduced disease risks and impact caused by Fusarium wilt disease**



## Framework of expected outputs and activities

|  |  |   |   |  |   |  |   |  |
|--|--|---|---|--|---|--|---|--|
| Outcome 1. Enhanced enabling environment and capacities to develop and implement strategies for improved prevention and management of banana Fusarium wilt disease worldwide |  |   | Outcome 2. Improved prevention of spread of Foc into non affected areas and countries                         |  |   | Outcome 3. Improved integrated management of the disease at field level  |   |  |
| OUTPUT 1:<br>International synergy, <b>collaboration</b> and knowledge sharing enhanced  | OUTPUT 2:<br><b>Policies, strategies</b> and awareness improved at all levels for effective prevention and management of the disease | OUTPUT 3:<br><b>Capacities</b> strengthened for improved management, containment and prevention                           | OUTPUT 4:<br><b>Surveillance</b> , early detection and monitoring approaches and systems improved             | OUTPUT 5:<br><b>Risks assessed</b> , and <b>phytosanitary</b> regulations and practices enhanced             | Output 6: <b>Effective containment</b> measures developed and introduced  | Output 7: <b>Collaborative</b> development and deployment of germplasm and varieties with <b>resistance</b> to Foc TR4 | OUTPUT 8:<br><b>Integrated management</b> practices and systems approach improved to suppress the disease at field level                  |  |
| 1.1. Promote and support <b>international</b> and regional collaboration and networking to manage Foc globally   | 2.1. Develop and promote global, regional and national <b>strategies</b> for improved prevention, preparedness and management        | 3.1. Strengthen technical capacities of regional and national <b>institutions</b> in disease prevention and management    | 4.1. Provide technical support and <b>guidance</b> for improved diagnosis, surveillance and monitoring        | 5.1. Conduct pest risk <b>analysis</b> and identify regional and national spread pathways                    | 6.1. <b>Assess</b> and document efficiency of containment <b>methods</b> , tools and measures                   | 7.1. Collect, characterize and conserve <b>genetic resources</b> in search for Foc TR4 resistance                      | 8.1. <b>Assess</b> and document best practices in disease management  |  |
| 1.2. Organize international and <b>regional</b> technical consultations, workshops and meetings  | 2.2. Develop national and regional <b>contingency plans</b> through improving coordination among stakeholders                        | 3.2. Improve <b>human resources</b> of national institutions in diagnosis, management and prevention                      | 4.2. Develop and introduce <b>early detection</b> and warning tools, approaches and mechanisms                | 5.2. Assess the status of national phytosanitary <b>regulations</b> and make necessary improvements          | 6.2. Develop <b>techniques</b> , tools and approaches for disinfection, eradication confinement and suppression | 7.2. Screen banana TR4 <b>resistance sources</b>   | 8.2. <b>Develop</b> and introduce plant and soil health practices and systems approach to suppress the disease and its impact             |  |
| 1.3. Support technical field study <b>exchanges</b> and south-south collaboration  | 2.3. Advocate and raise <b>awareness</b> among stakeholders including public institutions, farmers, NGOs and industry                | 3.3. Strengthen <b>infrastructure</b> of national institutions in surveillance, management and prevention                 | 4.3. Conduct national and regional <b>surveys</b> for updated disease mapping in affected and high-risk areas | 5.3. Assess current and potential socio economic <b>impacts</b> of the disease on production and livelihoods | 6.3. Introduce and implement measures and practices containment and suppression                                 | 7.3. <b>Develop</b> germplasm and varieties with resistance to Foc TR4   | 8.3. Improve <b>seed</b> systems to make fungus free planting materials accessible  |  |
| 1.4 Facilitate <b>knowledge</b> sharing and dissemination internationally and locally  | 2.4. Analyse and develop policy and <b>financial</b> arrangement options and requirements for management, prevention and containment | 3.4. Conduct training for <b>farmers</b> , farm workers and quarantine inspectors in diagnosis, management and prevention | 4.4 Facilitate information <b>sharing</b> on disease occurrence and impact                                    | 5.4. Support national institutions in <b>implementing</b> phytosanitary measures and standards               | 6.4. Develop and introduce farm / community level <b>contingency</b> plans for improved preparedness            | 7.4. <b>On-farm evaluation</b> and deployment of promising germplasm and varieties                                     | 8.4. <b>Promote</b> bio-diversification and integrated disease management practices to improve resilience in different production systems |  |

## 2.13 Global Program TR4 and IITA acceptance for leading global banana breeding

5<sup>th</sup> June, 2017

Dr. Hans Dreyer (AGP-Director@fao.org )  
Director  
Plant Production and Protection Division  
FAO  
Via Delle Terme Di Caracalla  
00153 Roma  
Italy

Dear Dr. Dreyer,

I refer to your letter of 26 May 2017 concerning the global programme on prevention and management of the Fusarial Wilt disease of banana. We are pleased to note that you consider IITA as one of the main partners in this global effort comprising eight major themes, and that you in addition propose that IITA leads the collaborative effort in banana breeding.

We accept this offer with pleasure as IITA can bring in its long-term banana research experience, which started in 1976, and its breeding efforts that started in 1987. IITA breeds plantains in Nigeria in two locations and East African cooking bananas in Uganda and Tanzania. The plantain breeding resulted in the high yielding plantain hybrids that received the CGIAR King Baudouin award in 1994. These plantain hybrids have now been distributed to West Africa (Benin, Ghana, Nigeria, Ivory Coast), Central (DR Congo) and Eastern Africa (Burundi, Comoros, Rwanda, Tanzania and Uganda), as well as Colombia, Puerto Rico, and the Caribbean (Guyana, St Vincent and the Grenadines). The high yielding cooking bananas are the result of a joint breeding effort between IITA and NARO, Uganda, started about 25 years ago, and are now tested in Uganda, Tanzania, Rwanda, Burundi, DR Congo.

The core banana team of IITA consists of four breeders, supported by one molecular breeder, one nematologist and two plant pathologists. Our breeding efforts built in end user preferences and focus on resistance against Fusarium, Black Sigatoka, nematodes and weevils. In addition, we have scientists working banana bunchy top virus, postharvest, and agronomy.

The IITA banana breeding team has developed a network across the globe to bring in the best partners to improve the banana and plantain production in Africa. The external core team in our network for banana breeding is currently NARO/Uganda and EMBRAPA/Brazil. These partners exchange their hybrids for a common purpose and all data are brought together in an electronic database (Musabase) with support from the Boyce Thompson Institute/USA. An agreement has been made to bring in NRCB/India along the same lines. These breeding efforts are supported by research on Fusarium, like survey, diagnostics, molecular marker development, and thereby we rely on partners in Brazil, Malaysia,

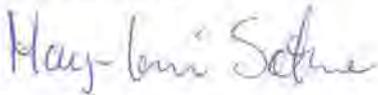
**International mailing address**  
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Australia and Uganda to complement our own efforts. For more information on our international banana breeding program spanning six continents I refer to the website: <http://bananabreeding.iita.org/>.

IITA is very pleased to lead this global effort in banana breeding and assist with setting up the strategy and search for funding. Your main contact will be Dr. Rony Swennen, a principal scientist at IITA, who leads the banana breeding at IITA and is associated with IITA since 1979. With the information explained above and the fact that he received several awards for his banana work beyond Africa, we can offer you a global player for banana breeding.

Yours sincerely,



May-Guri Saethre  
Deputy Director General (Research for Development)

## 2.14 Global Program TR4 Brochure



# GLOBAL PROGRAMME ON BANANA FUSARIUM WILT DISEASE

## PROTECTING BANANA PRODUCTION FROM THE DISEASE WITH FOCUS ON TROPICAL RACE 4 (TR4)

### THE DISEASE

Fusarium wilt disease has been a major constraint to banana production for more than a century. The disease is caused by the soil-borne fungus *Fusarium oxysporum f.sp. cubense* and it is one of the most destructive diseases of banana worldwide. Its new race Tropical Race 4 (TR4) has been causing serious losses in Southeast Asia resulting in abandonment of thousands of hectares of land. It has recently spread to the Middle East, Africa (Mozambique) and South Asia raising concerns that it may spread further.

### THE CROP

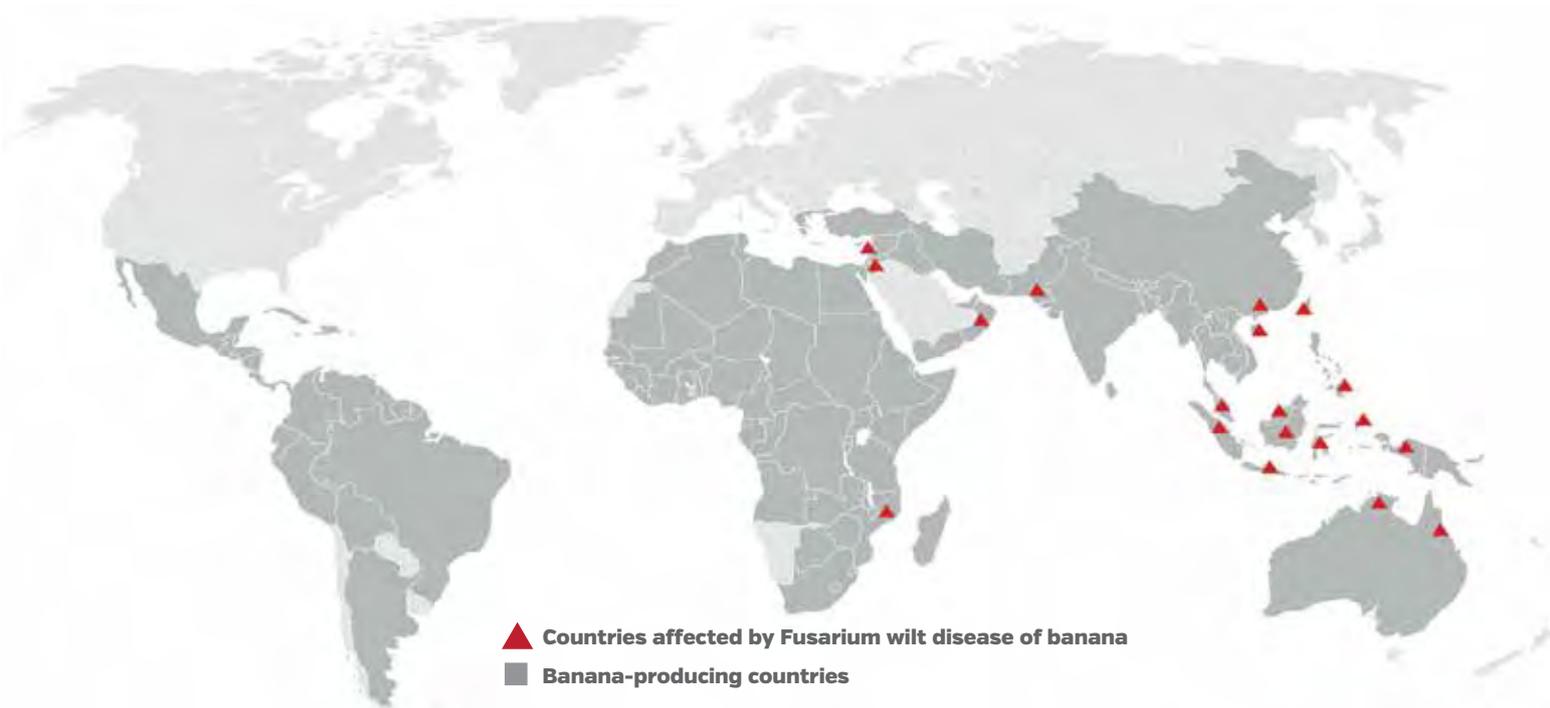
Banana, together with plantains, is the most exported fruit in the world and the fifth most produced food crop in least-developed countries. It is an important staple food or source of income for about 400 million people. TR4 poses a serious threat to production of this popular crop, with serious repercussions on livelihoods of smallholder producers, workers and the banana value chain. Cavendish bananas, representing around half of global banana production, are particularly affected by TR4.

### THE SPREAD AND IMPACT

The fungus spreads through infected plant materials and infested soil particles attached to any item such as farm tools, shoes, clothes, animals and vehicles. Irrigation and drainage water play also a critical role in its spread. Chemical control is currently not possible and once established, it remains viable in the soil for decades. Already 19 sites in ten countries are affected in Asia, the Near East and Mozambique. The disease could spread to new areas if no action is taken. Thus, a global programme is needed to prevent and manage this devastating disease.

# GLOBAL PROGRAMME ON BANANA FUSARIUM WILT DISEASE

## BANANA-PRODUCING COUNTRIES AND THOSE AFFECTED ALREADY BY TROPICAL RACE 4 OF THE FUNGUS



**135**  
countries



produce 145 million tonnes of bananas and plantains globally

**\$52**  
billion



represent the economic value generated by bananas

**400**  
million people



rely on banana as their staple food or as a source of income

**85%**  
of bananas



are consumed locally

**19**  
sites



in ten countries are already affected by TR4

**100%**  
yield loss



can be caused once established in a field

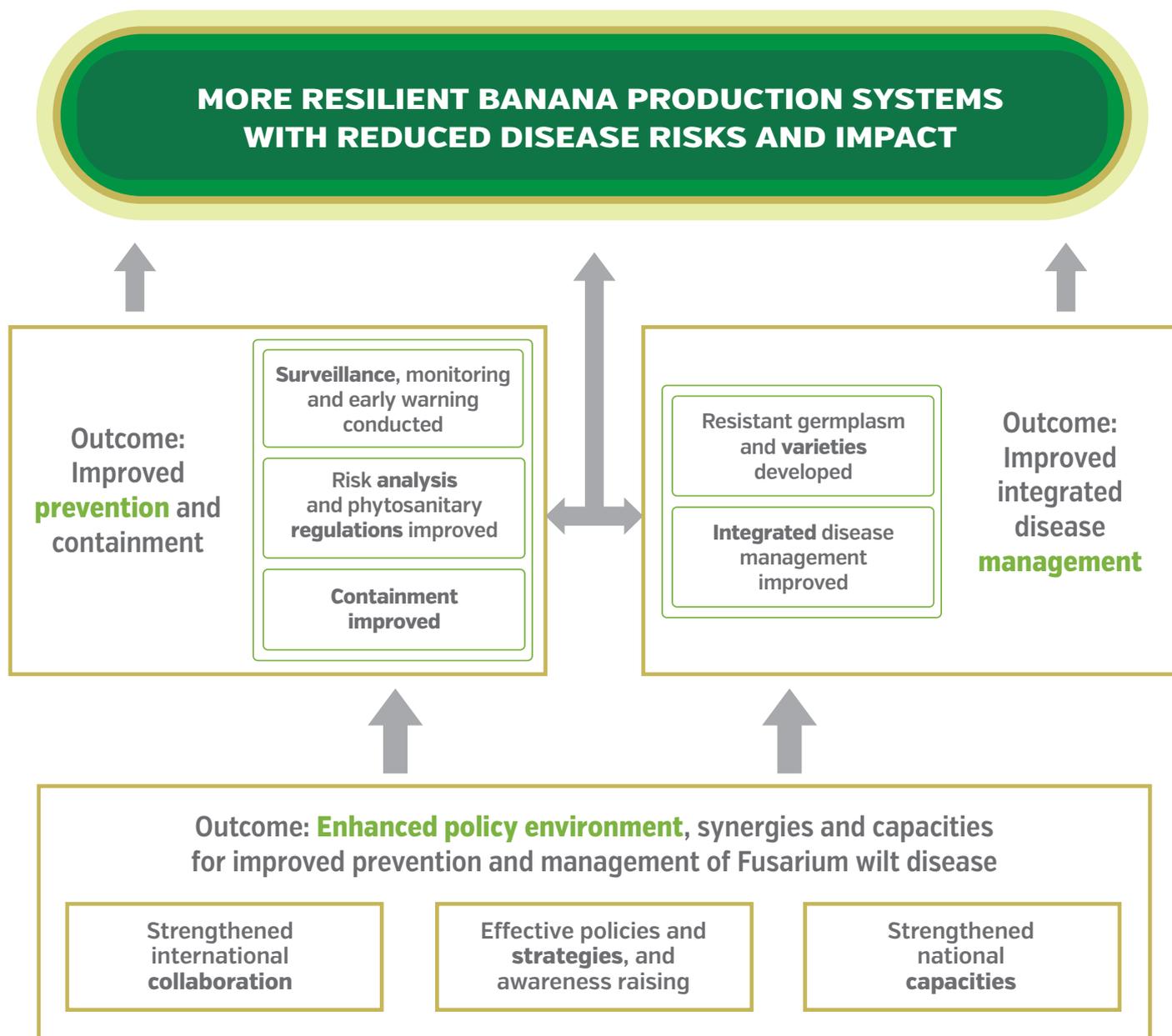


Banana is a **major staple food** and commercial crop in many countries in Asia, Africa, Latin America and the Caribbean



# GLOBAL PROGRAMME ON BANANA FUSARIUM WILT DISEASE

## PROGRAMME FRAMEWORK



The Global Programme on Banana Fusarium wilt (FW) disease is designed on three main fronts of action: preventing future outbreaks, managing existing cases, and strengthening international collaboration and coordination among institutions, researchers, governments and producers.

### KEY FACT

Fusarium wilt can cause **100% yield loss** in infested fields and affect the sustainability of its production

# GLOBAL PROGRAMME ON BANANA FUSARIUM WILT DISEASE



The programme aims to enhance international synergy and collaboration among the existing initiatives to assist countries in their efforts to prevent and manage this devastating threat more effectively. It is built on a multidisciplinary and coordinated action plan involving all the concerned stakeholders. It will be implemented through a partnership between FAO, Bioversity International, International Institute of Tropical Agriculture and the World Banana Forum, in collaboration with other international and national institutions. Collaborators will include, among others, national plant protection organisations, universities, regional networks, international institutions, industry and producer associations.

## KEY ACTIVITIES:

- ▶ Promoting and facilitating international and regional **collaborations** to develop and implement strategies and tools for disease management and prevention.
- ▶ Strengthening national **capacities** in implementing effective plant health legislation and **phytosanitary** standards.
- ▶ Supporting coordination among stakeholders for development and implementation of contingency plans and rapid **response**.
- ▶ **Awareness** raising and advocacy among decision makers and farmer communities.
- ▶ Assessing TR4 **risks** and impacts to banana production nationally, regionally and internationally based on scientific data.
- ▶ Supporting national and regional **surveillance** and monitoring mechanisms.
- ▶ Developing and deploying TR4 **resistant bananas** through international collaboration.
- ▶ Developing and promoting **integrated disease management** practices to prevent spread and to minimize damage by TR4.

## KEY FACT

The most effective way to control the disease is to take **preventive measures**. International **collaboration** and local actions are essential to manage the disease globally.

**Contact:** [Fazil.Dusunceli@fao.org](mailto:Fazil.Dusunceli@fao.org); [Food-Chain-Crisis@fao.org](mailto:Food-Chain-Crisis@fao.org)

[www.fao.org/food-chain-crisis/how-we-work/plant-protection/banana-fusarium-wilt/](http://www.fao.org/food-chain-crisis/how-we-work/plant-protection/banana-fusarium-wilt/)

## 2.15 Global Program TR4 Project Summary



Food and Agriculture Organization  
of the United Nations

# Global Programme on Banana Fusarium Wilt Disease (2018-2023)

Protecting banana production from the disease  
with focus on Tropical Race 4 (TR4)

(Programme Summary)



## Global Programme on Banana Fusarium Wilt Disease

### Programme in brief:

Banana is an important crop for food security and rural livelihoods particularly in Asia, Africa and Latin America and Caribbean. This popular crop is now threatened by a new race (Tropical Race 4 – TR4) of a soil borne disease known as Fusarium wilt (FW). Currently, this race is affecting 19 sites in 10 countries mostly in Southeast Asia, and 25 countries are considered at immediate risk in Asia, Near East and Africa. Preliminary assessment of scientists indicate that TR4 could potentially spread up to 1.6 million ha by 2040 if no significant interventions are instituted. This represents 17 percent of current area in production and corresponds to annual production potential of 36 million tonnes. Potential losses in these areas could have substantial socio economic impacts on livelihoods along the banana value chain.

This programme has been developed with the goal of enhancing sustainability and resilience of banana production under various crop production systems in different regions by preventing and managing the threats of the disease, in Asia, Africa, the Near East and Latin America and Caribbean. The programme aims to strengthen preventive measures and disease management efforts by enhancing international synergy and collaboration among the existing initiatives in order to provide the necessary technical support to countries affected by, and at risk of, this devastating threat. National capacities will be strengthened through mobilising resources and catalysing efforts of organisations and institutions of the public and private sectors.

The programme will be implemented through a partnership among the Food and Agriculture Organization of the United Nations (FAO), Bioversity International, International Institute of Tropical Agriculture (IITA) and the World Banana Forum (WBF), in collaboration with relevant international and national institutions. Initially 67 countries are targeted from different risk groups and regions. Implementation of the 32 activity packages will require an estimated budget of \$ 98 million over five years.

### The crop and the disease:

Banana, together with plantains, is the most exported fruit in the world and the fifth most produced food crop in the least-developed countries. Of the 145 million tonnes of production 85 percent is consumed locally and the rest is marketed internationally. Thus it is not only a valuable market commodity but also an important staple food or source of income in developing countries for about 400 million people. This crop is now threatened by the most recent race of the fungus *Fusarium oxysporum* f. sp. *Cubense* (Foc), the Tropical race 4 (TR4). This race is already affecting countries in Southeast Asia and posing a threat to production of this popular crop, with serious repercussions on the livelihoods of small holder producers and workers, and the banana value chain.

Fusarium wilt has been a major constraint to banana production for more than a century. It first gained prominence when it caused significant losses to Gros Michel bananas grown for export during the first half of the 20<sup>th</sup> century. To prevent the export industry from collapse, Gros Michel was replaced with Cavendish bananas that are resistant to the Race 1 of the fungus which caused this epidemic. However, in the last two decades Cavendish varieties succumbed to the disease due to TR4. Until early 2000s, TR4 had been restricted to some Cavendish-producing countries in the Asia-Pacific region, but it was recently discovered outside this region, as far as South Asia, the Middle East and Mozambique, bringing the total number of infested countries to ten. This shows that the disease can spread further and threaten bananas worldwide, endangering both the food security and livelihoods of smallholders

and banana trade. This global risk is exacerbated by the domination of world banana production by the highly susceptible Cavendish clones as well as many technical and socio economic factors contributing to disease spread.

### Root causes of the challenge

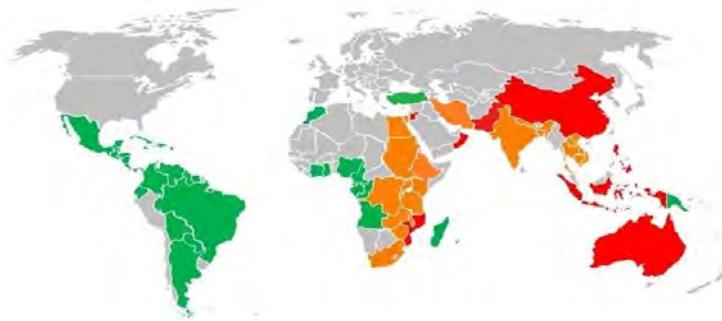
Fusarium wilt of banana is particularly difficult to control. The responsible fungus is soil-borne and can survive in the soil for decades in the absence of bananas. The fungus infects the plants through the roots and causes a lethal wilt. It can spread into new areas, close or far, through movement of infected planting materials or through contaminated soil particles attached to items such as shoes, clothes, farm tools and vehicles as well as in drainage and irrigation water. Control of the disease by fungicides is impossible and the only means to protect bananas is to prevent the fungus from spreading into disease-free fields through preventive measures, or by developing and planting resistant varieties. Because of the wide host-range of TR4, many bananas grown as food crop and for local or international markets, are potential targets.

Besides the knowledge gaps, genetic vulnerability of the cultivars and technical constraints, the lack of awareness, surveillance systems, contingency plans and comprehensive and programmatic strategies contribute to the amplification of the risks of spread of the disease and its impact. Weaknesses in national phytosanitary regulations, seed systems and research and extension capacities make countries concerned more vulnerable to the disease. A concerted effort is thus needed to enhance international collaboration and linkages among diverse stakeholders to assist countries to strengthen their ability to prevent and manage the disease effectively.

**A banana plant and field affected by *Fusarium oxysporum* f. sp. *cubense* TR4**



**Current risk levels of TR4: Already present (red), at high risk (orange) or at risk (green) for countries where banana is an important commodity.**



## Potential impact of the disease and benefits of the programme

Estimating the current and potential impact of the disease proves challenging due to the lack of structured reporting systems and insufficient confirmed information. However, past experiences have shown how damaging it could be to the economies and local livelihoods. The damage caused by race 1 of the fungus in the last century is estimated over \$ 2.3 billion. Recent outbreaks in countries already affected by TR4 are a cause of concern. Some unofficial reports refer to affected areas of 15 500 ha in the Philippines, 40 000 ha in China and 80 percent of production area in the Jordan Valley. Globally, the affected area by TR4 so far is estimated by scientists to be close to 100 000 ha (Ordonez *et al*, 2015). In terms of economic losses caused by TR4, certain reports refer to losses of \$ 121 million in Indonesia, \$253 million in Malaysia and 14.1 million in Taiwan province of China<sup>1</sup>. Considering also the indirect consequences and social effects on the livelihoods of the producers, the workers and the locals, the real socio economic impact would be significantly higher than estimates. It is feared that the disease may already be present in some other locations and with additional incursions TR4 could spread to other non-affected countries and regions as well.

Regarding the potential global impact, scientists have estimated that TR4 could spread up to 1.6 million hectares of current banana lands by 2040 if no significant interventions are instituted (Scheerer *et al*, 2016). This represents 17 percent of the current area under production. The annual production potential of this area is 36 million tons with an estimated value of around 10 billion dollars at current prices. Thus, it is feared that such extensive spread could cause significant socio economic impacts on banana productions and livelihoods along the banana value chain globally.

The programme, through wide international collaboration and engagement with the concerned countries and stakeholders, aims to minimize the risk of spread of the disease into new areas and to support the affected countries for its management and recovery supporting development and implementation of improved and novel technologies and practices. By targeting 67 countries in Asia, Africa, the Near East and Latin America and the Caribbean, the programme aims to reduce by 60 percent the potential area that can be affected by the disease at full impact rate, and by 30 percent at a moderate impact rate. The programme will also support countries and regions which are already affected by the disease to introduce and support improved disease management practices.

It is estimated that through effective implementation of the programme, an annual investment of \$1 today would bring in 10 and 20 years' time a return of around \$48 and \$196 at full impact rate, and \$24 and \$98 at a moderate impact rate, respectively. These estimations are made mostly based on expected outcomes of awareness raising campaigns and implementation of preventive phytosanitary measures and immediate management practices. The impact of longer term activities, such as research and breeding, would depend on the advancements, and the benefits would mostly be realized beyond the planned duration of the programme. However, these advancements and their implementation would be crucial for ensuring sustainability and resilience of banana production systems in the long term.

### The programme and its objectives

The programme is designed in view of the global magnitude of the risks posed to banana production by TR4, as well as other races. Considering the global mandate and strategic objectives of FAO and its partners Bioversity International, IITA, World Banana Forum, and others, the programme has been developed collaboratively with inputs of many institutions and experts. The programme aims to enhance international synergy and collaboration among the existing initiatives focusing on areas where further work is needed in order to provide the necessary technical support to countries affected

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<sup>1</sup> Source: [www.promusa.org](http://www.promusa.org)

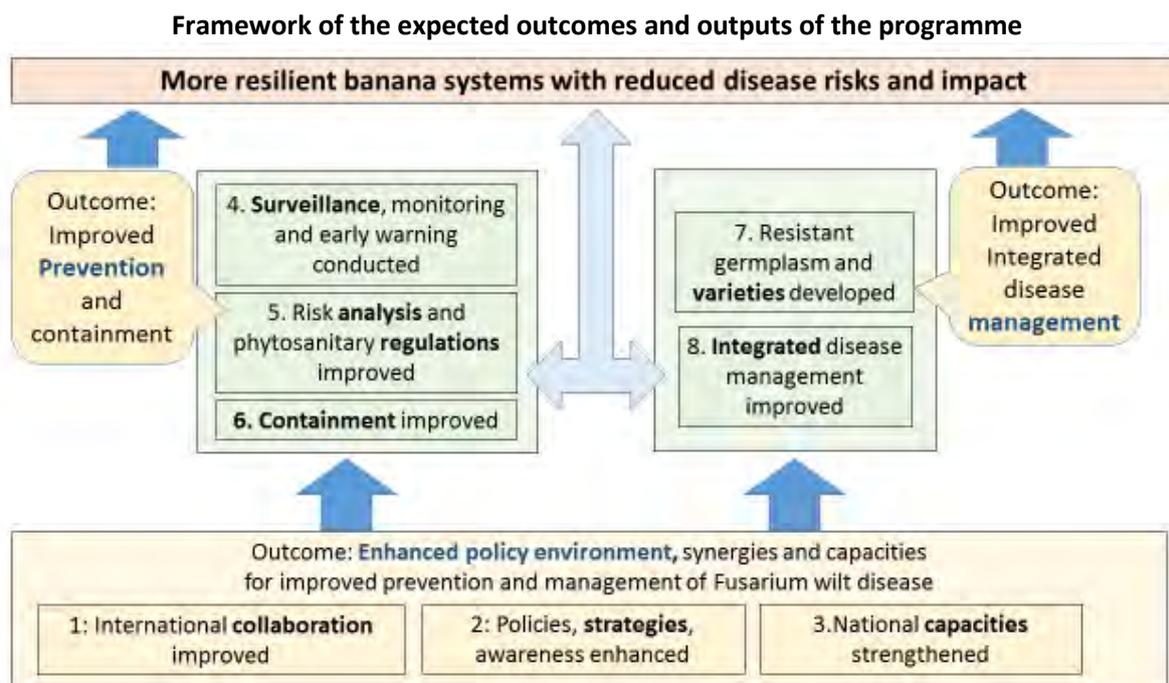
by, and at risk of, this devastating threat. National capacities will be strengthened through mobilising resources and catalysing efforts of organisations and institutions of the public and private sectors.

The programme also aims to fill in knowledge gaps by supporting the development and exchange of the science-based evidences, methodologies, experiences and tools in key priority areas, such as biology and epidemiology of the causal fungus, detection and surveillance, soil health and beneficial organisms, and development and adoption of resistant cultivars. These tools will help to adopt integrated disease management practices that help to prevent disease spread and suppression of the fungus to ensure more resilient banana cropping systems in the long-term. Bio-economic and spatial analyses will be carried out to assess the potential and real impacts of the different interventions.

### The framework and planned activities

The programme activities have been designed to achieve eight thematic outputs targeting three major expected outcomes. The basic expected outcome is to create an enabling environment through improved international collaboration, development of multi-sectoral strategies and improved national capacities to support actions to prevent and manage the threats. The outcome of improved prevention of disease spread will be achieved through supporting surveillance, phytosanitary measures and rapid containment focusing in countries where the disease is not present or just appeared. Improved disease management in countries where the disease already occurs will be achieved through developing and deploying resistant varieties and promoting long-term integrated disease management practises. The programme will be continuously monitored and evaluated periodically to assess progress and ensure learning across the different areas of interventions and stakeholders.

The activities will be prioritised for different regions and countries based on the status of the disease, risk levels and assessments of national institutions. Similarly, these will be differentiated also for different banana production systems such as smallholder farming, mixed cropping systems and monocultures. In total, 67 countries are considered as beneficiary countries in Asia, Africa, the Near East and Latin America and the Caribbean.





## **Coordination arrangements**

The programme will be hosted at FAO headquarters and implemented in close collaboration with its partners Bioversity International, IITA, World Banana Forum and others from various sectors, regions and countries. Regional priorities and work plans will be established at regional levels around regional platforms in Asia, the Near East, Africa and Latin America and the Caribbean. These platforms will involve FAO regional, subregional and country offices, global and regional networks coordinated by Bioversity International, IITA and WBF, as well as Regional Plant Protection Organizations (RPPOs) linked with the IPPC and other relevant institutions.

A programme steering committee consisting of members from FAO, Bioversity International, IITA, World Banana Forum and representatives from Asia, Africa, the Near East and Latin America and the Caribbean will guide and oversee the implementation and progress. This will be supported by a technical advisory panel which will consist of focal points of the collaborating institutions and scientific experts. Progress of planned activities will be monitored through biannual assessments and annual workshops. Lessons learnt will be assessed, and updates and adjustments will be made as appropriate taking into account the recommendations of the participating institutions, member countries and resource partners.

Programme activities will contribute greatly to the protection of the banana productions under different cropping systems from the threats of the Fusarium wilt outbreaks by enhancing international collaboration and strengthening national capacities. This will strengthen the resilience and sustainability of global banana production and livelihoods of around 400 million people for whom it is an important staple food crop or source of income. For implementation of this five year programme in full, a budget requirement of US\$ 98 million is estimated.

### **Key messages:**

- BANANA IS A MAJOR SOURCE OF STAPLE FOOD AND REVENUE IN MANY COUNTRIES IN ASIA, AFRICA, LATIN AMERICA AND THE CARIBBEAN
- FUSARIUM WILT DISEASE OF BANANA CAUSED BY TR4 IS AMONG THE MOST DESTRUCTIVE DISEASES OF BANANA
- TR4 AFFECTS PARTICULARLY CAVENDISH BANANAS, SUPPLYING AROUND HALF OF GLOBAL BANANA PRODUCTION
- EFFECTIVE ERADICATION IS CURRENTLY NOT POSSIBLE. THE PATHOGEN REMAINS VIABLE FOR DECADES IN THE SOIL
- ONCE ESTABLISHED IN A FIELD, IT CAN CAUSE 100 PERCENT YIELD LOSS
- ASSESSMENTS INDICATE THAT TR4 COULD POTENTIALLY SPREAD UP TO 1,6 MILLION HECTARE BY 2040
- PREVENTION AND PHYTOSANITARY MEASURES ARE THE MOST EFFECTIVE WAYS OF CONTROLLING THE DISEASE
- DIVERSIFICATION AND BETTER USE OF AVAILABLE GENETIC RESOURCES ARE KEY TO BUILDING RESILIENCE TO THE DISEASE IN THE LONG TERM

- SUPPORT IS NEEDED FOR DISEASE MANAGEMENT AND RECOVERY IN AFFECTED COUNTRIES
- INTERNATIONAL COLLABORATION AND LOCAL ACTIONS ARE ESSENTIAL

#### References:

1. Ordonez, N., Seidl, M.F., Waalwijk, C., Drenth, A., Kilian, A., Thomma, B.P.H.J., Ploetz, R.C. & Kema, G.H.J. 2015. Worse Comes to Worst: Bananas and Panama Disease—When Plant and Pathogen Clones Meet. *PLoS Pathog*, 11(11): e1005197.  
<http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005197>
2. Scheerer, L., Pemsli, D., Dita, M., Perez Vicente, L. & Staver, C. 2016. A quantified approach to project losses caused by Fusarium wilt Tropical race 4. In: *Proceedings of X International Symposium on Banana: ISHS-ProMusa Symposium on Agroecological approaches to promote innovative banana production systems*, Montpellier, France, 10-14 October 2016. Acta Horticulture. ISHS, Leuven, Belgium.

## Expected outcomes, outputs and work packages of the programme

| Outcome 1. Enhanced <b>enabling environment</b> , synergies and capacities to develop and implement strategies for improved prevention and management of banana Fusarium wilt (FW) disease worldwide |  |  | Outcome 2. Improved <b>prevention</b> of spread of TR4 into non affected areas and countries                 |  |   | Outcome 3. Improved integrated <b>management</b> of the disease at field level               |   |
|--|--|--|--|--|---|--|---|
| OUTPUT 1:  | OUTPUT 2:  | OUTPUT 3:  | OUTPUT 4:  | OUTPUT 5:  | OUTPUT 6:   | OUTPUT 7:  | OUTPUT 8:   |
| International synergy, <b>collaboration</b> and knowledge sharing enhanced   | <b>Policies, strategies</b> and awareness improved at all levels for effective prevention and management of the disease                    | <b>Capacities</b> strengthened for improved management, containment and prevention                                     | <b>Surveillance</b> , early detection and monitoring approaches and systems improved                         | <b>Risks</b> assessed, and <b>phytosanitary</b> regulations and practices enhanced                           | Containment & preparedness measures developed and introduced  | Germplasms, varieties and hybrids with resistance to TR4 developed collaboratively           | <b>Integrated management</b> practices and systems approach improved to suppress the disease at field level                               |
| 1.1. Promote and support <b>international</b> and regional collaboration and networking to manage FW globally  | 2.1. Develop and promote global, regional and national policies and <b>strategies</b> for improved prevention, preparedness and management | 3.1. Strengthen technical capacities of regional and national <b>institutions</b> in disease prevention and management | 4.1 Provide technical support and <b>guidance</b> for improved diagnosis, surveillance and monitoring        | 5.1. Conduct pest <b>risk analysis</b> and identify international, regional and national spread pathways     | 6.1. <b>Assess</b> and document efficiency of containment <b>methods</b> , tools and measures                   | 7.1. Screen banana genepool to identify TR4 <b>resistance sources</b>                        | 8.1. Improve <b>seed</b> systems to make pathogen free planting materials accessible  |
| 1.2. Organize international and <b>regional</b> technical consultations, workshops and meetings  | 2.2. Develop national and regional <b>contingency plans</b> through improving coordination among stakeholders                              | 3.2. Improve <b>human resources</b> of national institutions in diagnosis, management and prevention                   | 4.2. Develop and introduce <b>early detection</b> and warning tools, approaches and mechanisms               | 5.2. Assess the status of national phytosanitary <b>regulations</b> and make necessary improvements          | 6.2. Develop <b>techniques</b> , tools and approaches for disinfection, eradication confinement and suppression | <b>7.2. Develop</b> varieties and hybrids with resistance to TR4                             | 8.2. <b>Assess</b> and document best practices in disease management  |
| 1.3. Support technical field study <b>exchanges</b> and south-south collaboration  | 2.3. Advocate and raise <b>awareness</b> among stakeholders including public institutions, farmers, NGOs and industry                      | 3.3. Conduct training for <b>farmers and</b> farm workers in diagnosis, management and prevention                      | 4.3 Conduct national and regional <b>surveys</b> for updated disease mapping in affected and high-risk areas | 5.3. Support national institutions in <b>implementing</b> phytosanitary measures and standards               | 6.3. Develop and introduce farm / community level <b>contingency</b> plans for improved preparedness            | 7.3. On-farm <b>evaluation</b> and deployment of promising germplasm and varieties           | 8.3. <b>Develop</b> and introduce plant and soil health promoting practices and systems approach to suppress the disease and its impact   |
| 1.4 Facilitate <b>knowledge</b> sharing and dissemination internationally and locally  | 2.4. Analyse and develop <b>financial arrangement</b> options that can help prevention and management                                      | 3.4. Strengthen <b>infrastructure</b> of national institutions in surveillance, management and prevention              | 4.4 Facilitate information <b>sharing</b> on disease occurrence and impact                                   | 5.4. Assess current and potential socio economic <b>impacts</b> of the disease on production and livelihoods | 6.4. Introduce and <b>disseminate</b> and Implement measures and practices containment and suppression          | 7.4. Collect, characterize and conserve <b>genetic resources</b> in search for FW resistance | 8.4. <b>Promote</b> bio-diversification and integrated disease management practices to improve resilience in different production systems |

### 3. Work Package 2

#### 3.1 Distribution maps of Fusarium, nematodes, weevils and Sigatoka in Tanzania and Uganda

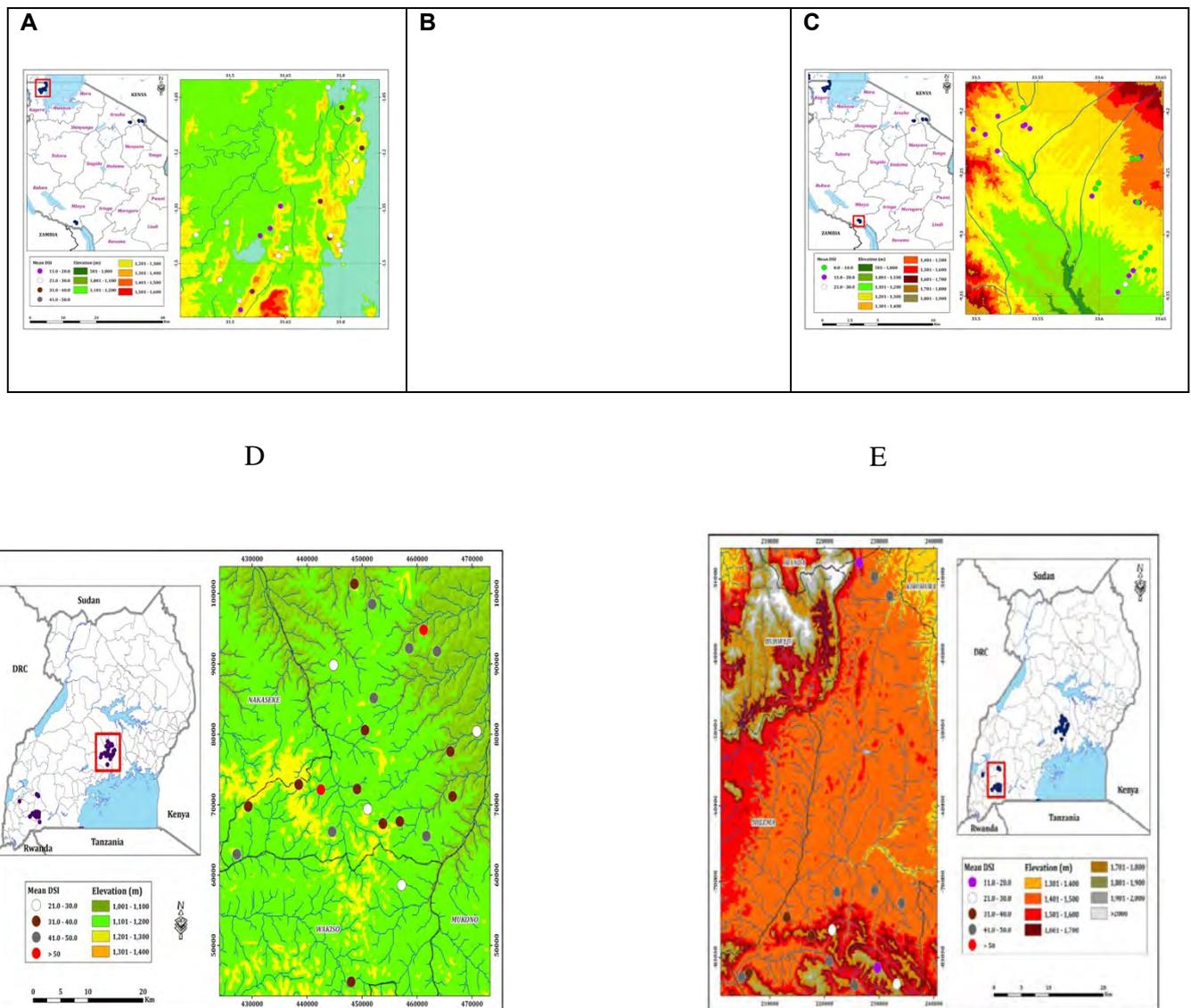
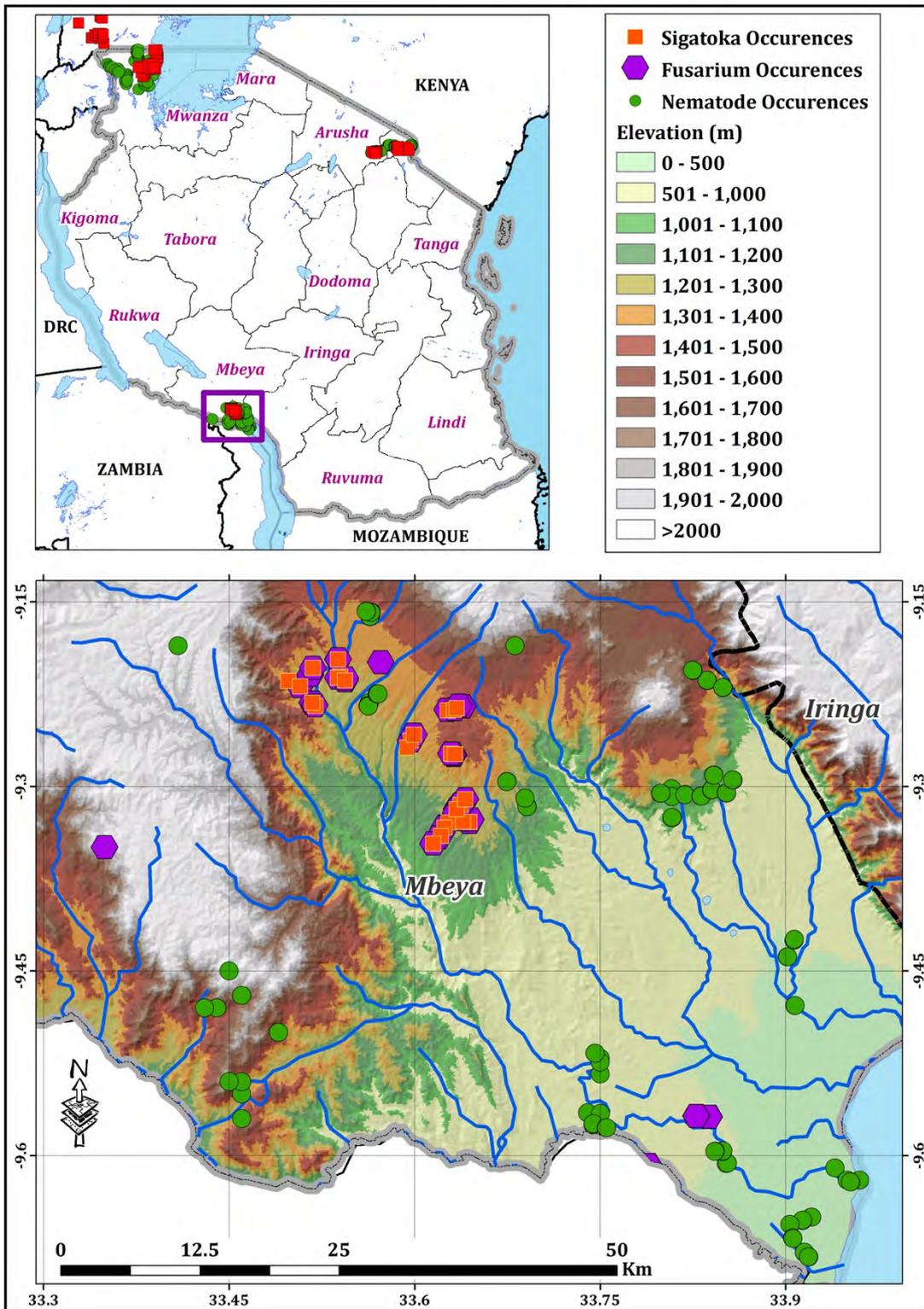


Figure: Distribution and severity of Sigatoka leaf spots in Uganda and Tanzania



**Figure:** Distribution map of Fusarium, nematodes and Sigatoka leaf spots in Mbeya, Tanzania

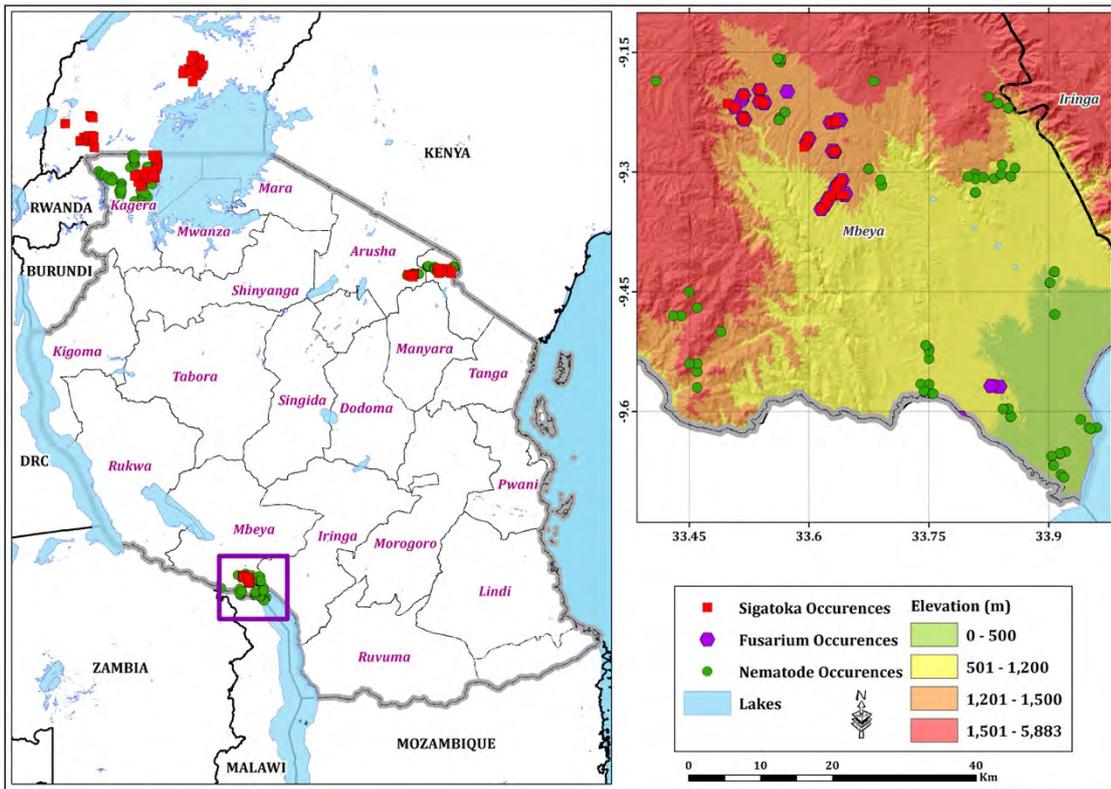


Figure: Distribution map of Fusarium, nematodes and Sigatoka leaf spots in Uganda and Tanzania

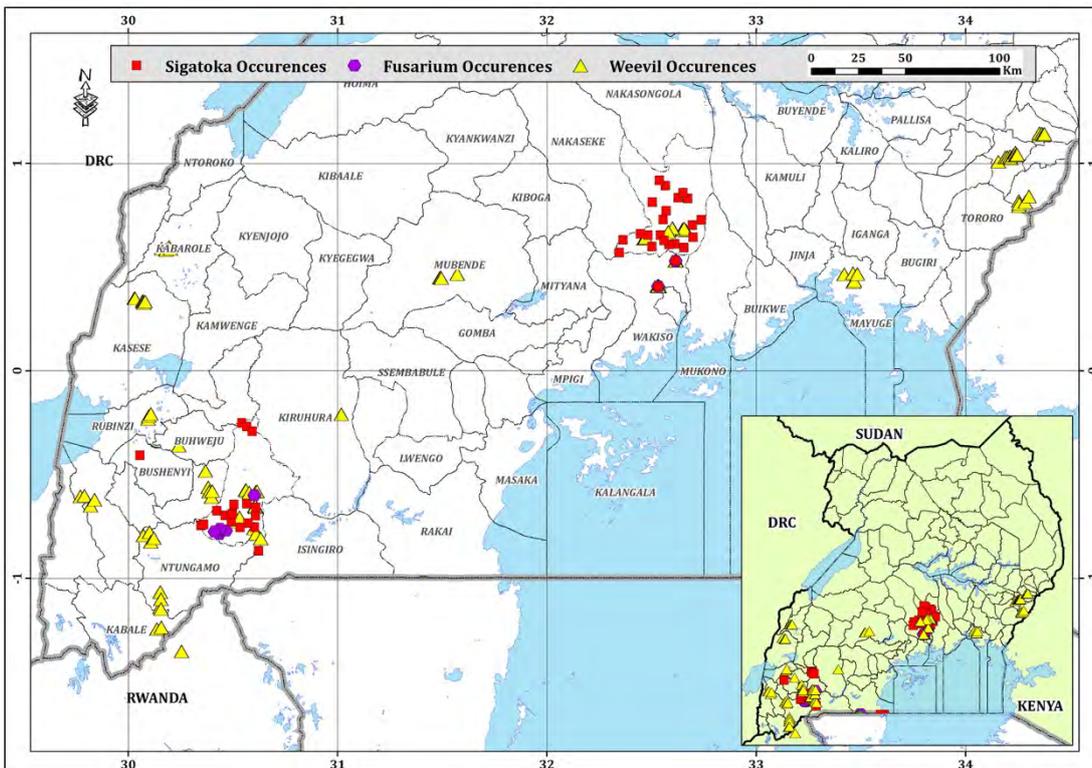


Figure: Distribution map of Fusarium, weevils and Sigatoka leaf spots in Uganda

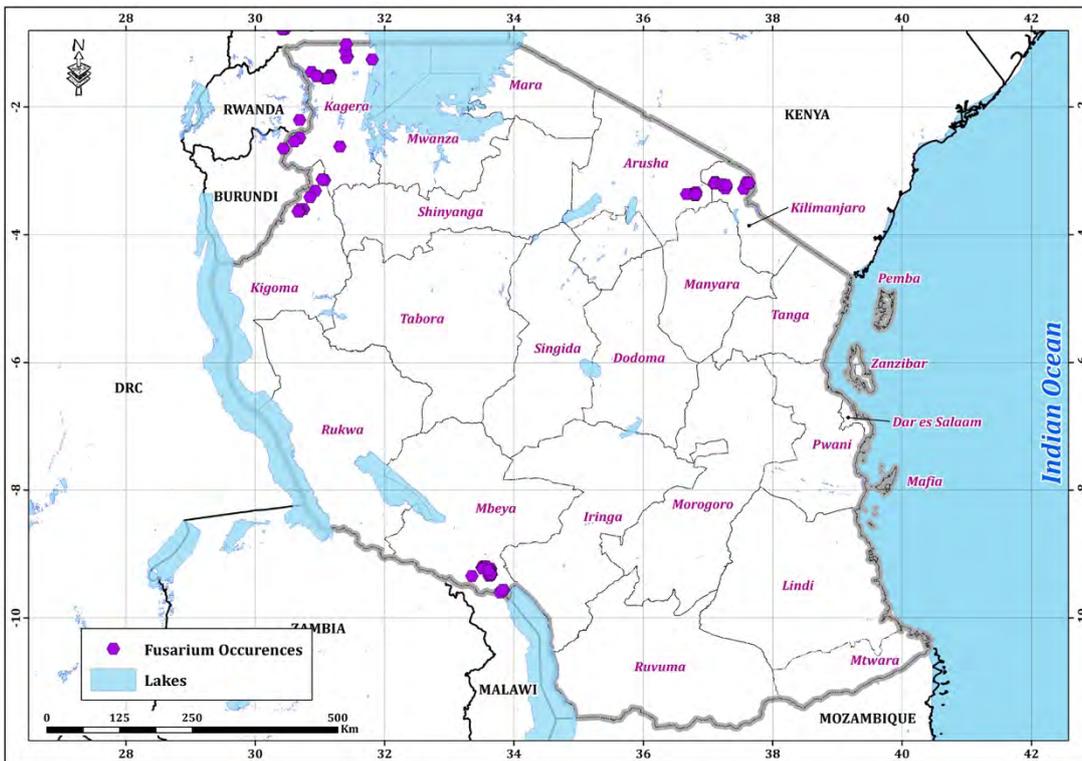


Figure: Distribution map of Fusarium in Tanzania

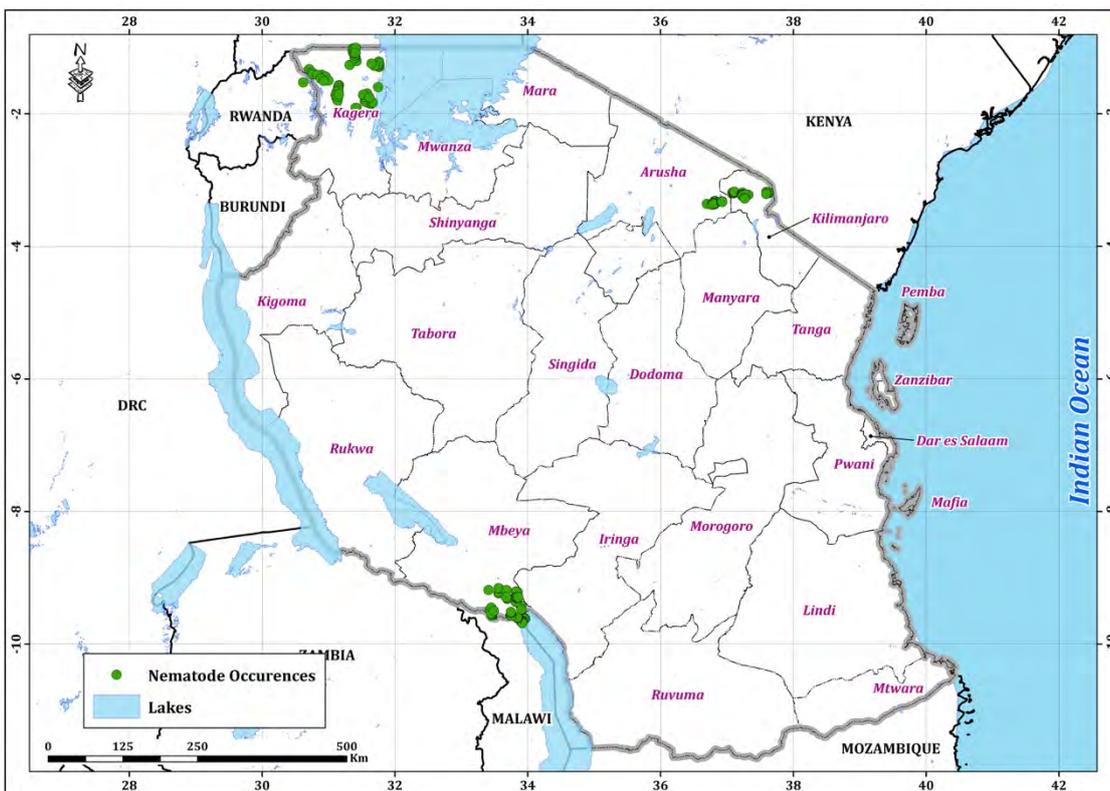


Figure: Distribution map of nematodes in Tanzania

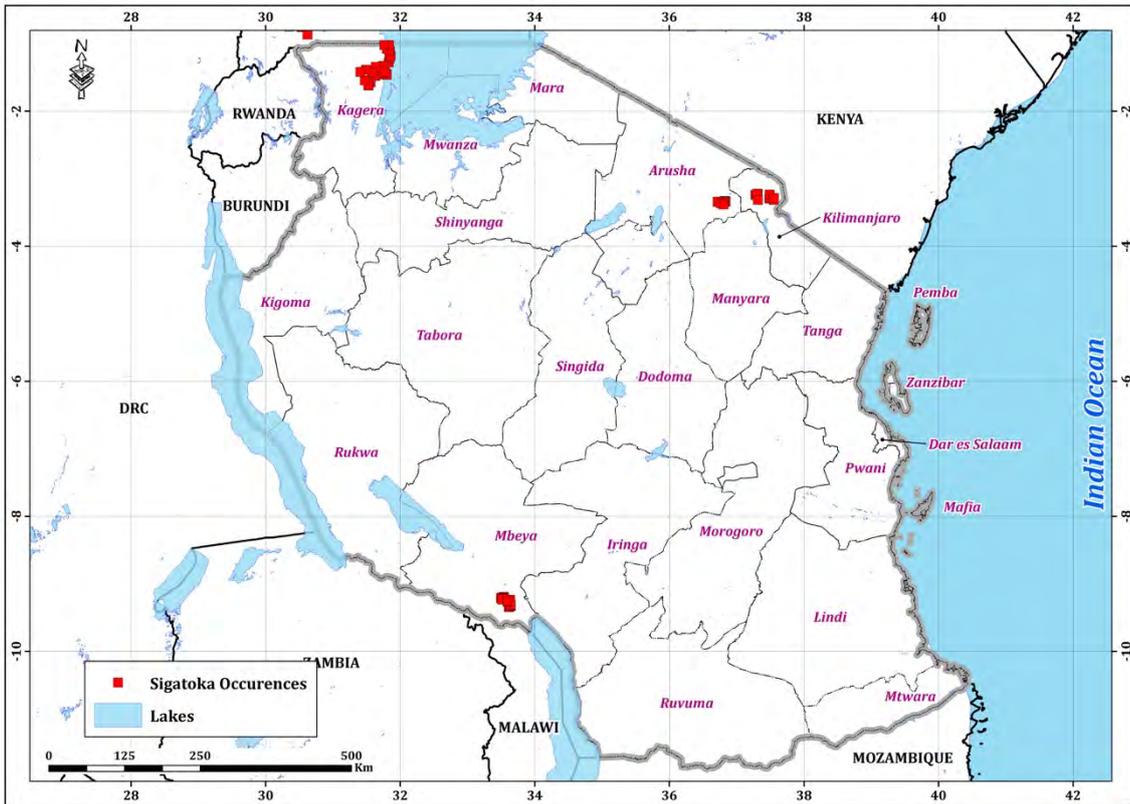


Figure: Distribution map of Sigatoka in Tanzania

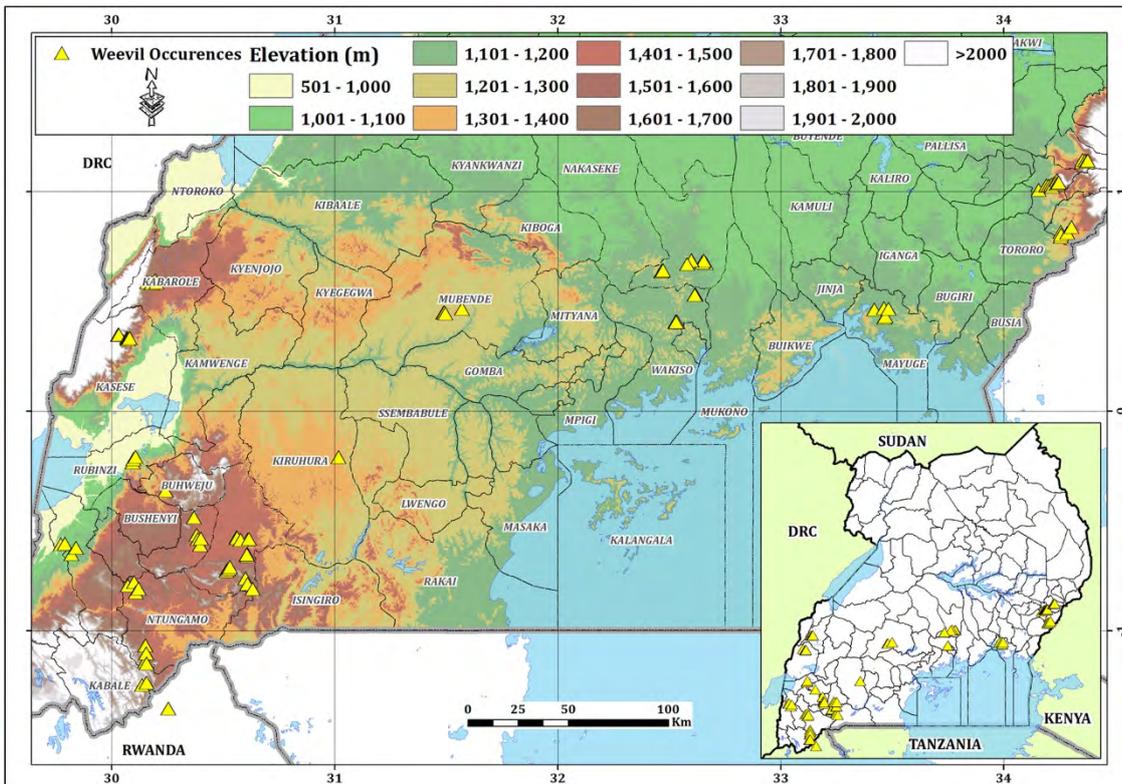


Figure: Distribution map of weevils and elevation in Uganda



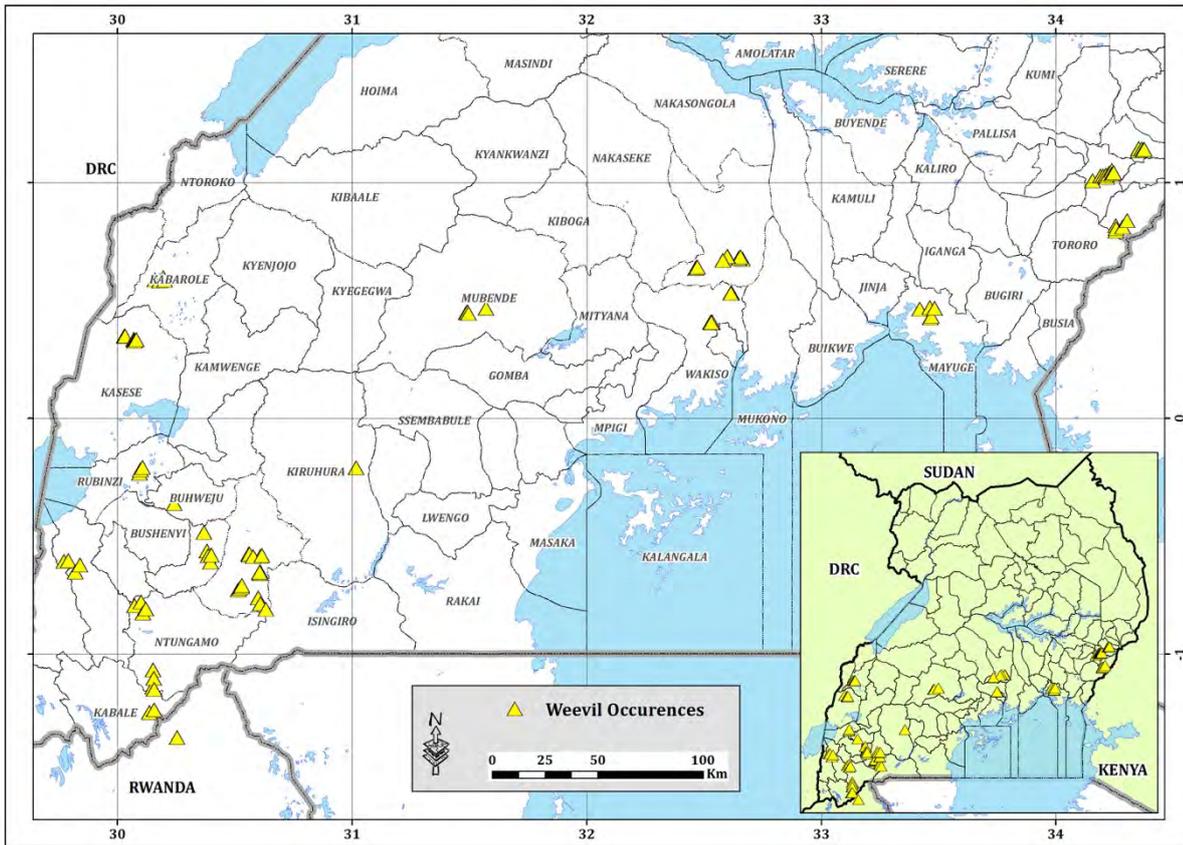


Figure: Distribution map of weevils in Uganda

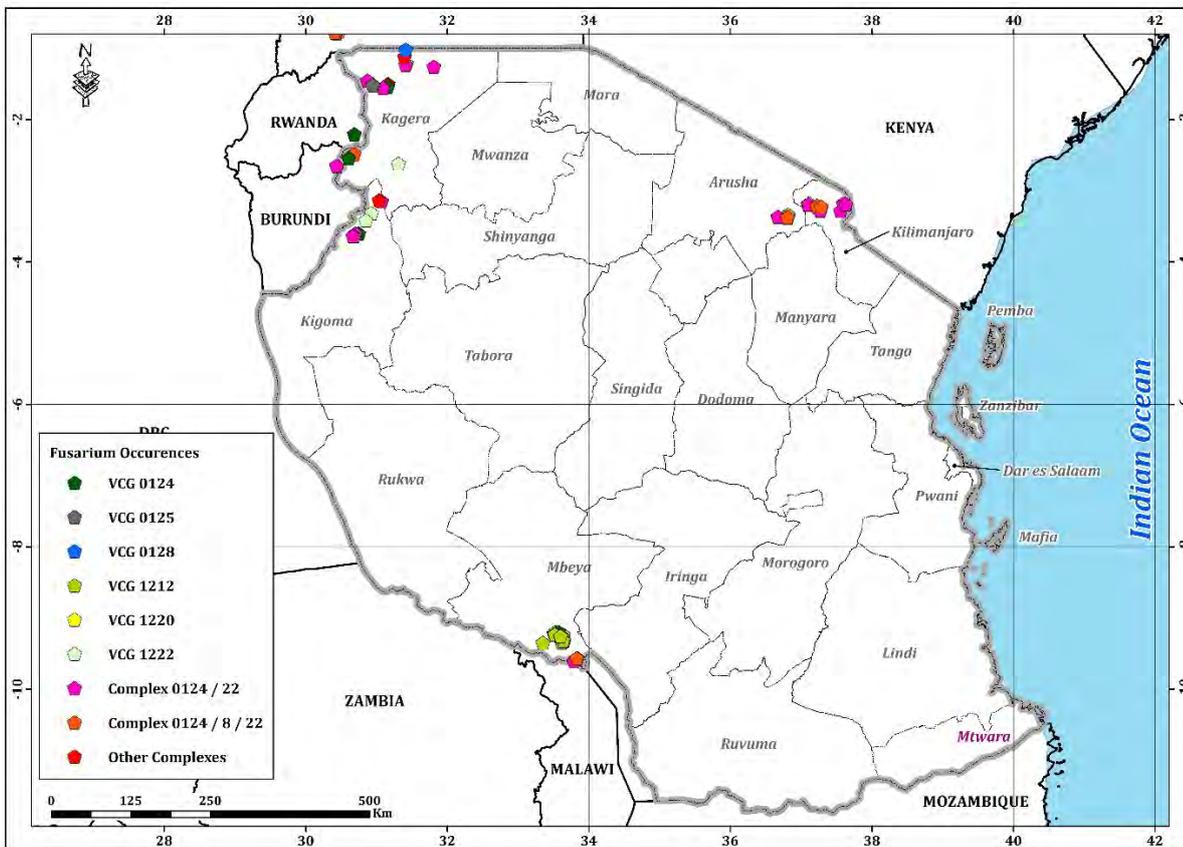
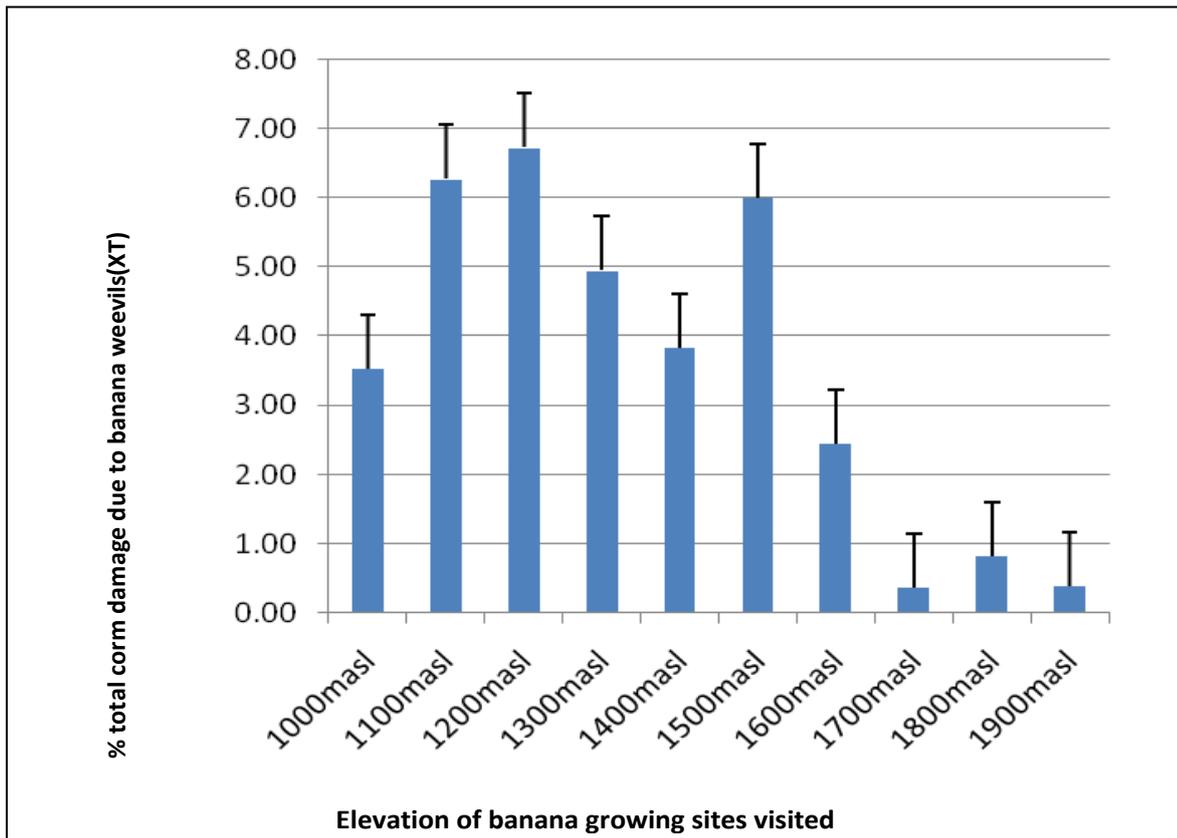
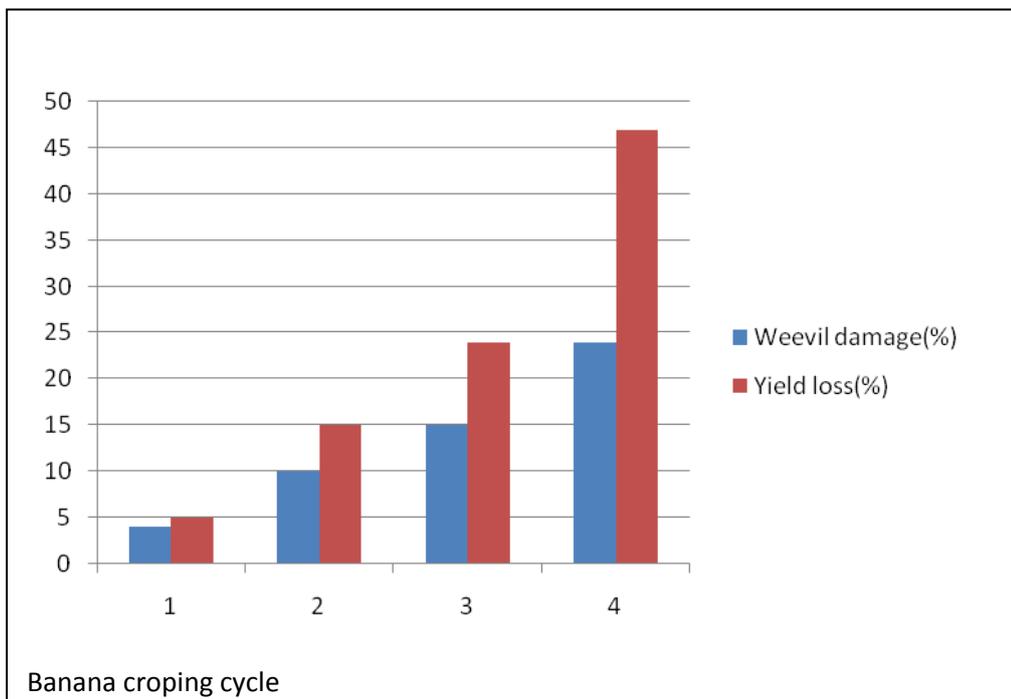


Figure: Distribution map of *Fusarium oxysporum* VCGs at Mbeya, Arusha and Kagera in Tanzania



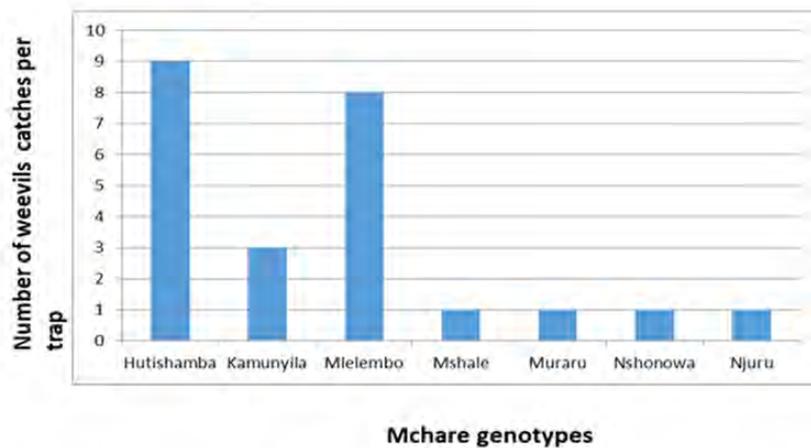
### 3.2 Banana weevil damage on East African Highland banana across different altitudes



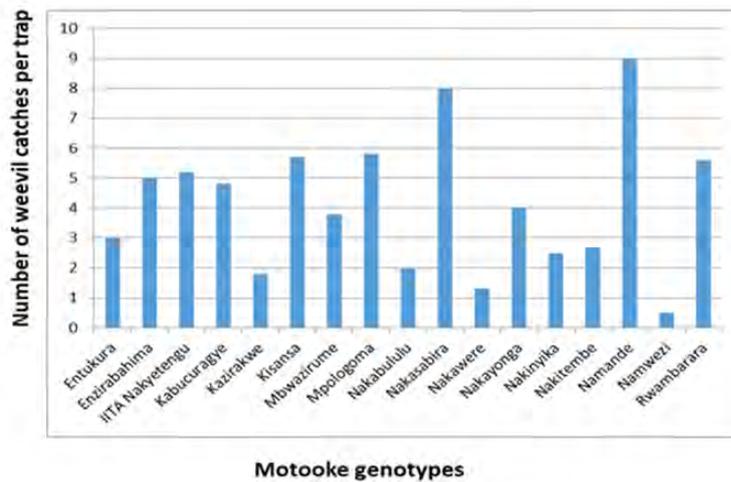
### 3.3 Cumulative Weevil damage and yield loss in banana in successive crop ratoons

-

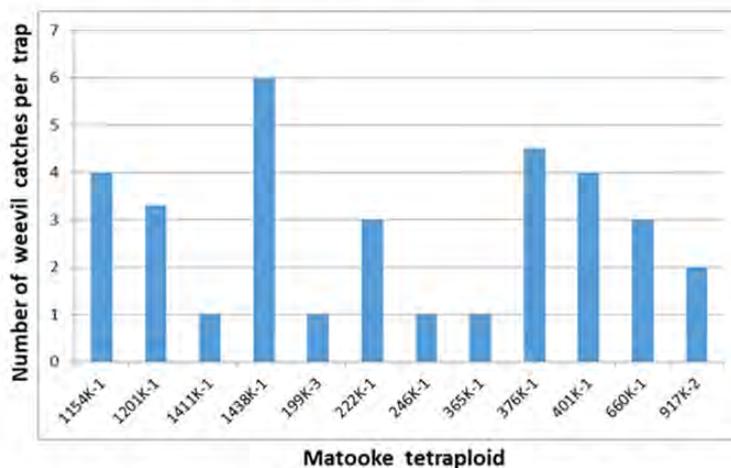
### 3.4 Number of weevil catches on at Kawanda (NARO) breeding site



**Figure A.** Number of weevil catches on Mchare at Kawanda (NARO) breeding site (Field established December 2014).

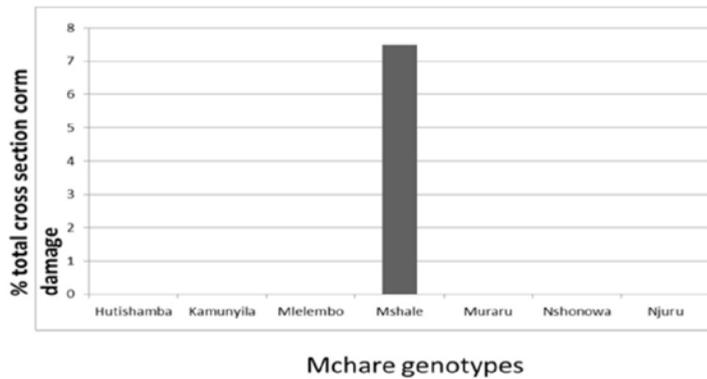


**Figure B.** Number of weevil catches on Motooke at Kawanda (NARO) breeding site (Field established October 2014).

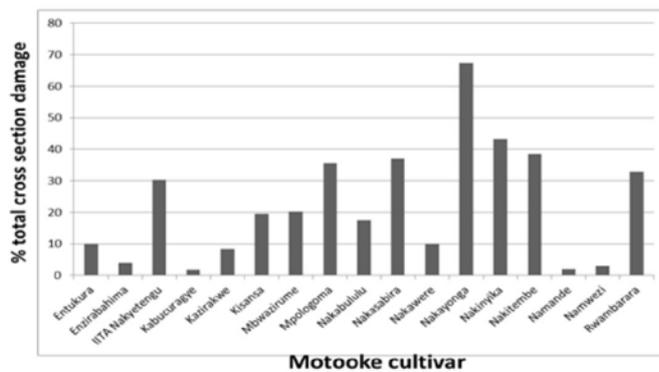


**Figure C.** Number of weevil catches on Motooke tetraploid hybrids at Kawanda (NARO) breeding site (Field established November 2014).

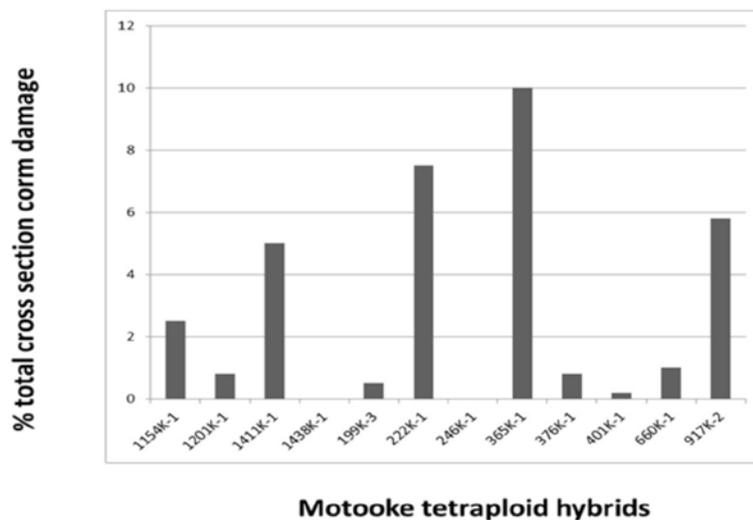
### 3.5 Banana weevil damage at Kawanda (NARO) breeding site



**Figure A.** Damage due to banana weevils (natural infestation) on Mchare genotypes at Kawanda (NARO) breeding site (Field established December 2014)



**Figure B.** Damage due to banana weevils (natural infestation) on Motooke genotypes at Kawanda (NARO) breeding site (Field established October 2014)



**Figure C.** Damage due to banana weevils (Natural infestation) on Motooke tetraploid hybrids at Kawanda (NARO) breeding site (Field established November 2014).

### 3.6 List of fungal isolates characterized between 1 April to 30 September 2017

| No | CAV no | Country  | Region/<br>Province | District where sample was collected | Name of cultivar | Foc Lin VI | VCG group or identity of the isolate |
|----|--------|----------|---------------------|-------------------------------------|------------------|------------|--------------------------------------|
| 1  | 3657   | Tanzania | Mbeya               | Rungwe                              | Sukari           | +          | 01212                                |
| 2  | 3665   | Tanzania | Mbeya               | Rungwe                              | Pisang           | +          | 01212                                |
| 3  | 3684   | Tanzania | Mbeya               | Rungwe                              | Sukari           | +          | 01212                                |
| 4  | 3706   | Tanzania | Mbeya               | Rungwe                              | Sukari           | +          | HSI                                  |
| 5  | 3708   | Tanzania | Mbeya               | Rungwe                              | Sukari           | +          | HSI                                  |
| 6  | 3713   | Tanzania | Mbeya               | Rungwe                              | Sukari           | +          | 01212                                |
| 7  | 3714   | Tanzania | Mbeya               | Rungwe                              | Sukari           | +          | HSI                                  |
| 8  | 3722   | Tanzania | Mbeya               | Rungwe                              | Sukari           | -          | Not <i>Fusarium</i> sp.              |
| 9  | 3727   | Tanzania | Arusha              | Tengeru                             | Mshare           | +          | 0124/22                              |
| 10 | 3731   | Tanzania | Arusha              | Tengeru                             | Sukari           | -          | Not <i>Fusarium</i> sp.              |
| 11 | 3742   | Tanzania | Arusha              | Meru                                | Sukari           | +          | 0124                                 |
| 12 | 3745   | Tanzania | Arusha              | Meru                                | Sukari           | +          | HSI                                  |
| 13 | 3802   | Tanzania | Arusha              | Siha                                | Sukari           | +          | HSI                                  |
| 14 | 3807   | Tanzania | Arusha              | Rombo                               | Pisang           | +          | 01222                                |
| 15 | 3811   | Tanzania | Arusha              | Rombo                               | Sukari           | +          | 0124                                 |
| 16 | 3818   | Tanzania | Arusha              | Moshi Rural                         | Mshare           | +          | 0124/22                              |
| 17 | 3822   | Uganda   | Mbarara             | Mbarara                             | Sukari           | +          | 01222                                |
| 18 | 3832   | Uganda   | Mbarara             | Mbarara                             | Sukari           | +          | 0124/22                              |
| 19 | 3846   | Uganda   | Kawanda             | Namulonge                           | Khom             | +          | 0124/5/8/22                          |
| 20 | 3850   | Uganda   | Kawanda             | Kawanda                             | Sukari           | +          | 0124/5/22                            |
| 21 | 3852   | Uganda   | Kawanda             | Kawanda                             | Sukari           | +          | 0124/22                              |
| 22 | 3862   | Uganda   | Kawanda             | Kawanda                             | Sukari           | +          | 0128/22                              |
| 23 | 3865   | Tanzania | Tengeru             | Tengeru                             | Mshare           | +          | 0124/22                              |
| 24 | 3868   | Tanzania | Arusha              | Hai                                 | Mshare           | -          | Not <i>Fusarium</i> sp.              |
| 25 | 3972   | Tanzania | Arusha              | TACRI                               | NARITA 9         | +          | 0124                                 |
| 26 | 3994   | Tanzania | Arusha              | TACRI                               | NARITA 9         | -          | Not <i>Fusarium</i> sp.              |
| 27 | 3995   | Tanzania | Arusha              | TACRI                               | NARITA 9         | +          | 0124/8/22                            |
| 28 | 3996   | Tanzania | Arusha              | TACRI                               | NARITA 9         | -          | Not <i>Fusarium</i> sp.              |
| 29 | 3997   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/22                              |
| 30 | 3998   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/22                              |
| 31 | 3999   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/22                              |
| 32 | 4000   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/22                              |
| 33 | 4001   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/5/22                            |
| 34 | 4002   | Tanzania | Arusha              | TACRI                               | Mshare           | -          | Not <i>Fusarium</i> sp.              |
| 35 | 4003   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/22                              |
| 36 | 4004   | Tanzania | Arusha              | TACRI                               | Mshare           | +          | 0124/22                              |
| 37 | 4005   | Tanzania | Arusha              | TACRI                               | Mshare           | -          | Not <i>Fusarium</i> sp.              |

HSI: Heterokaryon self-incompatible

### 3.7 List of 258 fungal isolates collected from Mbeya, Arusha and Kagera in Tanzania, Mbarara and Kawanda in Uganda.

| No | CAV no | Country  | Region/Province | Name of cultivar           | Foc Lin | VCG group                |
|----|--------|----------|-----------------|----------------------------|---------|--------------------------|
| 1  | CAV    | Tanzania | Arusha          | Obubit Ntanga              | -       | <i>Fusarium sacchari</i> |
| 2  | CAV    | Tanzania | Arusha          | Pisang Kepok-ITC0693       | +       | 0124/5/22                |
| 3  | CAV    | Tanzania | Arusha          | Pisang Raja-ITC0587        | -       | <i>Fusarium sacchari</i> |
| 4  | CAV    | Tanzania | Arusha          | Pisang Raja-ITC0587        | -       | <i>Fusarium sacchari</i> |
| 5  | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                  |
| 6  | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | <i>Fusarium</i>          |
| 7  | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | -       | <i>Fusarium sacchari</i> |
| 8  | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | -       | <i>Fusarium sacchari</i> |
| 9  | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 10 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                  |
| 11 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | -       | <i>Fusarium sacchari</i> |
| 12 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                  |
| 13 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                  |
| 14 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | -       | <i>Fusarium</i>          |
| 15 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 16 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 17 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 18 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 19 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 20 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 21 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 22 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>          |
| 23 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 24 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 25 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 26 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 27 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 28 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 29 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 30 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 31 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 32 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 33 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 34 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124/8/22                |
| 35 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>          |
| 36 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                    |
| 37 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                    |
| 38 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                    |
| 39 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | -       | not <i>Fusarium</i> sp.  |
| 40 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 41 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 42 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124/22                  |
| 43 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                    |
| 44 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                    |
| 45 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                    |
| 46 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                    |
| 47 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp.  |

| No | CAV no | Country  | Region/Province | Name of cultivar           | Foc Lin | VCG group               |
|----|--------|----------|-----------------|----------------------------|---------|-------------------------|
| 48 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                   |
| 49 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01212                   |
| 50 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 51 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 52 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp. |
| 53 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 54 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 55 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 56 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 57 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 58 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 59 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 60 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 61 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 62 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 63 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 64 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 65 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 66 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 67 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 68 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 69 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 0124/8/22               |
| 70 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 0124/8/22               |
| 71 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 72 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0128                    |
| 73 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 74 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 75 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 76 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 77 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | HSI                     |
| 78 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 79 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | HSI                     |
| 80 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 81 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 82 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 83 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 84 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 85 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | HSI                     |
| 86 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 0124/8/22               |
| 87 | CAV    | Tanzania | Mbeya           | Pisang Awak (ABB,          | +       | 01222                   |
| 88 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 89 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 90 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 91 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 92 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 93 | CAV    | Tanzania | Mbeya           | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp. |
| 94 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124                    |
| 95 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 01212                   |
| 96 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 97 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |

| No  | CAV no | Country  | Region/Province | Name of cultivar           | Foc Lin | VCG group               |
|-----|--------|----------|-----------------|----------------------------|---------|-------------------------|
| 98  | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 99  | CAV    | Tanzania | Arusha          | Mshare Mlelembo (AA,       | +       | 0124/22                 |
| 100 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 101 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp. |
| 102 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 103 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 104 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 105 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 106 | CAV    | Tanzania | Arusha          | MV1F1                      | +       | 01222                   |
| 107 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 01212                   |
| 108 | CAV    | Tanzania | Arusha          | Figue Pomme Geante         | +       | 0124/20/22              |
| 109 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 110 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 01222                   |
| 111 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 112 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | HSI                     |
| 113 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 114 | CAV    | Tanzania | Arusha          | Mshare, Ijihu Inkundu (AA, | +       | 0124/22                 |
| 115 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 116 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 117 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 118 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 01222                   |
| 119 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | HSI                     |
| 120 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 121 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 122 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | -       | <i>Fusarium</i>         |
| 123 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 01222                   |
| 124 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 01222                   |
| 125 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124                    |
| 126 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 127 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 128 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 129 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124                    |
| 130 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 131 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 132 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 133 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 134 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/8/22               |
| 135 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 136 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 137 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 138 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 139 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 01222                   |
| 140 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 141 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 142 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 143 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 144 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | -       | <i>Fusarium</i>         |
| 145 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 01222                   |
| 146 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 147 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |



| No  | CAV no | Country  | Region/Province | Name of cultivar           | Foc Lin | VCG group               |
|-----|--------|----------|-----------------|----------------------------|---------|-------------------------|
| 148 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 149 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 150 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 151 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 152 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 153 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 154 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 155 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 156 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 157 | CAV    | Uganda   | Mbarara         | Safet Velchi               | +       | 0124/22                 |
| 158 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 159 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 160 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 161 | CAV    | Uganda   | Mbarara         | Embu (MMC 402, NARO)       | +       | 0124                    |
| 162 | CAV    | Uganda   | Kawanda         | 2180K6-6                   | -       | <i>Fusarium</i>         |
| 163 | CAV    | Uganda   | Kawanda         | Khom                       | +       | 0124/5/8                |
| 164 | CAV    | Uganda   | Kawanda         | Pisang Awak (ABB,          | +       | 0124/8                  |
| 165 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 166 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 167 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/5/22               |
| 168 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 169 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 170 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 171 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/5/8/22             |
| 172 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 173 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/5/8/22             |
| 174 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/5/8/22             |
| 175 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 176 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 177 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp. |
| 178 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0124/5/8/22             |
| 179 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | +       | 0128/22                 |
| 180 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 181 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/8/22               |
| 182 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/22                 |
| 183 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/8/22               |
| 184 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 185 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | not <i>Fusarium</i> sp. |
| 186 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | not <i>Fusarium</i> sp. |
| 187 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | not <i>Fusarium</i> sp. |
| 188 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 01222                   |
| 189 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 190 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | -       | <i>Fusarium</i>         |
| 191 | CAV    | Uganda   | Mbarara         | Sukari Ndiizi (AAB,        | +       | 0124/8/22               |
| 192 | CAV    | Uganda   | Kawanda         | Khom                       | -       | not <i>Fusarium</i> sp. |
| 193 | CAV    | Uganda   | Kawanda         | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp. |
| 194 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | not <i>Fusarium</i> sp. |
| 195 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | not <i>Fusarium</i> sp. |
| 196 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/8/22               |
| 197 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | -       | not <i>Fusarium</i> sp. |

| No  | CAV no | Country  | Region/Province | Name of cultivar           | Foc Lin | VCG group               |
|-----|--------|----------|-----------------|----------------------------|---------|-------------------------|
| 198 | CAV    | Tanzania | Arusha          | Pisang Awak (ABB,          | -       | not <i>Fusarium</i> sp. |
| 199 | CAV    | Tanzania | Arusha          | Pisang Kepok-ITC0693       | -       | not <i>Fusarium</i> sp. |
| 200 | CAV    | Tanzania | Arusha          | Mshare (Variety not known) | +       | 0124/8/22               |
| 201 | CAV    | Tanzania | Arusha          | Sukari Ndiizi (AAB,        | -       | not <i>Fusarium</i> sp. |
| 202 | CAV    | Tanzania | Arusha          | not mentioned              | +       | 0124                    |
| 203 | CAV    | Tanzania | Arusha          | not mentioned              | -       | not <i>Fusarium</i> sp. |
| 204 | CAV    | Tanzania | Kagera          | Kijoge                     | +       | 0124/22                 |
| 205 | CAV    | Tanzania | Kagera          | Kikonjwa                   | +       | 0124                    |
| 206 | CAV    | Tanzania | Kagera          | Kikonjwa                   | +       | 01222                   |
| 207 | CAV    | Tanzania | Kagera          | Kikonjwa                   | +       | 01222                   |
| 208 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 209 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/5/8/20/22          |
| 210 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 211 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0128                    |
| 212 | CAV    | Tanzania | Kagera          | Sukari Ndiizi (AAB,        | +       | 0124                    |
| 213 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0125/8/20/22            |
| 214 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124                    |
| 215 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/5/20/22            |
| 216 | CAV    | Tanzania | Kagera          | Igyinja                    | +       | 0124/22                 |
| 217 | CAV    | Tanzania | Kagera          | Igyinja                    | +       | 0124                    |
| 218 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0128                    |
| 219 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124                    |
| 220 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 221 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0125                    |
| 222 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 01222                   |
| 223 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0125                    |
| 224 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 225 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0128                    |
| 226 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0125                    |
| 227 | CAV    | Tanzania | Kagera          | Sukari Ndiizi (AAB,        | +       | 01222                   |
| 228 | CAV    | Tanzania | Kagera          | Sukari Ndiizi (AAB,        | +       | 01212                   |
| 229 | CAV    | Tanzania | Kagera          | Home                       | +       | 0124/22                 |
| 230 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/8/22               |
| 231 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0125                    |
| 232 | CAV    | Tanzania | Kagera          | Gros Michel                | +       | 0124                    |
| 233 | CAV    | Tanzania | Kagera          | Home                       | +       | 0124                    |
| 234 | CAV    | Tanzania | Kagera          | Sukari Ndiizi (AAB,        | +       | 0124/22                 |
| 235 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124                    |
| 236 | CAV    | Tanzania | Kagera          | Kisubi                     | +       | 0124                    |
| 237 | CAV    | Tanzania | Kagera          | Kisubi                     | +       | 01222                   |
| 238 | CAV    | Tanzania | Kagera          | Kataraza                   | +       | 01212                   |
| 239 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0128                    |
| 240 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 01212                   |
| 241 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/22                 |
| 242 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0125/8                  |
| 243 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 01222                   |
| 244 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 01222                   |
| 245 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 01222                   |
| 246 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124                    |
| 247 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,          | +       | 0124/22                 |

| No  | CAV no | Country  | Region/Province | Name of cultivar    | Foc Lin | VCG group |
|-----|--------|----------|-----------------|---------------------|---------|-----------|
| 248 | CAV    | Tanzania | Kagera          | Sukari Ndiizi (AAB, | +       | 0124      |
| 249 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 01212     |
| 250 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 0124/22   |
| 251 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 01220     |
| 252 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 0128/20   |
| 253 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 0125      |
| 254 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 01212     |
| 255 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 01212     |
| 256 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 0124/22   |
| 257 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 0124/22   |
| 258 | CAV    | Tanzania | Kagera          | Pisang Awak (ABB,   | +       | 01222     |

\*HIS: Heterokaryon self-incompatible, a non-self recognition property of filamentous fungi during vegetative growth.

### 3.8 Disease severity of Mchare varieties to banana Fusarium wilt (Foc race 1) at Kawanda and Arusha.

| IITA – Arusha (Tanzania) |                 |          |               |                          |                             | NARO – Kawanda (Uganda) |                 |                      |               |                          |                                  |
|--------------------------|-----------------|----------|---------------|--------------------------|-----------------------------|-------------------------|-----------------|----------------------|---------------|--------------------------|----------------------------------|
| No                       | Name            | ITC code | Incidence (%) | RDI* means               | Response to Foc VCG 0124/22 | No                      | Name            | NARO code collection | Incidence (%) | RDI means                | Response to Foc VCG 0124/8/20/22 |
| 1                        | Huti-white      |          | 21            | 1,35 ± 0,14 <sup>b</sup> | Intermediate                | 1                       | Nshonowa        | MMC 423              | 33            | 1,70 + 0,09 <sup>a</sup> | Susceptible                      |
| 2                        | Huti green bell | ITC1559  | 12,5          | 1,29 ± 0,14 <sup>b</sup> | Intermediate                | 2                       | Mshare          | MMC 501              | 23            | 1,37 + 0,09 <sup>b</sup> | Intermediate                     |
| 3                        | Mshare          |          | 17            | 1,21 ± 0,14 <sup>b</sup> | Intermediate                | 3                       | Mshare Mlelembo | MMC 453              | 10            | 1,10 + 0,09 <sup>c</sup> | Intermediate                     |
| 4                        | Ijihu Inkundu   | ITC1460  | 12,5          | 1,17 ± 0,14 <sup>b</sup> | Intermediate                | 4                       | Muraru          | MMC 421              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 5                        | Makyughu I      | ITC1454  | 12,5          | 1,14 ± 0,14 <sup>b</sup> | Intermediate                | 5                       | Kahuti          | MMC 483              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 6                        | Mshare Mlelembo | ITC1455  | 8             | 1,13 ± 0,14 <sup>b</sup> | Intermediate                | 6                       | Kamunyila       | MMC 479              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 7                        | Makyughu II     | ITC1446  | 4             | 1,09 ± 0,14 <sup>b</sup> | Intermediate                | 7                       | Hutishamba      | MMC 486              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 8                        | Akondro Mainty  | ITC0281  | 8             | 1,08 ± 0,14 <sup>b</sup> | Intermediate                | 8                       | Njuru           | MMC 418              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 9                        | Nshonowa        |          | 0             | 1,00 ± 0,14 <sup>b</sup> | Resistant                   | 9                       | Sukari Ndiizi*  |                      | 57            | 1,73 + 0,09 <sup>a</sup> | Susceptible                      |
| 10                       | Kahuti          | ITC1468  | -             | -                        | Not tested                  | 10                      | Mbwazirume**    |                      | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 11                       | Gros Michel**   |          | 33            | 1,83 ± 0,14 <sup>a</sup> | Susceptible                 |                         |                 |                      |               |                          |                                  |
| 12                       | Grande Naine*** |          | 0             | 1,00 ± 0,14 <sup>b</sup> | Resistant                   |                         |                 |                      |               |                          |                                  |

\* RDI: rhizome discolouration index

\*\* Susceptible control

\*\*\* Resistant control

### 3.9 VCG groups or VCG complexes of *Fusarium oxysporum* f.sp. *cabense* collected from screening sites in Uganda and Tanzania.

| Site              | Variety         | VCG group/Complex |      |      |       |       |       |         |           |             |               | Total  | %   |      |
|-------------------|-----------------|-------------------|------|------|-------|-------|-------|---------|-----------|-------------|---------------|--|-----|------|
|                   |                 | 0124              | 0125 | 0128 | 01212 | 01220 | 01222 | 0124/22 | 0124/8/22 | 0124/5/8/22 | Other complex |  |     |      |
| Kawanda           | Pisang Awak     |                   |      |      |       |       |       |         |           |             |               | 1 (0124/8)                                       | 1   | 0,5  |
|                   | Sukari Ndizi    | 1                 |      |      |       |       |       | 6       | 1         | 4           |               | 2 (0124/5/22, 0128/22)                           | 14  | 6,5  |
|                   | Khom            |                   |      |      |       |       |       |         |           |             |               | 1 (0124/5/8)                                     | 1   | 0,5  |
| Mbarara           | Safeti Velchi   |                   |      |      |       |       |       | 1       |           |             |               |  | 1   | 0,5  |
|                   | Embu            | 1                 |      |      |       |       |       |         |           |             |               |  | 1   | 0,5  |
|                   | Sukari Ndizi    |                   |      |      |       |       | 2     | 5       | 10        |             |               |  | 17  | 7,9  |
|                   | Mshares         | 3                 |      |      | 2     |       |       | 17      | 5         |             |               |  | 27  | 12,6 |
| Arusha            | Mshare Mlelembo |                   |      |      |       |       |       | 1       |           |             |               |  | 1   | 0,5  |
|                   | Ijihu Inkundu   |                   |      |      |       |       |       | 1       |           |             |               |  | 1   | 0,5  |
|                   | Sukari Ndizi    | 3                 |      |      | 1     |       | 2     | 6       |           |             |               |  | 12  | 5,6  |
|                   | Pisang Awak     |                   |      |      |       |       | 3     | 9       |           |             |               |  | 12  | 5,6  |
|                   | Pisang Kepok    |                   |      |      |       |       |       |         |           |             |               | 1 (0124/5/22)                                    | 1   | 0,5  |
|                   | Figue Pomme     |                   |      |      |       |       |       |         |           |             |               | 1 (0124/20/22)                                   | 1   | 0,5  |
| Mbeya             | MV1F1           |                   |      |      |       |       | 1     |         |           |             |               |  | 1   | 0,5  |
|                   | Sukari Ndizi    | 3                 |      | 1    | 47    |       |       | 1       | 5         |             |               |  | 57  | 26,5 |
|                   | Pisang Awak     |                   |      |      | 8     |       | 1     |         | 3         |             |               |  | 12  | 5,6  |
|                   | Pisang Awak     | 4                 | 5    | 4    | 4     | 1     | 5     | 9       | 1         |             |               | 5 (4/5/8/20/22, 4/5/20/22, 5/8/20/22, 5/8, 8/20) | 38  | 17,7 |
|                   | Sukari Ndizi    | 2                 |      |      | 1     |       | 1     | 1       |           |             |               |  | 5   | 2,3  |
| Kagera            | Kisubi          | 1                 |      |      |       |       | 1     |         |           |             |               |  | 2   | 0,9  |
|                   | Kataraza        |                   |      |      | 1     |       |       |         |           |             |               |  | 1   | 0,5  |
|                   | Kikonjwa        | 1                 |      |      |       |       | 2     |         |           |             |               |  | 3   | 1,4  |
|                   | Gros Michel     | 1                 |      |      |       |       |       |         |           |             |               |  | 1   | 0,5  |
|                   | Home            | 1                 |      |      |       |       |       | 1       |           |             |               |  | 2   | 0,9  |
|                   | Igyinga         | 1                 |      |      |       |       |       | 1       |           |             |               |  | 2   | 0,9  |
|                   | Kijoge          |                   |      |      |       |       |       | 1       |           |             |               |  | 1   | 0,5  |
| <b>Total</b>      |                 | 22                | 5    | 5    | 64    | 1     | 18    | 60      | 25        | 4           |               | 11   | 215 | 100  |
| <b>Percentage</b> |                 | 10,4              | 2,4  | 2,4  | 30,3  | 0,5   | 8,5   | 28,4    | 10,0      | 1,9         | 5,2           |  | 100 |      |

## 4. Work Package 3

### 4.1 Mapping populations in IITA (Arusha and Sendusu) and NARO (Kawanda)

| S/n | Female parent          | Male parent            | Intended segregation   | Location | <i>R. similis</i> resistance                    | B Sigatoka resistance              | Person in charge | Plants in T/C | Plants in nursery | Plants in field | Remarks  |
|-----|------------------------|------------------------|--|----------|---|------------------------------------|------------------|---------------|-------------------|-----------------|--|
| 1   | ITC.0591<br>Kasaska    | ITC.0253<br>Borneo     | Weevil resistance  | Kawanda  | Borneo: S<br>Kasaska: R                         | Borneo: 2<br>Kasaska: 6            | Ivan             |               |                   | 253             | Genotypes not from 1 F1 (Kasaska x Borneo), the available Kasaska not the one used in the original cross |
| 2   | ITC.0249<br>Calcutta 4 | ITC.0966<br>Zebrina GF | Dwarfness, Bunch orientation, finger size, parthenocarpy, plant size<br><br>Probably segregating for weevil resistance too | Sendusu  | Calcutta 4: R (literature)<br><br>Zebrina GF: S | Calcutta 4: 2<br><br>Zebrina GF: 3 | Brigitte         |               | 189               | 160             | Each genotype to be checked using SSRs   |
| 3   | ITC.1179<br>Monyet     | ITC.1243<br>Kokopo     | Black sigatoka, plant size, weevil<br><br>Promising for Foc  | Sendusu  | Both parents:<br><br>inconclusive               | Monyet: 3<br><br>Kokopo: 5         | Brigitte         |               | 10                | 210             | The population is 3x, because Monyet is 4x   |

| S/n | Female parent          | Male parent            | Intended segregation   | Location | <i>R. similis</i> resistance                            | B Sigatoka resistance                   | Person in charge | Plants in T/C | Plants in nursery | Plants in field | Remarks   |
|-----|------------------------|------------------------|--|----------|---|---|------------------|---------------|-------------------|-----------------|---|
| 4   | ITC.0249<br>Calcutta 4 | ITC.1121 P.<br>Lilin   | Parthenocarpy,<br>bunch<br>orientation,<br>Foc, palatability           | Sendusu  | Calcutta 4:<br>R (literature)<br><br>Pisang lilin:<br>R | Calcutta 4: 2<br><br>P. Lilin: 3        | Brigitte         |               | 22                | 298             | Each genotype to<br>be checked using<br>SSRs                    |
| 5   | ITC.0249<br>Calcutta 4 | ITC.0249<br>Calcutta 4 | Foc  | Kawanda  | Calcutta 4:<br>R (literature)                           | Calcutta 4: 2                           | Ivan             |               |                   | 210             |   |
| 6   | ITC.0766<br>Paliama    | ITC.0253<br>Borneo     | Bunch<br>orientation<br><br>Nematode?<br>Black<br>Sigatoka?<br><br>Foc | Arusha   | Paliama: not<br>tested<br><br>Borneo: S                 | Paliama: not<br>tested<br><br>Borneo: 2 | Hassan           |               |                   | 296             |   |
| 7   | Malaccensis<br>(UQ)    | Malaccensis<br>(UQ)    | Foc  | Arusha   |   |   | Mohamed          | 82            |                   |                 | 2 batches sent so<br>far. Population<br>not doing well in<br>TC |
| 8   | Mchare                 | ITC.0249<br>Cacutta 4  | Black<br>Sigatoka/Foc  | Kawanda  |   |   | Ivan             | 135           |                   |                 | NEW CROSS   |

Sigatoka: Disease severity is recorded according to modified Gauhl's 0-6 scale.

## 4.2 Phenotyping for QTL mapping

| Population                     | Target number of genotypes | Foc     |         |          |                               | Weevil  |         |          |                               | Nematode |         |          |                               | Remarks  |
|--------------------------------|----------------------------|---------|---------|----------|-------------------------------|---------|---------|----------|-------------------------------|----------|---------|----------|-------------------------------|--|
|                                |                            | Site    | Parents | Complete | In progress (to be completed) | Site    | Parents | Complete | In progress (to be completed) | Site     | Parents | Complete | In progress (to be completed) |  |
| <b>Kasaska x Borneo</b>        | 200                        | Kawanda | T x T   | 62%      | Depending on nematode results | Kawanda | S x R   | 62%      | Depending on nematode results | Sendusu  | R x S   | 89%      | 11% (Nov 2017)                | The population is not from 2 parents, but 4 or 5, and it will be analysed with GWAS. Priority now given to C4xZGF) |
| <b>Calcutta 4 x Zebrina GF</b> | 180                        |         | -       | -        | -                             |         | -       | -        | -                             | Sendusu  | R x S   | 34%      | 51% (March 2018)              |  |
| <b>Monyet x Kokopo</b>         | 180                        | Kawanda | T x S   | 74%      | 26% (March 2018)              | Sendusu | R x S   | 23%      | 37% (March 2018)              |          | -       | -        | -                             |  |
| <b>Calcutta 4 x</b>            | 200                        | Kawanda | T x T   | 55%      | 0% (Feb 2018)                 |         | -       | -        | -                             |          | -       | -        | -                             | Plants in TC in preparati  |



|                                  |     |        |                      |     |                  |  |   |   |   |  |   |   |   |                       |
|----------------------------------|-----|--------|----------------------|-----|------------------|--|---|---|---|--|---|---|---|-----------------------|
| <b>Calcutta 4</b>                |     |        |                      |     |                  |  |   |   |   |  |   |   |   | on for screening      |
| <b>Paliama x Borneo</b>          | 200 | Arusha |                      | 34% | 64% (March 2018) |  | - | - | - |  | - | - | - | Segregation confirmed |
| <b>Malaccensis x Malaccensis</b> | 200 | Arusha | R x R (heterozygous) | 0%  | 35%              |  | - | - | - |  | - | - | - |                       |

#### 4.3 Phenotyping of the training population and heterosis (27 traits)

| Field                                       | Cycle 1   |            | Cycle 2   |            | Cycle 3   |            |
|---|-----------|------------|-----------|------------|-----------|------------|
|   | Flowering | Harvesting | Flowering | Harvesting | Flowering | Harvesting |
| <b>GS Training Population LIM - Sendusu</b> | 100%      | 98%        | 93%       | 87%        | 73%       | 67%        |
| <b>GS Training Population HIM - Sendusu</b> | 100%      | 96%        | 97%       | 93%        | 84%       | 76%        |
| <b>GS Training Population - Mbarara</b>     | 100%      | 93%        | 85%       | 66%        | 43%       | 11%        |
| <b>Validation (200 genotypes, Sendusu)</b>  | 54%       | 45%        | 20%       | 4%         | 0%        | 0%         |
| <b>Heterosis</b>                            | 94%       | 93%        | 86%       | 83%        | 60%       | 50%        |

## 4.4 Phenotyping for pests and diseases

### Phenotyping for Foc R1 and SR4

The populations developed last year were tested for segregation, and phenotyping was initiated in those populations segregating for Foc. Monyet x Kokopo population was evaluated in the screen house in Kawanda for Foc Race 1. Plants were first raised in tissue culture. In total 133 genotypes were screened together with the parents and the controls. Scoring was done on corm discolouration, yellowing of the leaves and splitting of the pseudostem. Based on corm discolouration, on a scale of 1 to 6 and the interpretation 42 hybrids were found resistant, 60 partially resistant, 19 susceptible and 12 highly susceptible. The other genotypes are in different stages of evaluation, some being in the screen house awaiting inoculation and others in tissue culture under multiplication.

*Interpretation of leaf system index (LSI) and corm discolouration index (RDI) using Muhamed et al., (1999) and Sutanto et al., (2011) indexes*

| RDI   | LSI   | Translation        |
|-------|-------|--------------------|
| 1     | 1     | Resistant          |
| 1.1-3 | 1.1-2 | Partial resistance |
| 3.1-5 | 2.1-3 | Susceptible        |
| 5.1-6 | 3.1-4 | Highly Susceptible |

Paliama x Borneo population was evaluated to confirm segregation for Foc Race 1 in Arusha. Sixty-seven were successfully screened. Using the scale 1 hybrid was found resistant, 15 partially resistant, 20 susceptible and 2 highly susceptible.

Calcutta 4 x Calcutta 4 population was tested for segregation to Foc R1. This was done on single plants after germinating the seeds. In total 111 genotypes were tested, 50 were found resistant, 36 partially resistant, 14 susceptible and 11 highly susceptible. The population is under multiplication for a replicated screening.

Furthermore, the second batch of the population from the University of Queensland in Australia was received in Arusha. This is from selfing 852, a Malaccensis genotype heterozygous for the QTL associated with resistance to Foc SR4 and TR4. The total number of genotypes sent is 135. However, this Malaccensis x Malaccensis population is not doing well in tissue culture; many genotypes are not proliferating after enough time in tissue culture. About 70 genotypes of this population are weaned to the screen house, getting ready to be inoculated.

To generate comparable results for screening for Foc R1, SR4 and TR4, genotypes are being screened for TR4. These genotypes include some genotypes of the Malaccensis x Malaccensis population, Calcutta 4, Pahang, SH-3362, Guadeloupe, Pisang Jari Buaya, ITC250 Malaccensis. Polyploids include FHIA 2, 3, 18, 23, 25, GCTCV119, gold finger. Part of the set will be inoculated with Foc R1 and the results will be compared with those to be generated in Arusha where the Malaccensis x Malaccensis population will be inoculated with Foc R1.

At UQ, work continued to fine-map the Foc SR4 resistance QTL, 345 individuals from the self-crossed F<sub>2</sub> population (852) were screened using 5 PCR markers in this QTL region. This screen identified 23 informative cross-over events which could be used to delimit the genetic interval. Currently the entire interval spans 157 kb sequence and covers a total genetic distance of 6.7cM. This distal region appears to have a very high gene density (one predicted gene model per 5.6kb of sequence). This is comparable to some of the gene rich regions or 'gene islands' observed in Arabidopsis and rice. Currently we are trying to multiply enough clones for these 23 individuals to perform disease assay against both tropical and sub-tropical race 4. To assess expression profiles of the candidate genes, a time course experiment at 0, 12h, 24h, 48h and 5 d time points was conducted. The genotypes include the resistant and susceptible Malaccensis parents. The plant roots were dipped into a 2 million conidia per mL spore suspension for 1 hour and then replaced back in soil. Entire roots of each plant were harvested at specific time points. Quantitative PCR systems (SYBER GREEN) were developed for 6 candidate genes. These include a small ribonucleoprotein involved in spliceosome function, Nuclear transcription factor subunit NF-YA1, a calcium-dependent protein kinase, a zinc finger CCCH domain containing protein, MATE efflux protein and WRKY20. WRKY20 gene seems to be constitutively expressed at a low level in the roots. Although its expression seems to be higher in the resistant line than in the susceptible line across all time points. Some genes such as the zinc finger CCCH gene seems to be up-regulated at day 1 to day 5

time periods in the resistant line but not in the susceptible line or the mock-treated control. This might suggest that they are up-regulated as part of the transcriptome response to race 4 during the early stages of infection. Since we haven't found any NBS-LRR genes in our candidate gene interval, we are looking at the expression of genes that play important roles in the systemic acquired resistance (e.g. NPR1 and PR1).

To examine the presence and absence variations of genes in our Malaccensis that could have been missed in the DH Pahang genome, Oxford Nanopore sequencing was performed to sequence the parental genotypes. However, the platform produced very long reads with the averages of anywhere between 5-9kb, resulting in poor quality and quantity of output, at a high price (1 GB of data at most per run at \$1000 USD). Considering that the Malaccensis genome is approximately 550MB, this type of sequencing might not be a cost effective way to sequence our target region. Steve Rounsley had a look along with the original Illumina data we had. Both datasets had very low coverage of 2-4 x which means that identifying regions where you could potentially get a presence or absence variation when aligned to the reference (DH Pahang) becomes really difficult due to the gaps and noisy background. So, to this end, we decided to use the Illumina HiSeq4000 platform to sequence the parents using 150 cycles and single read at 30x coverage each. The cost will be 2200 (AUD) and is a lot cheaper than the Nanopore. We are in the process of getting this done and will hopefully add more sequence depth to the reads mapped to the DH Pahang reference. If the 30x Illumina is insufficient to cover some of the large gaps then we will revisit the Nanopore again at a later date. We think that it will be a cost-effective approach to detect structural variations in this region. We will continue to work with Steve as himself and his lab has the bioinformatics tools to quickly analyse the data.

### **Phenotyping for nematode resistance (*Radopholus similis*)**

Phenotyping continued for the population Kasaska x Borneo. Eight experiments were conducted which concluded the evaluation of 177 hybrids together with the parents and controls (Valery as a susceptible check and Yangambi Km 5 as the resistant check). Based on the number of total nematode count, 33 genotypes were found resistant, 8 partially resistant, 35 susceptible, 101 inconclusive. Because of the pollination mistakes identified in this population using SSR markers, we started phenotyping the F<sub>1</sub> population from Calcutta 4 x Zebrina GF. Sixty-two hybrids of this population were successfully phenotyped, 30 were found resistant, 9 susceptible, 23 inconclusive. Ninety-two additional genotypes are currently in the screen house at various stages of being screened.

Despite the obvious pollination mistake in Kasaska x Borneo, phenotyping for this population will continue to completion. The inconclusive genotypes will be repeated. QTL mapping will be done as marker-trait association using Structure results as a kinship matrix.

The major challenge met while phenotyping for nematode resistance was the lack of suckers, as some genotypes are not producing enough suckers in the field. Macro-propagation units were established to increase the number of suckers in a relatively short period.

### **Phenotyping for banana weevil (*Cosmopolites sordidus*)**

Data for weevil resistance are available for 124 hybrids of the Kasaska x Borneo. Phenotyping in pot experiments was initiated for the Monyet x Kokopo population. Forty-two hybrids were successfully screened. Additional 67 genotypes are in a pot experiment. Some genotypes of the Monyet x Kokopo population don't produce suckers readily. These were taken to macro-propagation together with the population Calcutta 4 x Zebrina GF. Preparations are in progress to set up laboratory fast-screening screening experiments for weevil resistance using the same population (Monyet x Kokopo). This will be done on corms using the methodology developed in WP2. Each experiment is set up to run and be terminated in 2 weeks, hence enabling to screen many genotypes in a short period. The results will be compared with those from pot experiments.

## 4.5 Genotyping with SSR markers

In total, 526 accessions (546 samples) were genotyped during this period that represented several mapping populations including the parents of the progeny and other material for which the genetic diversity was analyzed by SSR markers.

A. Mapping populations: These were genotyped to check their true-to-typeness, relative to the intended crosses.

1) Mapping population Kasaska x Borneo (ITC0253):

- a. Target: 200 hybrids + parents
- b. Accomplished so far: 187 hybrids + parents (F<sub>1</sub>, Kasaska and Borneo)
- c. The population is being phenotyped for nematode resistance, and half of it has been phenotyped for weevil resistance.
- d. The intended population was F<sub>2</sub> from selfing one F<sub>1</sub> genotype generated by crossing Kasaska x Borneo. SSR results indicated that Kasaska available at Kawanda was not the Kasaska used to generate the population, since it doesn't share any allele with the population for 10 out of 13 markers reliably scored in the parents. Structure analysis suggested that the population resulted from crossing different F<sub>1</sub> genotypes from the available Borneo and an unknown Kasaska. It is not possible to generate a mapping population for such a population. However, QTL mapping will be done as marker-trait association using the Structure results as a kinship matrix.

2) Monyet (ITC1179) x Kokopo (ITC1243) population from Sendusu/Uganda:

- a. Target: 200 hybrids + parents
- b. Accomplished so far: 110 hybrids + parents
- c. The population is being phenotyped for resistance to Fusarium wilt race 1 and weevil.
- d. Based on SSR scores, the population was derived from Monyet x Kokopo (common alleles between the parents and the progeny).
- e. SSR results suggested that the hybrids might not be diploid, but triploid. Ploidy analysis of 94 hybrids showed that 97% were 3x, 2% were 4x and 1% was 2x. Ploidy analysis of the parents showed that Kokopo is 2x, and 2 ploidy levels for Monyet at Sendusu: out of 13 mats 11 were 4x and 2 were 2x. The tetraploidy of Monyet explains the presence of majority being triploid hybrids. SSR results suggest that Monyet is an autotetraploid, as it has only 2 alleles at each locus.

3) Calcutta 4 (ITC0249) x Zebrina GF (ITC0966) population from Sendusu/Uganda:

- a. Target: 180 hybrids + parents + new 189 hybrids
- b. Accomplished so far: 82 hybrids + parents
- c. The population is being phenotyped for nematode resistance
- d. 11 SSRs were used to genotype this population
- e. SSR results for the 82 hybrids suggest that Calcutta 4 is the mother for all the hybrids. However, Zebrina GF doesn't share the alleles with the hybrids for 9 markers. Further genotyping of 13 mats of the Zebrina GF confirmed that they were one single genotype. The 85 genotypes were from old crosses (from 2010) when attempts to generate different mapping populations based using Calcutta 4 as the mother were made.
- f. Genotyping of the remaining hybrids will shed more light on this population. The population now has 160 genotypes in the field and 189 additional hybrids were recently weaned. Optimistically we will get at least 180 genotypes from two parental combinations out of 349 available hybrids.

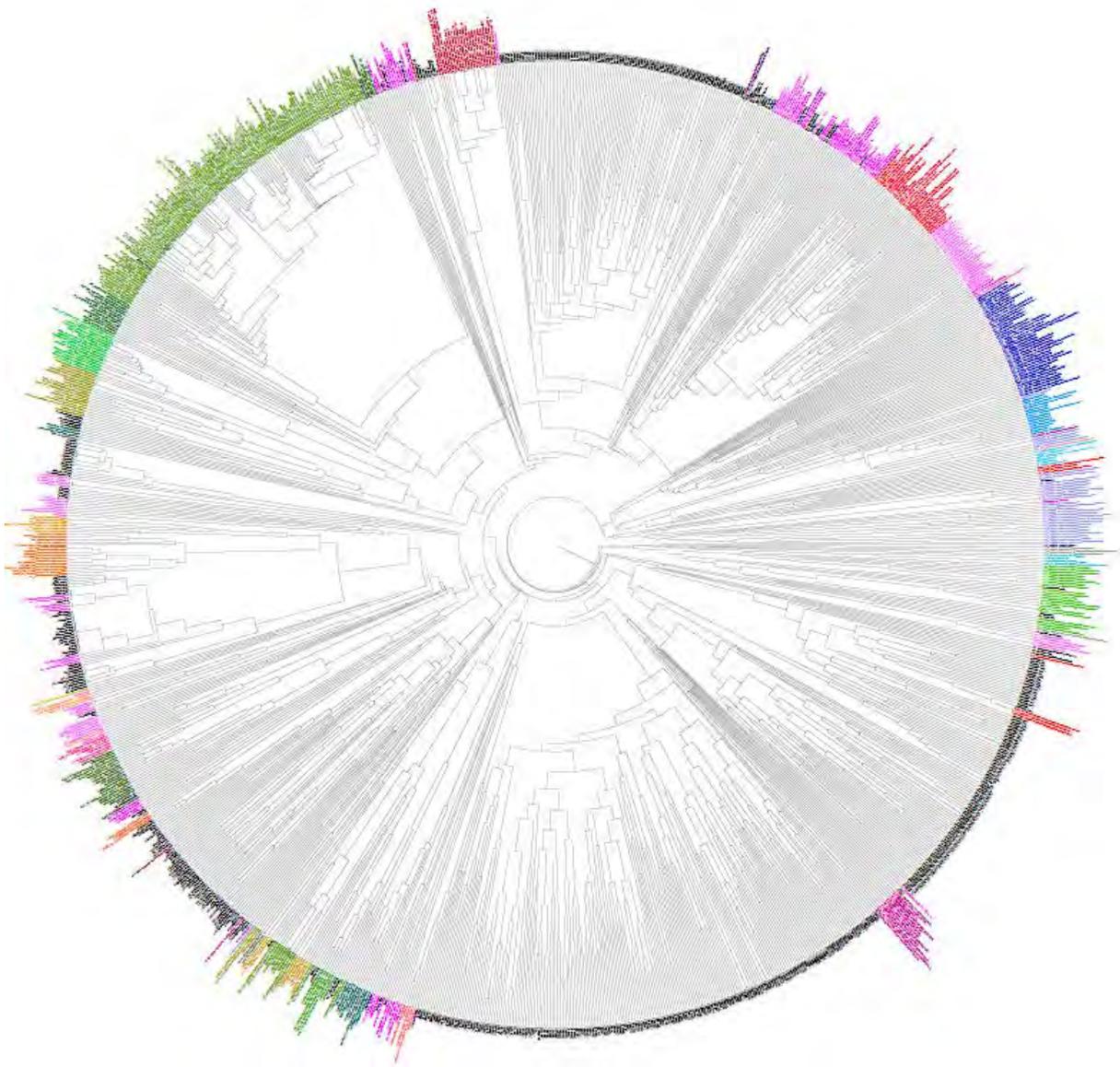
B. Other accessions: These were genotyped to check their diversity, compared to the core ITC set genotyped at IEB.

- 1) *M. velutina* x *M. acuminata* hybrids from Ibadan/Nigeria: 10 hybrids and 8 parents and grandparents
- 2) Mchare/Muraru/Mlali accessions from Arusha/Tanzania and Mchare from Kawanda and Sendusu – 21 accessions from the Mchare, Muraru and Mlali collection in Arusha and 10 mats of "Mshale"/Mchare from Uganda (Sendusu and Kawanda).
- 3) Set of accessions used for BXW resistance screening from Uganda – 92 accessions
- 4) Malaccensis genotypes from UQ used in the generation of the mapping population – 2 accessions

All these genotypes are shown on the dendrogram together with the ITC core collection genotyped at IEB.

The remaining genotypes to be genotyped using the 19 SSR markers include:

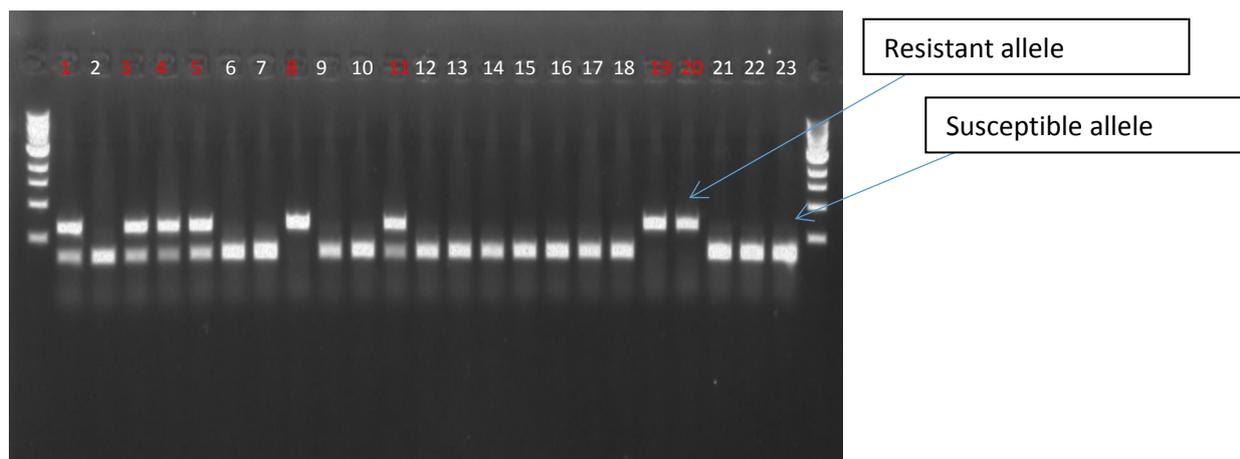
- Monyet x Kokopo: 90 hybrids
- Calcutta 4 x Zebrina GF: 201 genotypes (including 189 new hybrids)
- Paliama x Borneo: 250 genotypes
- Calcutta 4 x P. Lilin: 250 genotypes
- Calcutta 4 x Calcutta 4: 200 genotypes



**4.6 SSR marker dendrogram for IITA and ITC core collection genotyped at IEB**

## 4.7 Genotyping of Malaccensis and Malaccensis-derived genotypes for Foc SR4 QTL

A QTL for Foc SR4 has been identified on chromosome 3 by the team at UQ led by Dr. E. Aitken. The QTL has been fine-mapped to 157 kb nucleotide sequence. This region contains 15 candidate genes. The QTL has been converted into a PCR-based marker and confirmed in known Malaccensis resistant genotypes. DNA for 18 genotypes was sent to UQ for genotyping for this specific QTL. These included 7 diploid parents used at Sendusu which are Malaccensis or Malaccensis-derived (having a Malaccensis in their pedigree) and 11 accessions used in WP2 screening for Foc R1. These were run together with 2 known controls (resistant and susceptible).



*Gel photo of the analysis of the IITA material for the Foc SR4 QTL*

Among the Malaccensis and Malaccensis-derived diploid parents sent, only SH3217 has the Foc SR4 QTL in a homozygous resistant state. TMB2x7197-2, 5601S-1, SH3362 and Malaccensis-250 are heterozygous and the rest of the tested genotypes were homozygous susceptible. SH3362 and Malaccensis 250 have been screened and found to be resistant. TMB2x8075-7 does carry the resistant allele. Mbwazirume, a Matooke known to be resistant to Foc Race 1 (used as resistant control in WP2 experiments) is homozygous susceptible for this QTL. This suggests that Mbwazirume is either susceptible to Foc SR4, or it has another source of resistance on the genome, other than this specifically mapped QTL. SH3217 is the female parent of SH3362 and the source of resistance seems to have carried through in this cross. Most of the NARITAs (derived hybrids, 3x ploidy) seem to have either SH3362 or SH3217 as its male parent. This increase the chance that these lines are resistant against Foc race 4. The PYT and AYT\_TP collections have a significant portion of lines that were derived from 5610S-1 as either a male or female parent or Malaccensis (Malaccensis 250) as the male parent. SH3362 and SH3217 were also the male parents of half of the genotypes in advanced stages of selection. Resistance against Foc race 4 should be expected from some of these genotypes. The team at UQ has shared with IITA the primer and cutting enzyme information for this marker. Further screening will be carried out in Arusha and Sendusu.

Furthermore, QTL for resistance for Foc SR4 was tested in 16 Malaccensis ITC accession. DNA was provided by Prof Jaroslav at the IEB. Out of the 16 accessions, the marker detected resistance in 5 genotypes. These include Malaccensis lines (ITC0399, ITC0250), Pahang (ITC0609), Pa Mysore no2 (ITC0668), Klui Pal (ITC0979) and DH Pahang (ITC1511). Pahang and DH Pahang are known to carry resistance. The draft genome of Malaccensis is sequenced using DH Pahang. We have so far assessed the phenotypes of ITC0250 and DH Pahang against sub-tropical race 4 and both genotypes showed resistant phenotypes. The resistant genotypes are additional sources of resistance for banana breeding programmes.

**Interpretation of the gel photo above for the material sent from Sendusu for genotyping for the Foc SR4 QTL**

| Slot number (gel) | Accession                | Parents             | Type                | QTL genotype | UQ SR4 phenotype |
|-------------------|--------------------------|---------------------|---------------------|--------------|------------------|
| 1                 | TMB2x7197-2              | SH3362 x Long Tavoy | 2x parent           | RS           |                  |
| 2                 | TMB2X8075-7              | SH3362 x Calcutta 4 | 2x parent           | SS           |                  |
| 3                 | 5610S-1                  | Kabucuragye         | 2x parent           | RS           |                  |
| 4                 | SH3362                   | SH3217 x SH3142     | 2x parent           | RS           | Resistant        |
| 5                 | Malaccensis_250 (ITC250) |                     | 2x parent           | RS           | Resistant        |
| 6                 | Hutishamba               |                     | 2x parent           | SS           |                  |
| 7                 | Mchare Laini             |                     | 2x parent           | SS           |                  |
| 8                 | SH3217                   | SH2095 x SH2766     | 2x parent           | RR           | Being tested     |
| 9                 | CV Rose                  |                     | 2x parent           | SS           |                  |
| 10                | Mularu                   |                     | WP2 genotype        | SS           |                  |
| 11                | SH-3361                  | Error               | Error (SH3362)      | SR           |                  |
| 12                | Kamunyiya                |                     | WP2 genotype        | SS           |                  |
| 13                | Mlelembo                 |                     | WP2 genotype        | SS           |                  |
| 14                | Njuru                    |                     | WP2 genotype        | SS           |                  |
| 15                | Kahuti                   |                     | WP2 genotype        | SS           |                  |
| 16                | Mbwazirume               |                     | WP2 genotype        | SS           |                  |
| 17                | Sukari Ndiizi            |                     | WP2 genotype        | SS           |                  |
| 18                | Nshonowa                 |                     | WP2 genotype        | SS           |                  |
| 19                | 851                      |                     | Resistant control   | RR           | Resistant        |
| 20                | 851                      |                     | Resistant control   | RR           | Resistant        |
| 21                | 845                      |                     | Susceptible control | SS           | Susceptible      |
| 22                | 846                      |                     | Susceptible control | SS           | Susceptible      |
| 23                | 846                      |                     | Susceptible control | SS           | Susceptible      |



## 4.8 Leaf archiving

IITA has started leaf archiving of all genotypes being phenotyped under WP1, WP2, WP3 and WP4. Samples have been collected at Sendusu, Kawanda and Arusha. The leaf samples are kept at -80°C while waiting to be freeze-dried.

Each genotype is archived in duplicate or triplicate. A documentation system has been created to locate any sample. Leaf samples of 2688 genotypes were archived (see table). The samples from Kawanda are being freeze-dried because the machine is available at the station. Those at Sendusu will be freeze-dried after the machine has been purchased. The used protocol for sampling and archiving has been shared with the team in Arusha and the work has also started.

**Error! Reference source not found.** The sampled material constitutes about 80% of the material to be archived at Sendusu, 25% of the material in Kawanda and 50% of the material in Arusha. Leaf sampling will continue in the first half of Year 4 of the project to have all the genotypes under phenotyping archived.

### *Summary of genotypes whose leaf samples have been archived*

| Type of material/Trial  | Location | Number of genotyped collected |
|---|----------|-------------------------------|
| 2x, 3x, 4x parents  | Sendusu  | 16                            |
| EET 22  | Sendusu  | 259                           |
| EET 24  | Sendusu  | 123                           |
| EET 25  | Sendusu  | 116                           |
| EET 26  | Sendusu  | 285                           |
| EET 27  | Sendusu  | 336                           |
| PYTs  | Sendusu  | 95                            |
| NARITA hybrids  | Sendusu  | 23                            |
| PITAs and BITAs   | Sendusu  | 36                            |
| Collection (black Sigatoka scoring)                           | Sendusu  | 72                            |
| Black Sigatoka field experiment                               | Sendusu  | 9                             |
| Calcutta 4 x Zebrina GF population                            | Sendusu  | 127                           |
| Calcutta 4 x P. Lilin population                              | Sendusu  | 178                           |
| Calcutta 4 x P. Lilin extension                               | Sendusu  | 125                           |
| Training population of genomic selection                      | Sendusu  | 231                           |
| Heterosis trial   | Sendusu  | 31                            |
| EET 13  | Kawanda  | 255                           |
| EET 15  | Kawanda  | 164                           |
| Paliama x Borneo population                                   | Arusha   | 170                           |
| GWAS panel/collection/pollination                             | Arusha   | 21                            |
| Other (diversity study, controls for Foc, nematode screening) | Arusha   | 16                            |
| <b>Total</b>  |          | <b>2688</b>                   |

## 5. Work Package 4

### 5.1 Banana Products Preferred Traits and Descriptors

Table 1. Banana Product descriptions

| Product (local language)                                  | Product nature   |
|---|--|
| ensaano   | flour  |
| kiburu  | mixture of banana and beans  |
| kideri  | mashed banana mixed with fresh milk  |
| kitawa  | mixture of banana, skimmed milk, yoghurt and/or maize; banana and milk smash, porridge           |
| loshoro   | mix of maize and banana smash  |
| machalari/mbalaga   | chopped bananas boiled with meat and other ingredients   |
| makashi   | bananas dried in the sun   |
| mangolo   | dried banana (sun or smoked)   |
| matoke/ ebitoke   | banana boiled with meat, fish or beans, banana leaves used to cover                              |
| matooke   | banana steamed in banana leaves then mashed  |
| mchemsho  |  |
| memba   | boiled banana mixed with caustic soda  |
| mtori   | banana mixed with meat and smashed after cooking   |
| ng'ande   | bananas are cooked and later squeezed to make heavy porridge, bananas mixed with meat and mashed |
| shiro   | bananas mixed with beans and caustic soda  |
| supu  | soup, banana mixed with meat to make stew  |
| ugali (unyangwa)  | stiff porridge from banana flour   |
| kabaragara, kabalagala, balagala, vitumbua (vibama)       | snack, pancake, bun (mashed ripe banana mixed with wheat flour and fried)                        |
| naasha  | smashed banana mixed with skimmed milk   |
| eminekye, yokurya, tugalya, menu, mbivu, ebihise, matunda | ripe dessert fruits  |
| gonja, ndizi yakuchoma, ndizi roast                       | roasted banana   |
| etekyere  | cooked gonja   |
| chipusi   | chips  |
| omwokya   | fried banana   |
| kukaranga   | deep fried banana  |
| choma mafuta  | oil fried banana   |
| choma majiva  | ash roasted  |
| choma mkaa  | charcoal roasted   |
| kitafunwa   | breakfast snack  |
| unga  | flour  |
| biskuti   | biscuit  |
| pombe, mbege, rubisi, tonto                               | local beers made from banana and mixed with sorghum or millet                                    |
| waragi, konyagi, gongo                                    | local gin  |
| omubisi, mubisi, togwa, juisi, eshande                    | local juice  |

Table 2. Products from different banana types in parts of Tanzania and Uganda

| Banana type     | Uganda   |   |   | Tanzania  |  |  |
|-----------------|--|---|---|---|--|--|
|                 | Mbarara  | Luwero  | Bukoba  | Meru  | Moshi  | Mbeya  |
| <b>Cooking</b>  | <b>1. matooke</b><br>2. <i>ensaano</i> – flour<br>3. wine  | <b>1. matooke</b>   | <b>1. matoke/ ebitoke</b><br>2. <i>omusongo</i> - mashed bananas<br>3. <i>supu</i> – soup                                   | <b>1. machalari</b><br><b>2. mtori</b><br><b>3. kideri</b><br><b>4. loshoro</b><br><b>5. kitawa</b><br>6. <i>mangolo</i>          | <b>1. machalari</b><br><b>2. mtori</b><br><b>3. kiburu</b><br>4. <i>shiro</i><br>5. <i>ng'ande</i><br>6. <i>kitawa</i><br>7. <i>mchemsho</i><br>8. <i>makashi</i><br>9. <i>memba</i> | <b>1. mbalaga (machalari)</b><br>2. <i>ugali (unyangwa)</i><br>3. <i>mtori</i><br>4. <i>supu</i>   |
| <b>Dessert</b>  | <b>1. eminekye yokurya – fruits</b><br>2. <i>kabaragara</i> – pancakes                               | 1. <i>tugalya, menu</i> – fruits<br><b>2. kabalagala - pancakes</b> | 1. <i>mbivu, ebihise</i> -fruits<br><b>2. balagala – snack/bun</b>  | <b>1. matunda - fruits</b><br>2. <i>naasha</i>  | 1. <i>matunda</i>  | <b>1. fruits</b><br>2. <i>vitumbua (vibama)</i> – buns, snack<br>3. juice<br>4. wine   |
| <b>Roasting</b> | 1. <i>gonja</i><br>2. <i>etekyere</i> -cooked <i>gonja</i>   | 1. <i>gonja</i>   | 1. <i>gonja</i><br>2. <i>chipusi</i> – chips<br>3. <i>omwokya</i> – fried banana<br>4. <i>kukaranga</i> – deep fried banana | 1. <i>kitafunwa</i> – breakfast snack<br>2. <i>ndizi yakuchoma</i> - roasted banana<br>3. crisps<br>4. chips<br>5. <i>biskuti</i> | 1. <i>ndizi roast</i> – roasted banana<br>2. crisps  | <b>1. choma mafuta - oil fried banana</b><br><b>2. choma majiva - ash roasted</b><br>3. <i>choma mkaa</i> - charcoal roasted<br>4. <i>unga</i> - flour |
| <b>Beverage</b> | <b>1. tonto – local beer</b><br>2. <i>eshande</i> - juice<br>3. <i>waragi</i> – local gin<br>4. wine | <b>1. mubiisi – juice</b><br><b>2. waragi</b><br>3. <i>tonto</i>    | <b>1. rubisi – local beer</b><br><b>2. konyagi, gongo - gin</b><br>3. <i>togwa</i> - juice                                  | 1. <i>pombe, mbege</i> - local beer<br>2. <i>juisi</i> - juice  | <b>1. mbege</b><br>2. <i>juisi</i><br>3. <i>ndizi kuchoma</i>  | 1. <i>vitumbua (vibama)</i> – snack  |

\*bold = most important products in area (preliminary analysis), \*not in order

**Table 3. Variety and product traits for a 'good' end product**

| Product        |                     | Varieties that make good product   | Traits of varieties that make good product/ traits before preparation   | Traits/characteristics of good product  |
|----------------|---------------------|--|---|---|
| <b>Mbarara</b> | Matooke             | Entaragaza<br>Mbazilume<br>Enjagata<br>Embururu<br>Kibuzi<br>Enyeru<br>Butobe                                      | at least one finger ripens<br>mature banana<br>yellowish when peeled<br>big fingers<br>easy to peel and cook<br>yellow when cooked<br>falling of tips on fingers<br>makes good matooke even if they have not ripened<br>should have some dry leaves<br>bursting of finger [not all varieties] | yellow when cooked and peeled<br>good aroma (can be brought by leaves)<br>keeps together when mashed<br>soft<br>smooth on tongue and throat like sweet banana<br>slippery on the fingers<br>should be prepared in banana leaves   |
| <b>Luweru</b>  | Matooke             | Nakitembe<br>Mpologoma<br>Kisansa<br>Mbwazirume<br>Nakabululu  | mature fruits<br>smooth peeling skin, soft peel<br>big fingers<br>not diseased  | good smell<br>soft<br>colour [orange after peeling and before cooking]<br>yellow when cooked<br>texture when cooked [pliable like chewing gum]<br>taste [no feeling of sap]<br>feeling in the hand [soft like a sponge and like desert banana]<br>taste [smooth on tongue]  |
| <b>Bukoba</b>  | Matoke/<br>ebitoke  | Inyoya<br>Entobe<br>Ensika<br>Enjunjuzi<br>Enchoncho<br>Kintu<br>Empigi<br>Ensikira<br>Enshansha<br>Enyitabunyonyi | mature early<br>big bunches<br>big fingers<br>should be elastic when cooked<br>do not separate when cooked<br>low water content<br>high carbohydrate content<br>should stay longer in the stomach<br>turns yellow when cooked   | moderate soft, smooth and soft<br>yellow in colour<br>good taste<br>good aroma, good flavour<br>well ripened<br>elasticity<br>cooked for min time [30 min]<br>should be mixed with beans<br>should satisfy people when taken<br>has enough starch<br>small amount of water<br>bananas from farm with manure<br>makes good matooke |
| <b>Meru</b>    | Machalari           | Mshare<br>Ndizi Uganda<br>Jamaica  | banana/bunch should be medium mature<br>not hard, soft<br>not small, large finger size  | soft when cooked<br>not too sweet, good taste<br>good smell - 'kahawiya'  |
| <b>Moshi</b>   | Machalari           | Mshare<br>Mnyenyele<br>Bukoba<br>[Matooke]   | medium soft, not to hard<br>well or moderately matured bananas<br>straight fingers<br>smell good for example Mnyenyele and Bukoba   | yellowish colour<br>taste - not sour, depends on the ingredients<br>smell depends on the ingredients<br>white/cream/milk colour but turns brown when mixed with other ingredients<br>slightly soft texture<br>salt and oil on average high quality of meat particularly gastrointestinal  |
| <b>Rungwe</b>  | Mbalaga (machalari) | Plantain (Mzuzu)<br>Uganda/Bukoba  | should not be too soft<br>well mature<br>when cooked should not turn like porridge  | slight sugar taste<br>texture- has a bit of viscosity<br>texture in hands -soft and slide in hand   |

| Product | Varieties that make good product               | Traits of varieties that make good product/ traits before preparation       | Traits/characteristics of good product  |
|---------|--|---|---|
|         | Mshare<br>Ndiali<br>Jamaica<br>Malindi<br>FHIA | should not have ulcers<br>bananas not produced using industrial fertilizers | slightly yellow due to mixture of many ingredients; have orange colour but faint<br>'natural smell' of mbalaga<br>'natural taste' of mbalaga, not sour<br>should be mixture of numerous ingredients such as meat; oil moderate salt |

**Table 4. Variety and product traits for a 'bad' product**

| Product       | Varieties that make bad product   | Characteristics of varieties make bad products  | Traits/characteristics of a bad product   |
|---------------|---|---|---|
| <b>Bukoba</b> | Ebitoke<br>Entobe<br>Eshakara<br>FHIA<br>Enkila<br>Enchoncho<br>KM5<br>Enubo<br>KM5 [kadaba]<br>Egonjwa<br>Nshskana | produce big bunches, big finger [length and width]<br>matoke is hard, watery<br>high water content when cooked hence too soft<br>no good flavour<br>sour taste, taste not good<br>no elasticity<br>they are good for business/sales (+ve) | banana is too soft or too hard<br>has a lot of salt or no salt  |
| <b>Meru</b>   | Machalari<br>Ngumade  | very hard when cooked   | bananas are too soft or too hard<br>has a lot of salt or no salt<br>mixing banana varieties when cooking can make bad machalari   |
| <b>Moshi</b>  | Machalari<br>Mchare<br>ngumadu<br>Ndizi ng'ombe   | makes sour machalari  | banana is too soft or too hard-<br>depends on the banana type<br>little fire when starting  |
| <b>Rungwe</b> | Mbalaga/<br>machalari   | -   | white colour<br>hard<br>sour taste, astringent, may have bad taste if boiled without meat e<br>turns black if left for a long time after cooking<br>if removed from fire before well-cooked<br>if banana which is not well mature is used<br>lack ingredients |

\*No data for Mbarara and Luwero

**Table 5. Preferred traits for each banana type**

|                                  | Trait category                        | Trait  | Cooking                  | Beverage                 | Dessert                  | Plantain                 |
|----------------------------------|---------------------------------------|--|--------------------------|--------------------------|--------------------------|--------------------------|
| PHYSICAL DESCRIPTORS             | <b>Agronomic</b>                      | Suckering ability <sup>w</sup>                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Time to maturity -early, quick                           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Lifespan of the corm/mats, cultivar longevity            | <input type="checkbox"/> | <input type="checkbox"/> |                          | <input type="checkbox"/> |
|                                  |                                       | Fruit during the dry season                              | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Ripening period  |                          |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Yield (as determined by bunch mass or weight)            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Yield stability  | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Growth habit   |                          |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Plant height   | <input type="checkbox"/> |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Strength of pseudostem                                   |                          |                          | <input type="checkbox"/> |                          |
|                                  |                                       | Hardiness  | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Resistance to pests – weevils <sup>b</sup> , nematodes   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Resistance to diseases, fusarium wilt*, BLS <sup>b</sup> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Tolerance to lodging                                     | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Resistance to toppling                                   |                          | <input type="checkbox"/> |                          | <input type="checkbox"/> |
|                                  |                                       | Tolerance to drought                                     | <input type="checkbox"/> | <input type="checkbox"/> |                          | <input type="checkbox"/> |
|                                  |                                       | Tolerance to wind  | <input type="checkbox"/> | <input type="checkbox"/> |                          |                          |
|                                  |                                       | Tolerance to hailstorm                                   | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Adaptation to poor soil fertility <sup>w</sup>           | <input type="checkbox"/> | <input type="checkbox"/> |                          |                          |
|                                  |                                       | Intercropping ability                                    | <input type="checkbox"/> |                          |                          |                          |
|                                  | Labour requirements                   | <input type="checkbox"/>                                 | <input type="checkbox"/> |                          |                          |                          |
|                                  | <b>Size and shape attributes</b>      | Bunch size <sup>b</sup>                                  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Bunch size after maiden crop                             | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Bunch length   |                          |                          |                          |                          |
|                                  |                                       | Bunch compactness  | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Finger (fruit) size                                      | <input type="checkbox"/> |                          | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Finger length  |                          |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Finger thickness/girth                                   |                          |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Finger weight  |                          |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Finger uniformity  | <input type="checkbox"/> |                          |                          |                          |
|                                  |                                       | Finger shape   | <input type="checkbox"/> |                          | <input type="checkbox"/> |                          |
|                                  |                                       | Number of fingers per bunch                              | <input type="checkbox"/> |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Number of fingers per hand                               | <input type="checkbox"/> |                          | <input type="checkbox"/> |                          |
| Hand size                        |                                       | <input type="checkbox"/>                                 |                          |                          |                          |                          |
| Number of hands per bunch        | <input type="checkbox"/>              |  |                          | <input type="checkbox"/> |                          |                          |
| Pulp: peel ratio                 |                                       |  |                          | <input type="checkbox"/> |                          |                          |
| SENSORY/ORGANOLEPTIC DESCRIPTORS | <b>Appearance (before processing)</b> | Freshness  | <input type="checkbox"/> |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Peel colour (ripe/unripe)                                | <input type="checkbox"/> |                          | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Peel appearance  | <input type="checkbox"/> |                          | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Pulp colour (ripe/unripe)                                | <input type="checkbox"/> |                          | <input type="checkbox"/> |                          |
|                                  | <b>Appearance (after processing)</b>  | Pulp firmness  | <input type="checkbox"/> |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Pulp appearance when cooked                              |                          |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Colour when cooked                                       | <input type="checkbox"/> |                          |                          |                          |
|                                  | <b>Texture attributes</b>             | Texture of cooked pulp                                   | <input type="checkbox"/> |                          |                          | <input type="checkbox"/> |
|                                  |                                       | Texture of peeled pulp                                   |                          |                          | <input type="checkbox"/> | <input type="checkbox"/> |
|                                  |                                       | Uniformity   | <input type="checkbox"/> |                          |                          |                          |
| <b>Flavour</b>                   | Flavour                               | <input type="checkbox"/>                                 | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |                          |

|  | Trait category               | Trait  | Cooking                                      | Beverage                 | Dessert                  | Plantain                 |
|--|------------------------------|--|--|--------------------------|--------------------------|--------------------------|
|  | <b>attributes</b>            | Aroma/smell  | <input type="checkbox"/>                     |                          | <input type="checkbox"/> | <input type="checkbox"/> |
|  |                              | Taste of ripe fruit  |  |                          | <input type="checkbox"/> | <input type="checkbox"/> |
|  |                              | Taste (after cooking)  | <input type="checkbox"/>                     | <input type="checkbox"/> |                          | <input type="checkbox"/> |
|  |                              | Juice flavour  | <input type="checkbox"/>                     |                          |                          |                          |
| <b>PROCESSING AND PRODUCT RELATED TRAITS</b> | <b>Processing attributes</b> | Shelf life/perishability                                       | <input type="checkbox"/>                     |                          |                          | <input type="checkbox"/> |
|  |                              | Ease of peeling  | <input type="checkbox"/>                     |                          | <input type="checkbox"/> |                          |
|  |                              | Characteristics after peeling                                  | <input type="checkbox"/>                     |                          |                          |                          |
|  |                              | Finger detachability   |  |                          | <input type="checkbox"/> |                          |
|  |                              | Cooking quality  | <input type="checkbox"/>                     |                          |                          | <input type="checkbox"/> |
|  |                              | Traits after cooking   | <input type="checkbox"/>                     |                          |                          |                          |
|  |                              | Degree of ripeness/maturity                                    |  |                          |                          | <input type="checkbox"/> |
|  |                              | Cookability  | <input type="checkbox"/>                     |                          |                          |                          |
|  |                              | Palatability   | <input type="checkbox"/>                     |                          |                          | <input type="checkbox"/> |
|  |                              | Poundability   |  |                          |                          | <input type="checkbox"/> |
|  |                              | Cooking time   | <input type="checkbox"/>                     |                          |                          | <input type="checkbox"/> |
|  |                              | Suitability for matooke  | <input type="checkbox"/>                     |                          |                          |                          |
|  |                              | Suitability for production of beverage products (multipurpose) | <input type="checkbox"/>                     | <input type="checkbox"/> |                          |                          |
|  |                              | Suitability for production of food                             |  | <input type="checkbox"/> |                          |                          |
|  |                              | Yield of processed beverage product (e.g. juice productivity)  |  | <input type="checkbox"/> | <input type="checkbox"/> |                          |
|  |                              | Flavour of processed beverage product                          |  |                          | <input type="checkbox"/> |                          |
|  |                              | Taste of processed beverage product                            |  |                          | <input type="checkbox"/> |                          |
|  |                              | Quality of processed product                                   | <input type="checkbox"/>                     | <input type="checkbox"/> | <input type="checkbox"/> |                          |
|  |                              | <b>SOCIO-ECONOMIC DESCRIPTORS</b>                              | <b>Commercial and market life attributes</b> | Market demand, prices    | <input type="checkbox"/> | <input type="checkbox"/> |
| Rate of sheen loss                           | <input type="checkbox"/>     |  |  |                          |                          |                          |
| Bruising                                     | <input type="checkbox"/>     |  |  |                          |                          |                          |
| Hand or finger drop                          | <input type="checkbox"/>     |  |  |                          |                          |                          |
| Ripening traits                              | <input type="checkbox"/>     |  |  |                          |                          |                          |
| Non-presence of female flower buds           | <input type="checkbox"/>     |  |  |                          |                          |                          |
| <b>Cultural attributes</b>                   | Cultural uses <sup>b</sup>   |  | <input type="checkbox"/>                     |                          |                          |                          |
|  | Uses of other plant parts    |  | <input type="checkbox"/>                     |                          |                          |                          |
| <b>OTHER</b>                                 | <b>Other attributes</b>      | Number of consumption uses                                     | <input type="checkbox"/>                     |                          |                          |                          |
|  |                              | Health benefit   | <input type="checkbox"/>                     |                          |                          | <input type="checkbox"/> |
|  |                              | Accessibility of planting material                             | <input type="checkbox"/>                     |                          |                          |                          |
|  |                              | Availability of planting material                              |  | <input type="checkbox"/> |                          |                          |
|  |                              | Type of biotechnology used to produce planting material        | <input type="checkbox"/>                     |                          |                          |                          |

<sup>w</sup>= women specifically mentioned trait; <sup>m</sup> = men specifically mentioned trait, <sup>b</sup>= both men and women specifically mentioned trait;

\* = study indicated differences but does not specify if men or women prefer the trait

## 5.2 Varietal release guidelines

### Document 1

|                  |   |
|------------------|---|
| <b>Source</b>    | Received from Cornell when asked for Varietal Release Guidelines in Tanzania  |
| <b>Document</b>  | Varietal Release Guidelines Tanzania<br>From UPOV: <a href="http://www.upov.int/portal/index.html.en">http://www.upov.int/portal/index.html.en</a>  |
| <b>url</b>       | <a href="http://www.upov.int/edocs/tgdocs/en/tg123.pdf">http://www.upov.int/edocs/tgdocs/en/tg123.pdf</a><br><a href="https://cgiar-my.sharepoint.com/personal/p_marimo_cgjar_org/_layouts/15/guestaccess.aspx?docid=127885aa23cfe490b9bbc1738beb58778&amp;authkey=AWTYs0CSkDHDyJp3r9ZtWmA">https://cgiar-my.sharepoint.com/personal/p_marimo_cgjar_org/_layouts/15/guestaccess.aspx?docid=127885aa23cfe490b9bbc1738beb58778&amp;authkey=AWTYs0CSkDHDyJp3r9ZtWmA</a>  |
| <b>Country</b>   | Tanzania  |
| <b>Summary</b>   | <p><b>Guidelines for the conduct of tests for distinctness, uniformity and stability</b></p> <ul style="list-style-type: none"> <li>• Planting materials: corms, rhizomes or TC / variety</li> <li>• Min. 2 growing cycles (harvests), not counting mother crop</li> <li>• One location; if more, check document TGP/9</li> <li>• Min. 20 starting materials, resulting in min. 15 plants</li> <li>• Assess distinctness, uniformity (and stability)</li> <li>• Something about grouping of varieties??</li> <li>• Characteristics to be measured, their possible states of expression and example varieties given in Section 7 (Table)</li> <li>• Extra info given in Section 8</li> <li>• Forms to be filled out Section 10</li> <li>•</li> </ul>   |
| <b>Notes</b>     | Should be read in conjunction with General Introduction (document TG/1/3), and its associated TGP documents<br><a href="http://www.upov.int/resource/en/dus_guidance.html">http://www.upov.int/resource/en/dus_guidance.html</a><br><a href="http://www.upov.int/en/publications/tg-rom/tg001/tg_1_3.pdf">http://www.upov.int/en/publications/tg-rom/tg001/tg_1_3.pdf</a><br><a href="http://www.upov.int/edocs/tgpdocs/en/tgp_8.pdf">http://www.upov.int/edocs/tgpdocs/en/tgp_8.pdf</a><br><a href="http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf">http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf</a><br><a href="http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf">http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf</a><br><a href="http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf">http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf</a> |
| <b>Questions</b> | This seems to be an international document. Is the same document used for Uganda?<br>Who conducts the DUS tests? Is there a varietal release committee or the like? Or do NARS conduct these tests? Who approves these?   |



Document 2

|                 |  |
|-----------------|--|
| <b>Source</b>   | Received from Robooni when asked for Varietal Release Guidelines in Uganda   |
| <b>Document</b> | Uganda Seed Sector Baseline Study  |
| <b>url</b>      | <a href="https://cgiar-my.sharepoint.com/personal/p_marimo_cgiar_org/_layouts/15/guestaccess.aspx?docid=10c347fef5d6c44bca91e3712354514d4&amp;authkey=AXMZEu7uJSGmQ82Ntsw_g5E">https://cgiar-my.sharepoint.com/personal/p_marimo_cgiar_org/_layouts/15/guestaccess.aspx?docid=10c347fef5d6c44bca91e3712354514d4&amp;authkey=AXMZEu7uJSGmQ82Ntsw_g5E</a>  |
| <b>Country</b>  | Uganda   |
| <b>Summary</b>  | <p><b>Baseline survey of seed sector in Uganda, in relation to regional harmonization of seed legislation</b></p> <p><u>p.21-23 Section 3: Variety evaluation, release and registration:</u></p> <ul style="list-style-type: none"> <li>• Body in charge of variety evaluation, release and registration is the National Seed Certification Services (NSCS), in the Department of Crop Protection, Ministry of Agriculture, Animal Industry and Fisheries (MAAIF)</li> <li>• Before a variety can be recognized and entered in the National List of varieties, it has to be tested both for agronomic value and for Distinctness, Uniformity and Stability (DUS). The testing for agronomic value (*see below) is carried out by the breeders while the DUS testing is the responsibility of the NSCS.</li> <li>• NSCS ensures that only those varieties which undergo National Variety Performance Trials (NVPT) for two seasons are released for commercial production. <i>[Where a variety is already released in another country, such variety undergoes national variety performance trials for at least one main growing season before release provided that the breeder of such variety provides data used for release in similar agro-ecological zones.]</i></li> <li>• Any person or institution wishing to have their variety tested in National Performance Trials and DUS applies to the NSCS by filling Form SR 1 which is accompanied by the required seed sample and the prescribed fees.</li> <li>• While applying for NVPT, the applicant must show evidence of high performance of the variety in yield trials while on farm trials may be undertaken simultaneously with the NVPT.</li> <li>• The law, which is still in the making, states that NSCS shall independently carry out NVPT in accordance with the established standards/protocol. However, due to limited resources, the trials are conducted by NARO breeders. NSCS then uses these trials to carry out DUS testing. <i>[Note: MAAIF recognizes the short comings brought about by having NVPT conducted by breeders in NARO. It is the wish of MAAIF therefore that if resources ever allow, these trials will be conducted by NSCS.]</i></li> <li>• After evaluation of the candidate varieties in the NVPT, the Institution/individual wishing to have the variety released applies to NSCS by filling Form SR 2.</li> <li>• The National Variety Release Committee (NVRC), upon receipt of the application acknowledges it by filling Form SR 3.</li> <li>• The NVRC considers the applications and release of new varieties through meetings during which breeders and NSCS make presentations of the NVPT and DUS testing respectively. Any variety with superior agricultural value is released by the NVRC and registered onto the National Variety List. The applicant is informed of the decision of the NVRC through a notification on Form SR 4.</li> <li>• If a variety is released in more than one country of the East African Community, it qualifies to be on the East African Catalogue.</li> <li>• The law states that on evaluation of the candidate varieties in the NVPT, NSCS will present its recommendations to the NVPT Technical Committee before the varieties are presented to the National Variety Release Committee (NVRC) that approves the varieties for release. At the moment, the NVPT Technical Committee is not in place and after evaluation of candidate varieties, the breeders and NSCS present their reports to the NVRC in a variety release committee meeting.</li> <li>• * Testing for agronomic value: The performance of the variety is as follows:             <ul style="list-style-type: none"> <li>○ (i) It is suitable for ..... agronomic zones</li> <li>○ (ii) Yields are .....mt/ha</li> <li>○ (iii) Requires .....mm of rainfall distributed over .....days</li> <li>○ (iv) It is resistant to .....</li> </ul> </li> </ul> |

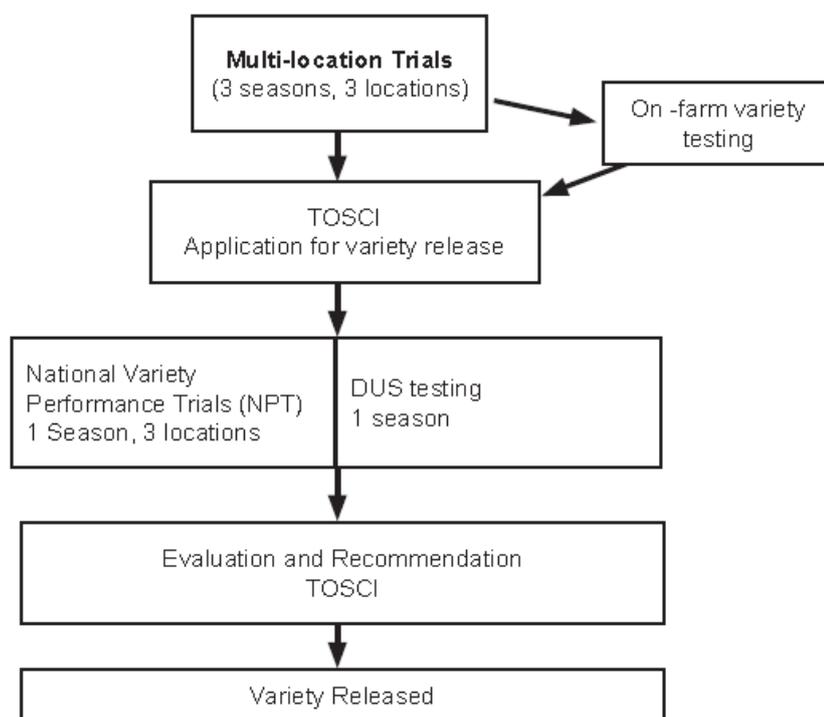
| Source    | Received from Robooni when asked for Varietal Release Guidelines in Uganda   |
|-----------|--|
|           | <ul style="list-style-type: none"> <li>o (v) Matures within ..... days</li> <li>o (vi)The variety stores best under ..... conditions</li> </ul>  |
| Notes     |  |
| Questions | <p>Dates from 2010 – still relevant?</p> <p>Does a similar document exist for Tanzania?</p> <p>Testing for agronomic value is responsibility of breeders – ok, what needs to be tested? Just the list* above?</p> <p>DUS testing is responsibility of NSCS – so they need to do this? They use the agronomic trials for this. So do they come to the field to measure? Does this follow rules of UPOV?</p> |

### Document 3

| Source    | Google search – varietal release Uganda   |
|-----------|---|
| Document  | National Crop Variety List for Uganda (2015)  |
| url       | <a href="http://tasai.org/wp-content/themes/tasai2016/info_portal/Uganda/National%20Crop%20Variety%20List%20for%20Uganda%20(2015).pdf">http://tasai.org/wp-content/themes/tasai2016/info_portal/Uganda/National%20Crop%20Variety%20List%20for%20Uganda%20(2015).pdf</a> |
| Country   | Uganda  |
| Summary   | <p>List of released crop varieties in Uganda</p> <p>Banana mentioned on p.34-36</p>   |
| Notes     |   |
| Questions |   |

## Document 4

|                 |   |
|-----------------|---|
| <b>Source</b>   | Google search – varietal release Uganda   |
| <b>Document</b> | Variety Testing and Release Approaches in DTMA Project Countries in Sub-Saharan Africa  |
| <b>url</b>      | <a href="http://dtma.cimmyt.org/index.php/publications/doc_view/84-variety-release-and-testing-approaches-in-dtma-project-countries-2009">http://dtma.cimmyt.org/index.php/publications/doc_view/84-variety-release-and-testing-approaches-in-dtma-project-countries-2009</a>   |
| <b>Country</b>  | Tanzania, Uganda  |
| <b>Summary</b>  | <p>DTMA project focuses on maize, but document describes general varietal testing and release procedures in the selected countries.</p> <p>For new crop varieties to be marketed they must be registered. The registration process requires that tests for distinctiveness, uniformity and stability (DUS) and value for cultivation and use (VCU) be conducted first before registration. The registration establishes legal ownership of the new variety. The DUS and the VCU tests can take between one and three years before sufficient data are available for variety registration. The seed laws for variety testing and release govern seed production, certification, marketing, import and export of seed. The variability and inconsistency of the seed laws between countries make it costly for seed companies to release and market new varieties. A new variety must be tested each time it is to be marketed in the respective countries, even if it is developed for sale across a wide range of agro-ecologies. In each country, a National Variety Release Committee (NVCR) makes a decision to release or to reject a new variety based on the data compiled in the release proposal.</p> <p>Tanzania member of Plant Breeders' Rights (UPOV), Uganda not; Neither member of ISTA or OECD.</p> <p>Published guidelines for DUS in Tanzania, not in Uganda (though table 4 says yes). The DUS tests are mostly conducted by National Seed Authorities (NSAs). In Tanzania and Uganda, the DUS tests are conducted at a fee.</p> <p>Published guidelines for VCU in Tanzania and Uganda. In Tanzania, the National Performance Trials (NPTs) are conducted by NSA at a given fee. In Tanzania, VCU data from other countries with similar agro-ecological zones may be used to complement in-country data.</p> <p><b>Tanzania</b></p> <p>In Tanzania, maize breeding is carried out by the various agricultural research institutes under the MoA. For new varieties to be released, they need to be tested by the breeder for VCU for a minimum of three seasons. Once testing is complete, the grain is submitted to the Tanzania Official Seed Certification Institute (TOSCI) for VCU and DUS tests for a minimum of one season. The VCU and DUS tests are conducted by TOSCI in selected areas depending on the recommended areas for the variety. Once the tests are complete, the Variety Release and Seed Certification Committee evaluate the data in order to make recommendations for release (Figure 12). The release committee is composed of the breeder of the variety, a pathologist, an entomologist, an economist, Director of Crop Development as Chairperson, Assistant Deputy Director of Research, and one member from the Seed Inspection Unit and TOSCI who also presents the DUS certificate. The rate of variety release has been acceptable, given that there are only a few seed companies in the country (Table 6). Unlike in Kenya, the NPT trials are conducted for a single season, which may hasten the rate of release.</p> |



**Figure 12. Variety release channel in Tanzania.**

#### Uganda

Uganda has a maize breeding program. For new maize varieties to be released and marketed in the country they must pass the DUS and VCU tests. The DUS tests are conducted by the National Seed Certification Service Unit of the Ministry of Agriculture. The DUS data must be collected for a minimum of two seasons across two locations. The NARS breeder is responsible for collecting the VCU data for a minimum of three seasons across five locations. The charges for collecting VCU data are US\$150 per variety while those for DUS data are US\$200 per variety. Once the data are ready, the breeder prepares a variety release proposal. The variety release proposal comprises the following:

- introduction,
- pedigree of the variety,
- description of the variety,
- site description,
- results and discussion,
- agronomic package, and
- variety maintenance.

Once the release proposal is ready, it is presented to the NVRC which is composed of three breeders (grain, forestry, and propagated crops), an agronomist, a biotechnologist, a representative of the private sector, three seed inspectors, DG National Agricultural Research Organization (NARO), and Director of Research NARO. The NVRC is appointed by the Commissioner of Crop Protection guided by the Seed Act. The variety release channel for Uganda is shown in Figure 13.

The rate of variety release has been poor in Uganda (Table 6).

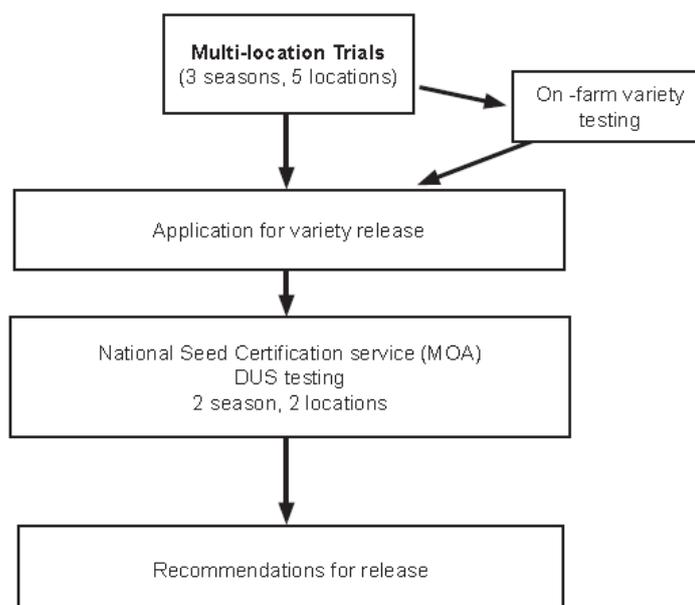


Figure 13. Variety release channel in Uganda.

|                  |                                     |
|------------------|-------------------------------------|
| <b>Notes</b>     | Interesting recommendations p.iv-v. |
| <b>Questions</b> |                                     |

## Document 5

|                 |   |
|-----------------|---|
| <b>Source</b>   | Google search – varietal release Uganda   |
| <b>Document</b> | Rice Varietal Release Systems in Africa   |
| <b>url</b>      | <a href="http://www.africarice.org/publications/rice_promise/Chap6%209781845938123.pdf">http://www.africarice.org/publications/rice_promise/Chap6%209781845938123.pdf</a>   |
| <b>Country</b>  | Tanzania, Uganda  |
| <b>Summary</b>  | <p>Common features of varietal release regulations are the establishment of:</p> <ul style="list-style-type: none"> <li>(i) a mandatory procedure for testing varieties proposed for release;</li> <li>(ii) a national varietal release committee (NVRC), which recommends or rejects release based on test results; and</li> <li>(iii) an official register of released varieties, recording names and main agronomic characteristics of varieties that have successfully passed the tests and have been recommended for release.</li> </ul> <p>An officially released variety is a new variety that has been tested according to the standards of a country and recommended by the NVRC of that same country to be of proven value, registered and made available to the public.</p> <p>In practice, some African countries keep a register of varieties that are ‘adopted’ in their country, because they do not operate a varietal release mechanism covering the three features mentioned above. In that case, ‘adopted varieties’ are those that are widely cultivated and for which the country has deemed it important to include them in the national crop register.</p> <p>If international standards (UPOV, 1978, 1991) are followed, new varieties can only be registered if they satisfy four criteria: novelty or value for cultivation and use (VCU), distinctness, uniformity and stability (DUS). Novelty means that the new rice variety performs better than the existing varieties for one or more traits of agronomic or</p> |

|                  |   |
|------------------|---|
|                  | <p>technological importance. The VCU of a new variety is tested and compared to local check varieties using standard protocols measuring key agronomic data such as yield, growth duration, grain quality, and resistance to biotic and abiotic stresses. Distinctness means that the variety is visually distinguishable from existing registered varieties in one or more morphological (shape, colour, height, leaf length, etc.) and agronomic (disease resistance, growth duration, etc.) traits. Uniformity or homogeneity means that at any development stage all individual plants are identical for all plant characteristics. Stability means that the variety remains identical to its initial description in its essential characteristics after repeated cycles of reproduction or propagation.</p> <p>We classified countries into four groups based on:</p> <ul style="list-style-type: none"> <li>(i) the existence of a varietal release system (comprising varietal testing, regular NVRC meetings to judge test results and official varietal registration);</li> <li>(ii) functionality of the varietal release system; and</li> <li>(iii) existence of at least a varietal register in the absence of a varietal release system.</li> </ul> <p>Tanzania and Uganda in group 1: varietal release system existent and functional.</p> <p>Both Tanzania and Uganda have DUS guidelines published. Tanzania has VCU guidelines published, Uganda not. Data from other countries are allowed in Uganda, not in Tanzania. Data from PVS trials not allowed in either country.</p> <p>In most countries, the national seed board is responsible for assembling and conducting national performance trials (NPTs) from which VCU and DUS data are obtained. Once the VCU and DUS data have been recorded, they are then submitted to the NVRC for consideration. In Tanzania and Uganda, the NPTs are conducted by the national seed boards for a set fee.</p> <p>To complement the VCU data from NPTs, independent trials, grown on farmers' fields by the farming community are required. Uganda accepts VCU and DUS data from other countries with similar agro-ecological conditions to complement in-country data. Agronomic data collected by breeders and socio-economic information from participatory varietal selection (PVS) are not acceptable as credible VCU data for varietal release in Tanzania and Uganda.</p> |
| <b>Notes</b>     |   |
| <b>Questions</b> | <p>Uganda accepts VCU and DUS data from other countries → so do we need a third location in Uganda, or can we take one of the locations from Tanzania as a reference?</p> <p>Agronomic data collected by breeders and socio-economic information from participatory varietal selection (PVS) are not acceptable as credible VCU data for varietal release in Tanzania and Uganda → so we will not be able to use the data from our trials? Someone else will have to do additional trials?</p>  |

## Document 6

|                  |   |
|------------------|---|
| <b>Source</b>    | Google search – varietal release Tanzania   |
| <b>Document</b>  | Establishment of Plant Breeders' Rights System In Tanzania: Achievements and Challenges   |
| <b>url</b>       | <a href="http://www.wipo.int/edocs/lexdocs/laws/en/tz/tz010en.pdf">http://www.wipo.int/edocs/lexdocs/laws/en/tz/tz010en.pdf</a> |
| <b>Country</b>   | Tanzania  |
| <b>Summary</b>   |   |
| <b>Notes</b>     | No info on varietal release per se; focus on plant breeders' rights   |
| <b>Questions</b> |   |

## Document 7

|                  |   |
|------------------|---|
| <b>Source</b>    | Google search – varietal release Tanzania   |
| <b>Document</b>  | Changing Seed and Plant Variety Protection Laws in Tanzania   |
| <b>url</b>       | <a href="http://acbio.org.za/wp-content/uploads/2016/05/Tanzania-Seed-Law-2016.pdf">http://acbio.org.za/wp-content/uploads/2016/05/Tanzania-Seed-Law-2016.pdf</a>   |
| <b>Country</b>   | Tanzania  |
| <b>Summary</b>   | <p>Overview of Tanzania's seed sector</p> <p>Tanzania Official Seed Certification Agency (TOSCA)</p> <p>Tanzania National Seed Company (Tanseed)</p> <p>Tanzania Association of Seed Traders (TASTA)</p> <p>Role of AGRA</p> <p>The National Seed Committee functions as an advisory body to the government and also provides the regulations for compulsory seed certification, laboratory seed testing, variety evaluation and registration under the Tanzania Official Certification Institute (TOSCI), which is a semi-autonomous institute, responsible for seed certification and quality seed control (The Legal Unit, Ministry of Agriculture, Food Security and Cooperatives (MAFSC), 2014).</p> |
| <b>Notes</b>     | No info on varietal release per se  |
| <b>Questions</b> | <p>Difference between TOSCI and TOSCA?</p> <p>Google search: Tanzania Official Seed Certification (TOSCI) is a government institute under the Ministry of Agriculture Food Security and Cooperatives (MAFC) established by the Seed Act No. 18, 2003. TOSCI is a result of transformation from Tanzania Official Seed Certification Agency (TOSCA) which was established by the Seed Act 1973. The transformation of TOSCI from TOSCA was a result of government reforms initiatives to increase efficiency of public institutions.</p>   |

## Summary

### IN GENERAL

It seems that in both countries: (1) new varieties need to be registered before they can be marketed; and (2) the registration process requires that tests for distinctiveness, uniformity and stability (DUS) and value for cultivation and use (VCU) be conducted.

### IN UGANDA

#### NATIONAL VARIETY PERFORMANCE TRIALS

Both VCU and DUS testing happen in National Variety Performance Trials (NVPT), by NARO breeders and the National Seed Certification Services (NSCS) respectively.

To apply for NVPT, Form SR 1 needs to be filled out and submitted to the NSCS, in the Department of Crop Protection, Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). This needs to be accompanied by evidence of high performance of the variety in yield trials. On farm trials may be undertaken simultaneously with the NVPT.

Testing for agronomic value (VCU) is responsibility of NARO breeders. VCU data need to be collected in National Variety Performance Trials (NVPT), for a minimum of three seasons across five locations:

- It is suitable for ..... agronomic zones
- Yields are .....mt/ha
- Requires .....mm of rainfall distributed over .....days
- It is resistant to .....
- Matures within ..... days
- (vi)The variety stores best under ..... conditions

The law states that the shall independently carry out NVPT for DUS testing, in accordance with the established standards/protocol. However, due to limited resources, NSCS uses the NARO NVPT trials to carry out DUS testing. This involves collecting data for a minimum of two seasons across two locations:

- Not clear from documents exactly what data are collected.

#### VARIETAL RELEASE

Once the VCU and DUS testing are complete, the NARO breeders prepare a variety release proposal (Form SR 2) that is submitted to the National Variety Release Committee (NVRC).

This proposal includes:

- Introduction
- Pedigree of the variety
- Description of the variety
- Site description
- Results and discussion
- Agronomic package
- Variety maintenance

The NVRC, upon receipt of the application, acknowledges it by filling Form SR 3.

The NVRC considers the applications and release of new varieties through meetings during which breeders and NSCS make presentations of the NVPT and DUS testing respectively\*. Any variety with superior



agricultural value is released by the NVRC and registered onto the National Variety List. The applicant is informed of the decision of the NVRC through a notification on Form SR 4.

*\*Note: The law states that on evaluation of the candidate varieties in the NVPT, NSCS will present its recommendations to the NVPT Technical Committee before the varieties are presented to the NVRC that approves the varieties for release. At the moment, the NVPT Technical Committee is not in place and after evaluation of candidate varieties, the breeders and NSCS present their reports to the NVRC in a variety release committee meeting.*

## IN TANZANIA

### VCU TESTING

This is the responsibility of the breeder, for a minimum of three seasons.

### NATIONAL VARIETY PERFORMANCE TRIALS

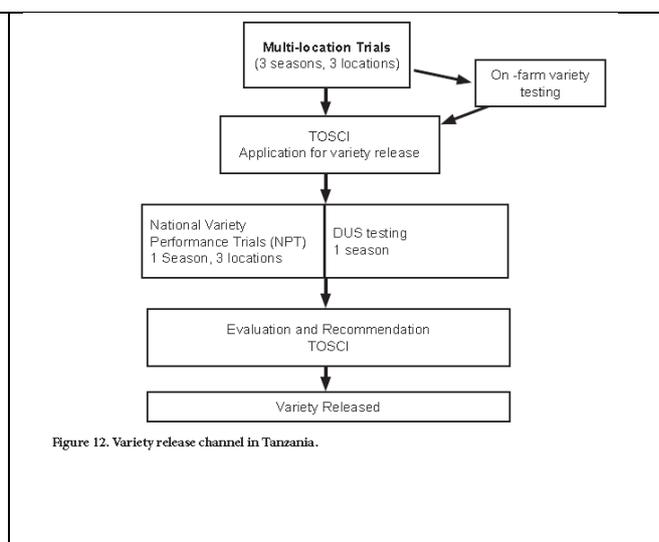
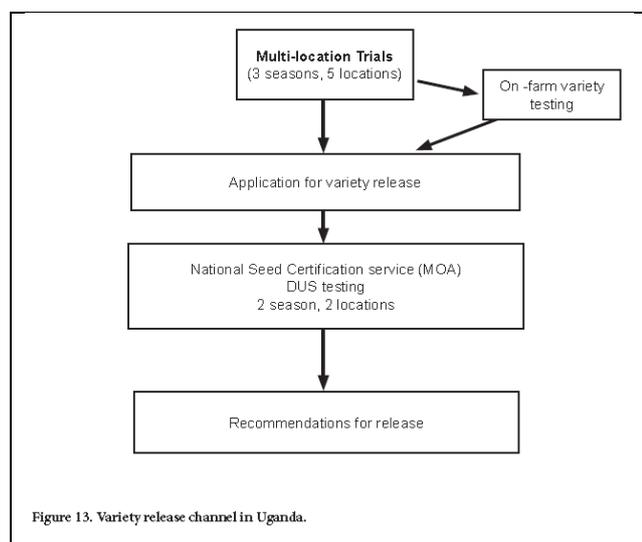
Once VCU testing (step above) is complete, the variety is submitted to the Tanzania Official Seed Certification Institute (TOSCI) for VCU and DUS tests. VCU and DUS tests are conducted by TOSCI in selected areas, depending on the recommended areas for the variety.

For VCU testing, data need to be collected for a minimum of 1 season across three locations. It's not clear from the documents what exactly the VCU tests entail.

For DUS testing, data need to be collected for a minimum of 1 season in one location. The DUS tests are described clearly in document 1.

### VARIETAL RELEASE

Once the test are complete, the Variety Release and Seed Certification Committee (VRSCC) evaluate the data in order to make recommendations for release.



## 5.3 Gendered Analysis of Seed Systems

### IN TARGET REGIONS FOR ADOPTION OF NEW BANANA CULTIVARS IN EAST AFRICA

#### BACKGROUND

The International Institute of Tropical Agriculture (IITA) and the National Agricultural Research Organisation, Uganda (NARO) have jointly developed 27 hybrid East African Highland Bananas (EAHB), called NARITAs, that have good tolerance/resistance to pests and diseases. As part of a Bill and Melinda Gates Foundation (BMGF) and Roots, Tubers and Bananas (RTB) funded project “Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa” running from 2014-2019, Bioversity International and partners are evaluating the NARITAs for their agronomic performance and consumer acceptance in a range of expected end-user environments and target markets in five regions in Uganda and Tanzania, using a Participatory Varietal Selection (PVS) methodology. The ultimate objective is to identify NARITAs that are well adapted to, and can be integrated into, existing EAHB farming systems.

In order to facilitate the successful integration of the selected NARITAs into the existing production systems in the target regions and increase their chances of wide-scale adoption, a better understanding of the seed systems is needed. Small-scale farmers obtain their seed<sup>7</sup> from various sources<sup>1</sup>, which are loosely grouped into what are called formal and informal seed systems, the latter also referred to as local, traditional or farmer seed systems. The *formal system* provides farmers with ‘modern’ varieties. It involves a chain of activities, usually – but not always – starting with plant breeding and ending with the official release of finished varieties. The *informal seed system* centers on local or farmer varieties, and includes most of the ways in which farmers themselves produce, disseminate, and procure seed: directly from their own harvest, through exchange among friends, neighbors and relatives, and through local seed markets or traders. Seed is produced, and often sorted, as an integral part of farmers’ production rather than as a discrete seed production enterprise. Because of its ability to meet local needs and preferences, the informal system provides most of the seed farmers use. Worldwide, this amounts to between 80% and 90% of seed stocks. There are however many flows between these two systems. For instance, new ‘modern’ varieties, though launched by the formal system, may move into informal channels quickly, and be disseminated farmer-to-farmer or even sold in local markets. Sometimes local varieties, or landraces, are brought into the formal system and then released officially.

#### OVERALL OBJECTIVE

Within the framework of the above-mentioned project, we aim to better understand the existing mechanisms and key actors for seed exchange in the target regions for adoption of the NARITAs, in order to identify opportunities and constraints for the introduction of new varieties in the banana production system, and the critical factors for success. At the start of the project, a gender-differentiated baseline study was conducted in the five project regions to characterize the target population environments in terms of agro-ecological and socio-economic conditions and existing production systems. The participatory rural appraisal (PRA) tools developed include an intra-household survey, seasonal and daily calendar exercises, a community wealth ranking exercise, and a banana trait preferences exercise (Annexes 1-5). The household survey included a range of questions on current banana varieties grown and their uses, varieties not grown anymore and the underlying reasons, the source of planting materials, and agricultural extension services. However, a more detailed assessment of the seed systems in the target regions is still needed. We seek to make a gendered assessment of the seed security situation in the target regions, in terms of availability of planting materials, access to planting materials and quality of planting materials (which includes both aspects of plant health and varietal attributes). More specifically, we want to understand where our target end users source their materials for new banana plantings, how they access information about quality planting materials of diverse varieties, and how they make decisions about which varieties and which materials to use for their new plantings. Emerging knowledge will be used to inform upcoming activities on the dissemination of selected NARITAs in the target regions.

<sup>7</sup> In the case of banana, ‘seed’ refers to the different types of vegetative planting materials.

## RESEARCH QUESTIONS

1. What are the considerations that farmers take into account when deciding which planting material to use:
  - a. Which varietal traits are important? What is e.g. the relative importance of agronomic traits and host resistance, consumption-related traits for fresh or processed foods, product development and economic value, cultural value, etc.
  - b. Which quality aspects do farmers consider? Are they aware of what clean planting material is? What are the motivations to use tissue culture or other types of planting materials?
  - c. What are farmers willing to pay for different cultivars and for different types of planting materials? How do farmers make decisions about cost/benefit trade-offs when choosing new planting materials?
  - d. How do these preferences and interests differ for different social groups/categories of farmers, taking into account sex, age, ethnicity, status, role in the banana value chain, etc?
2. Where/How do farmers source planting materials, and associated information?
  - a. Where do they source planting materials for local cultivars? Do they have access to clean planting material of local cultivars? What factors affect their choice for one source or another?
  - b. Where do they source planting materials for 'new' cultivars (and 'new' can refer to landraces from other locations (exotic) or improved cultivars)? Where do they access information about such new cultivars?
  - c. How do prices for different cultivars and for different types of planting materials differ in the target region?
  - d. How does access to different planting materials (including varietal and plant health aspects) differ for different social groups/categories of farmers?
3. Which formal and informal networks exist for seed exchange in the target regions, and how do they contribute to farmers' seed security?
  - a. Who are the main actors in the exchange of banana planting materials, and associated information? Who participates at what stage, and what role do they play? How are the key actors connected to each other?
  - b. Are different (formal and informal) networks working in parallel? What are the differences between them? Do they provide access to different types of planting materials and cultivars? Are interactions taking place between different networks?
  - c. What factors motivate different social groups/categories of farmers to participate in or use certain seed networks and not others? How does this affect the seed security of these different groups/categories?
  - d. What steps can be taken to improve the seed security of the most vulnerable social groups/categories of farmers? How can dissemination programs for new banana cultivars use this knowledge to ensure that new materials and associated information reach the target users?

Policy aspects? Link to varietal release process??

## PROPOSED ACTIVITIES / METHODS

### FOUR-SQUARE ANALYSIS

The four-square analysis is a participatory method that helps obtain greater detail on the crops grown, at the village and the farm level. The method classifies into four classes varieties identified in a given village based on the relative size of the area devoted to the variety (small or large) and on the relative number of households cultivating it (few or many)<sup>i</sup>.

A group of farmers brings a sample of each variety of the crop of interest they are growing. A large table is drawn to distinguish four categories or squares: varieties cultivated by many households on large areas; varieties cultivated by many households on small areas; varieties cultivated by few households on large areas, and varieties cultivated by few households on small areas (Fig. 1). For each sample, the group discusses the variety and decides where to place it in the four squares table.

|  |  |
|--|--|
| <p><b>Square I</b></p> <p><b>Large area</b></p> <p><b>Many households</b></p>  | <p><b>Square II</b></p> <p><b>Small area</b></p> <p><b>Many households</b></p> |
| <p><b>Square III</b></p> <p><b>Large area</b></p> <p><b>Few households</b></p> | <p><b>Square IV</b></p> <p><b>Small area</b></p> <p><b>Few households</b></p>  |

**Fig. 1. Four squares table**

After all the varieties have been placed in the four squares table, the group discusses the results and the different varieties by answering a number of questions, such as:

- What is the name of the variety?
- When was it first used?
- What is it used for?
- Positive traits of the variety?
- Negative traits of the variety?
- Where did you source the variety?
- How was the variety first obtained (initial source)?
- How much did you pay for the planting material?
- Why are some varieties only grown by a few households on large areas (Square III)?
- Why do many farmers maintain only small areas for certain varieties (Square II)?
- Why are some varieties grown maintained by only a few households on small areas (Square IV)?

The exercise would primarily contribute to answering the different sub-questions of RQ1, and some sub-questions of RQ2. The specific FGD questions could be adapted to complement (but not duplicate) the information already obtained through the household survey and FGD on banana trait preferences.

#### CHOICE EXPERIMENTS AND WILLINGNESS TO PAY

A choice experiment approach, adapted from consumer studies, will be used to understand farmers perceptions and motivation for preferring particular varieties and particular planting materials. People's willingness to pay for these varieties and planting materials will be investigated, by looking at their choices related to spending (fake) money on (fake) purchases of planting materials.

Possibility to also work on "games" to understand choices:

<http://www.tandfonline.com/doi/full/10.1080/15427528.2017.1303801>

The exercise will primarily contribute to answering RQ1c.

#### REPLACEMENT RATES AND SUCKER PLANTING

Through a survey, the number of suckers that farmers planted in the last year (in relation to the size of the farm, or number of banana trees) will be recorded. More information will be gathered about how often farmers replant their banana plants, for the different types and varieties. The survey will include questions on the reasons for

replacement of plants, the source of new materials, how decisions on which variety to replant with are taken, who takes these decisions, access to information about new varieties, etc.

The exercise will primarily contribute to answering the different sub-questions of RQ2.

### SEED SYSTEMS NETWORK ANALYSIS

Social network analysis (SNA) is the process of investigating social structures through the use of network and graph theories<sup>iii</sup>. It characterizes networked structures in terms of nodes (individual actors, people or things within the network) and the ties, edges or links (relationships or interactions) that connect them. We will use SNA to better understand the flows of banana planting materials in and between farming communities, and identify the critical nodes, i.e. the key players in the seed system that the project needs to work with to ensure the large-scale dissemination and adoption of the NARITAs in the project regions (and beyond), and the (lack of) interactions between these key players.

Different starting points could be used for the SNA:

- Farmers, to find out where they source their materials, and then snowball up through the network to identify the original sources of planting materials and varieties
- Breeding programs, to understand how they “push” their materials out, and then snowball down through the network to identify how new varieties reach (or don’t reach) the target farmers
- So-called “middle-men” in the seed system, such as (certified) seed multipliers, nurseries, NGOs, etc, to identify how they interact with other stakeholders in the seed system

The exercise will primarily contribute to answering the different sub-questions of RQ3.

### MEANS-END-CHAIN

Means – End – Chains theory, which is common in the field of consumer studies, will be adapted to study how for banana farmers’ motivations vary and affect the preference for particular sources, varieties and types of propagation materials.

The exercise will primarily contribute to answering the different sub-questions of RQ1.

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## 6. Work Package 5

### 6.1 Digital data capture in Banana: A system for tracking seed, monitoring progress and reporting results in Banana breeding programs

BTracT : Banana Tracking Tool

Trushar Shah, Margaret Karanja and Allan Brown: design and development of the tool

Rony Swennen: domain expertise on the Banana varieties.

Guillaume Bauchet, Nick Morales and Lukas Mueller: integration with Musabase.

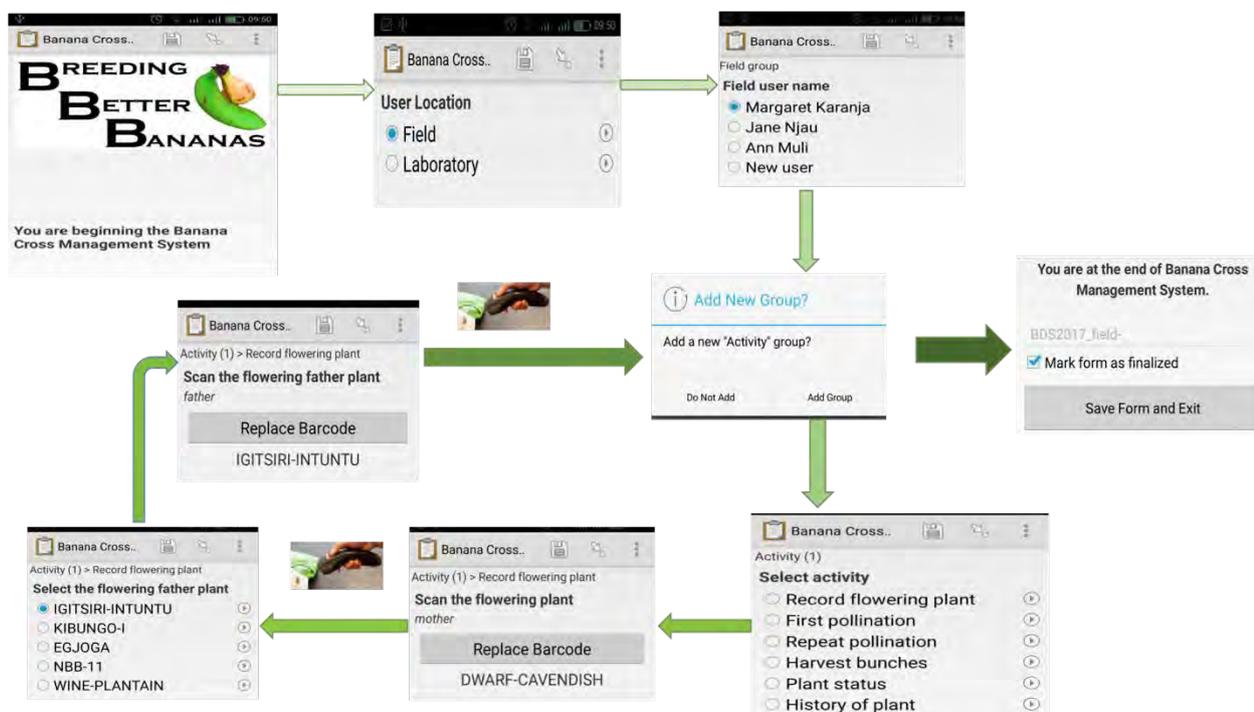
#### Introduction

Banana breeding programs face a number of technical challenges such as ploidy and sterility of banana cultivars, slow propagation, space requirements and the time required for breeding. To overcome some of the logistical and management constraints in this long-winded process, we have come up with a data management system that is complementary and fully integrated with Musabase. This system allows accurate, timely and efficient data collection, management, analysis and interpretation that are crucial at all stages of the crop improvement cycle in Banana. Such information is not only important in monitoring progress but also identifying bottlenecks, providing biological insight and in providing alerts for situations where immediate intervention is required eg: plant death or disease outbreaks.

The salient features of the system were envisioned by the Banana breeding team, whose aim was to use an on-line data management system that will see reduced to zero data collection errors in the field, laboratory and screenhouses while providing instant access to information at any given time and place. The design and development of the system involved gathering of user requirements, mapping all the activities from the field to the laboratory and back to the field. The Open Data Kit (ODK) framework was used to develop the handheld-device based tools that help to manage and integrate mobile data collection activities remotely.

#### Activity mapping

Banana pipeline is a large and complex process that uses an advanced form designed to capture all information regarding a banana plant in our field trials. The general idea in each step is to capture the plant/bunch/plantlet ID and the date of action. Once a cross is captured, it is followed throughout its life from pollination, harvesting, seed extraction, tissue culture and back to the open field as a plantlet. Figure 1 below illustrates the activities captured under the field based activities as displayed on the mobile application.



**Figure 1.** Screenshot of mobile application and workflow for handheld application

### Technical Methods Overview

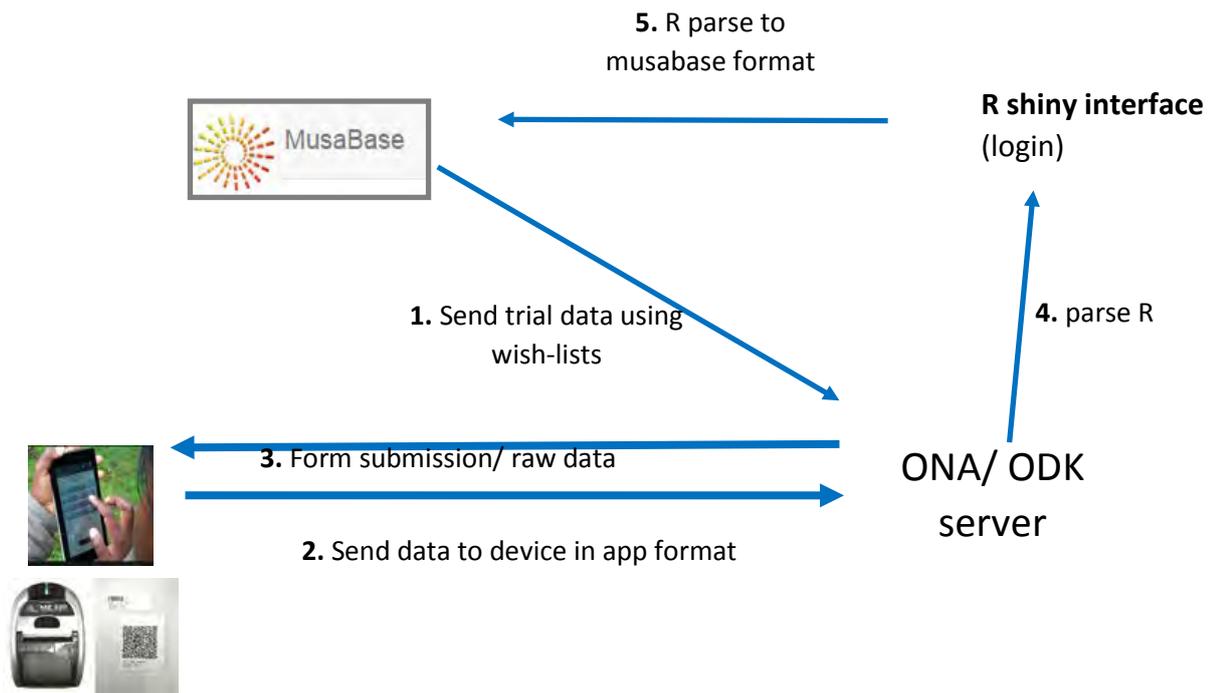
The digital data capture system is built on a case management process that is integrated with Musabase, a server platform (Ona) that simultaneously aggregates data from the various users of the system and R (a statistical package used for data manipulation). This is illustrated in Figure 2.

If a breeder is interested in making crosses, he/she starts by creating a cross wish list to be used in pollination, from MusaBase where he selects the female and their respective male parents. Once this list is generated, it is immediately sent to Ona platform (mobile aggregation platform) and to the specific form as a media file after which the information is availed to the users. The field layout information is also provided from Musabase to the mobile application.

When the data is collected through the mobile phone, it is submitted to Ona platform and aggregated with its time stamps and geo-points. Using a daily scheduler, the dataset is pulled from Ona to R using Ona.R package. In the R environment, these data are structured and organized into the required formats and then pushed back to Ona and to the specific form as well as back to Musabase. This whole process ensures efficient tracking where an 'identifier' will proceed to the next step only if it has passed the previous one. ODK functionality such as relevance, constraints if any, and pull-data functions have made this process easy in ensuring data quality control.

Reports are generated in R as email alerts and also available through an R shiny dashboard view (Figure 3). These reports are accessible to authenticated users at any time and place. From the dashboard one can filter the reports to know the number of crosses made at any given day, bunches in the ripening shed, how many are at a particular stage and so on. Data sets can be filtered and downloaded for their intended use.



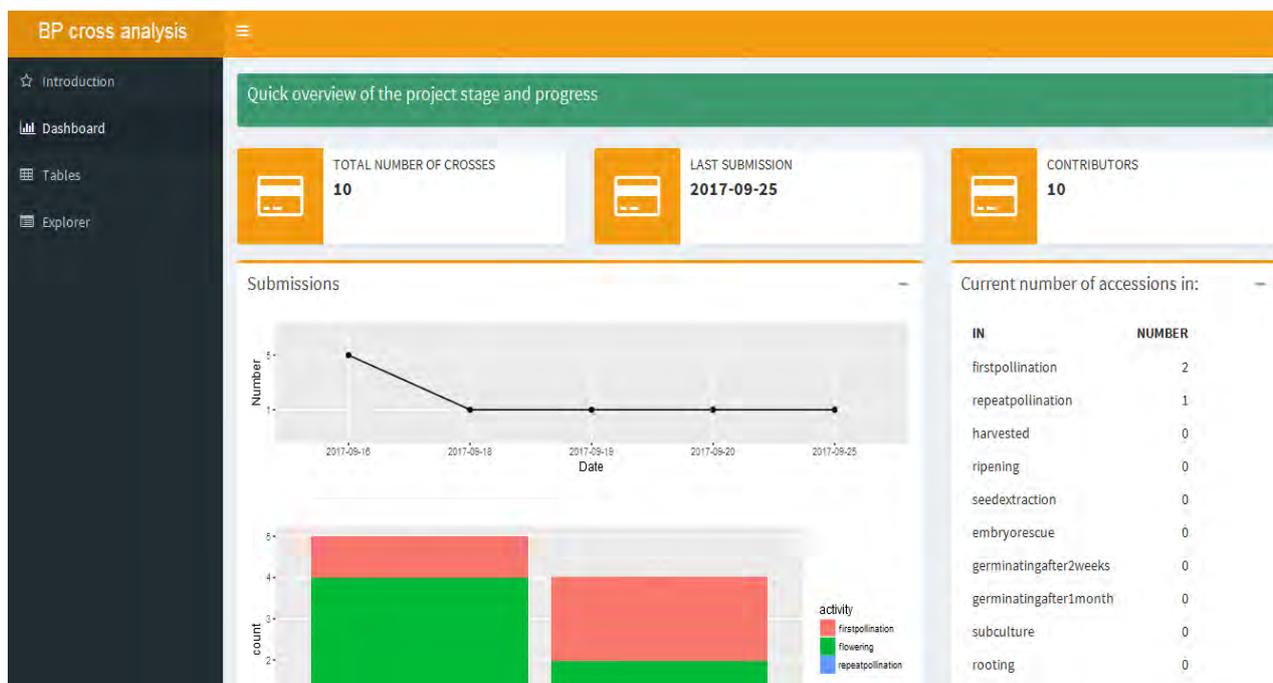


**Figure 2:** Technical overview of the system and data flow

During the project we have investigated different IT equipment and peripherals that are required. We have already identified recommendations for android handheld devices, mobile printers and barcode scanner.

The integration for Musabase has been done for obtaining data from Musabase (crossing wishlist and field layout) but is in progress for posting back to Musabase after the crossing and tissue culture workflows.

This system is now tested at the IITA banana breeding program in Arusha in October 2017 and we plan to have it operational by January 2018. Thereafter it will be transferred to the banana breeding activities of NARO and IITA, Uganda by March 2018.



**Figure 3.** R-Shiny dashboard for reports and real-time visualization

As the data in the Musabase has been accumulated from different sources mainly in Excel spreadsheets we have also tried to use fuzzy searches to identify duplications, misspelling and mislabeling of varieties in the field. We are streamlining the naming of varieties to that existing in the ProMusa database as well as records from the International Transit Centre (ITC). This has been a very involving data curation exercise, but is essential to bring harmonization and standardization across breeding locations.

### Future improvements

In future additional features such as alerts, 'travelling salesman' algorithm for efficient pollinations in the field and improved a customized reports for users will be made available.

## 6.2 MusaBase Training at BTI, Cornell University

1<sup>st</sup> - 30<sup>th</sup> August 2017

### Summary Report

*By Violet Akech, Ringo Sifuel and Henry Mwaka*

*Banana Breeding Data managers - IITA Uganda, IITA Arusha and NARO, Uganda*



From left to right: Ringo Sifuel (IITA-Arusha), Nick Morales, Guillaume Jean Bauchet (BTI), Violet Akech (IITA-Uganda), Lukas Mueller (Mueller Lab, BTI), Henry Mwaka (NARO-Uganda)

## Introduction

### Trainees

Violet Akech - IITA, Uganda  
 Ringo Sifuel - IITA, Arusha  
 Henry Shykins Mwaka – Banana breeding programme, NARO

We were nominated by the banana breeding programs of IITA and NARO to participate in the MusaBase training organized by project collaborators at the Boyce Thompson Institute in Cornell because of the role of data management that was allocated to us.

The Boyce Thompson Institute is an independent research institute devoted to using plant sciences to improve agriculture, protect the environment, and enhance human health. The institute is located in Cornell University, at 533 Tower Road, Ithaca, New York.

The training instructor was Guillaume Bauchet and the training was supervised by Lukas Mueller and supported by different personnel at BTI. The training period run from 1<sup>st</sup> to 31<sup>st</sup> August.

The main objectives of the course were set as follows:

- To learn more about the MusaBase Sol genomics Platform
- To understand the features available in MusaBase
- Practice curation and management of accessions (adding new accessions, editing, etc)
- Understand database formats compatible with MusaBase including trials, traits, field plans/layouts
- Learning how to prepare new and existing trials for uploading into MusaBase
- Understanding the relationship and interphase between field book and MusaBase
- Working with barcodes
- Learn how to set up crosses using the wish list feature

It was therefore expected that on completion of the course the new expertise gained would be extended to existing Banana breeding fields at IITA stations and NARO.

### Course Content

The course content as administered at BTI comprehensively covered the MusaBase platform. The objectives were exhaustively tackled using a hands-on practical approach. An opportunity was given to practice skills gained using a replica (test version) of the production site hosted at the url <https://musabase-test.sgn.cornell.edu/>. Practice areas included

- Creating, editing and merging lists such as accessions, traits, etc.
- Curation of traits, accessions
- Uploading of trials
- Adding trait files to existing traits
- working with barcodes and field book

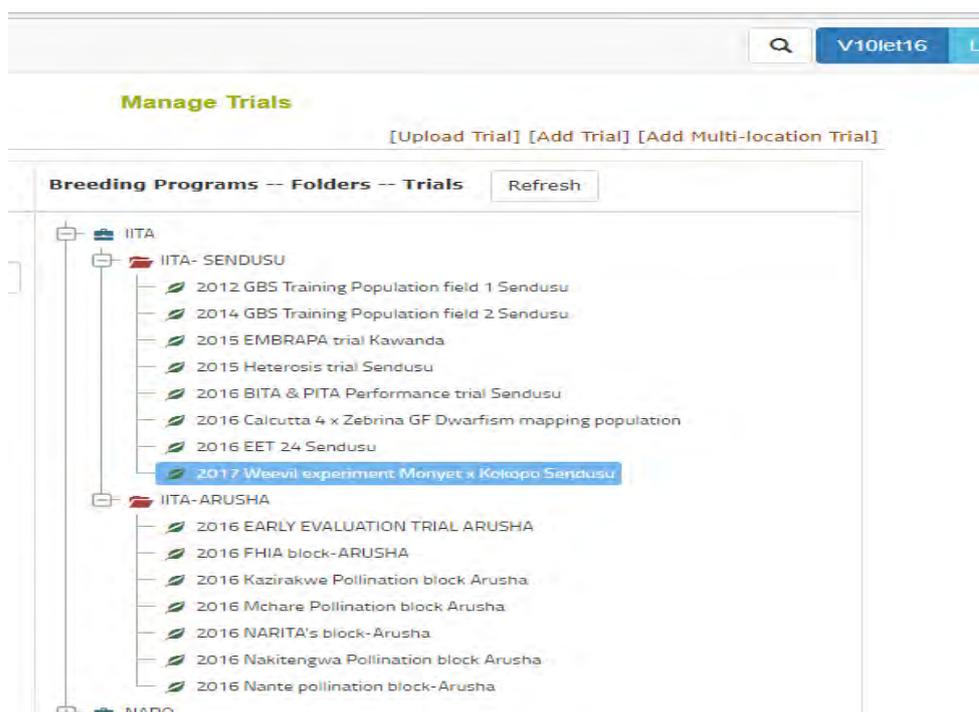
### Achievements

- We had an over-view of the features of MusaBase database with a hands-on complete training on use of the MusaBase with sufficient practice and building confidence with the test sites, an opportunity was given to work on the production site (musabase.org) with close supervision.
- We are well versed with the curation and management of accessions, which includes but is not limited to creating accession lists, add in new accessions and linking them to their different synonyms if they exist, creating and uploading the pedigree files. Collectively we updated the accession list to 5,037 accessions now stored in the data base. This can be viewed on <https://musabase.org/search/stocks>



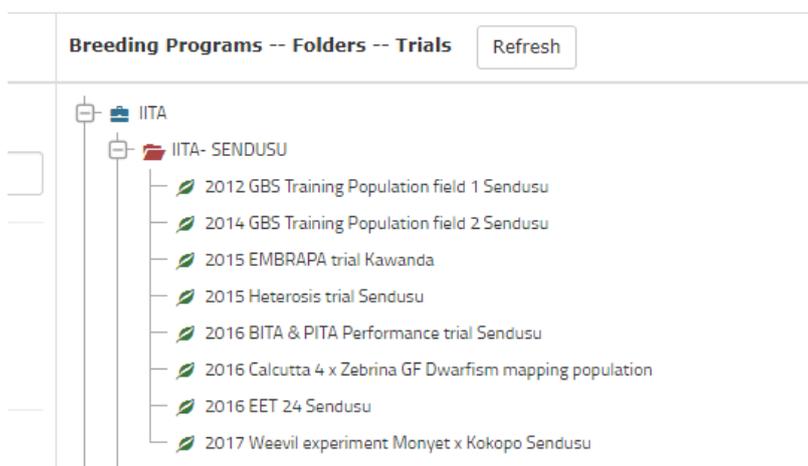
Figure 1 Updated accessions in Musabase as at 31.08.2017

- We developed uniform formats for naming trials (Year of establishment, Purpose of trial, location of trial), Plot unique id (Last two digit of the year of trial-Accession name\_Row, column, Plot No.)

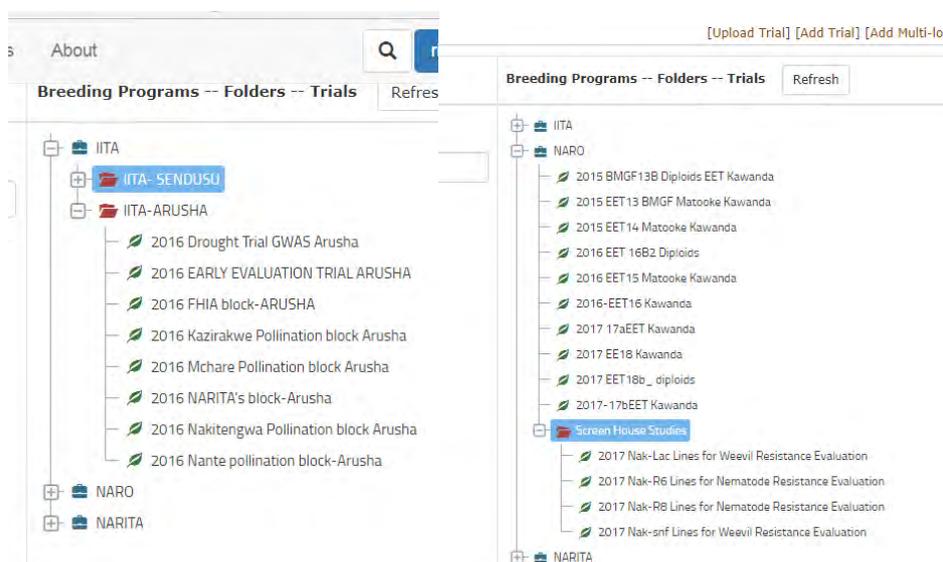


- Knowledgeable on field book integration with MusaBase which includes creation and download of field book templates for trials right from the database. This also ensures that the right trait codes and names are used to minimize errors when uploading phenotypic data files
  - Management of crosses using the Wish list feature, this will be helpful once we start using the IBP system to manage our breeding activities.
  - Ability to design future trials right from the data base (MusaBase)
  - Converted the already existing trials to the MusaBase format and successfully uploaded eight existing trials run at Sendusu listed below and as seen in the picture below and link below
    - ❖ 2012 GS Training Population
    - ❖ 2014 GS Training population
    - ❖ 2015 EMBRAPA trial at Kawanda
    - ❖ 2015 Heterosis trial at Sendusu
    - ❖ 2016 BITA & PITA performance trial at Sendusu
    - ❖ 2016 Calcutta x Zebrina GF Mapping population
    - ❖ 2016 EET 24 Sendusu
    - ❖ 2017 Weevil experiment on Monyet x Kokopo population
- <https://www.musabase.org/breeders/trials/>

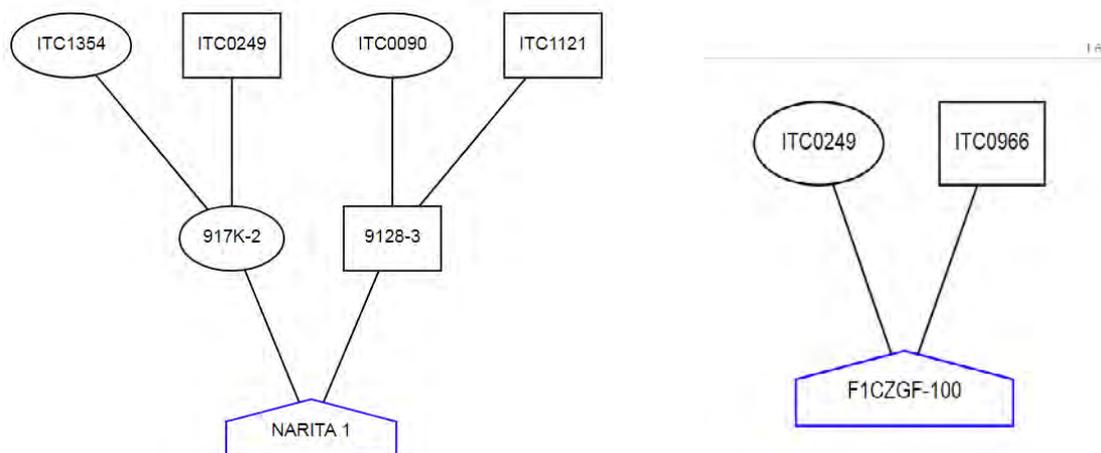
[Upload Trial] [Add Trial] [Add Multi-location Trial]



Eight trials were uploaded from IITA, Arusha and 14 trials at NARO, Kawanda as seen in the pictures below



- Uploading pedigree files; creating the pedigree files in the right database format using the data base spreadsheet format. This allows for more information about the accessions to be known to all database users.  
 The challenge is still that the pedigree flow chart displays the accessions using the ITC codes only for accessions that have ITC codes. Therefore it is important that we include both the ITC code (to be sure what is unique) PLUS the common name (so we have a good idea what the genotype is). This should be done whenever possible as this is not always possible because there are also genotypes without ITC code.



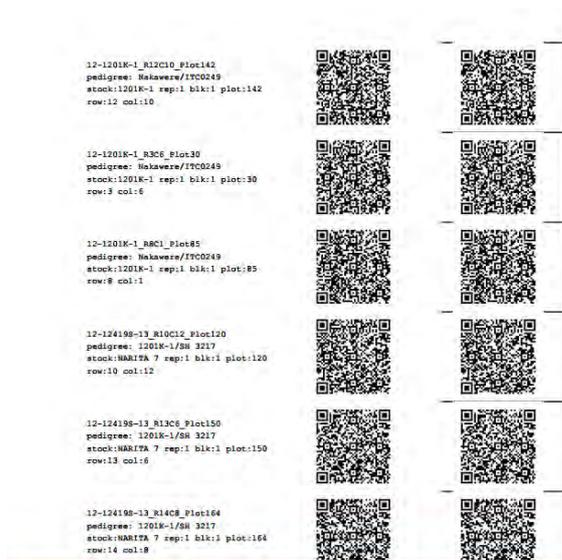
In the example above, ITC0249 is Calcutta 4 and ITC0966 is Zebrina GF, it would be ideal to have them displayed as ITC 0249 Calcutta 4 and ITC 0966 Zebrina GF. We have pointed this out to Guillaume and the team and they are looking into its possibility.

- Updated ontology: We were able to removed duplicate traits (same code for different traits), duplicate codes (different code for same traits), added missing traits and defined them their observation scales.
- Bar coding

A good example for improvement of quality control is in the field where plots/plants are identified with barcodes. The barcodes developed provide compatibility for identification of a given plant using the field book application and accurate data capture eliminating the hazards associated with mix ups caused by human error. A Musa printing format was developed by the BTI team for different use case scenarios which included the following;

- Laboratory (petri dishes, falcon tubes & other small vessels )
- Labelling of pots & potting bags (in screen house/ green house )
- Field Labelling (plots, plants, bunches, seedlots/seed bags, sample bags)

We are now able to generate 2D bar codes for trials as seen below for the training population at Sendusu. We agreed that where possible the bar code for the fields should contain accession name, pedigree, plot coordinates-row and column numbers and location of trial (please zoom out the picture to see more details clearly). A bar code format named Musa format (<https://www.musabase.org/breeders/trial/340?format=>) was created by Alex Ogbonna with standard settings that generated the barcodes below. A new format was created for the sample printing paper purchased (32 labels per A4 page). However on trying the paper out it was noted that the barcodes are too small for effective scanning from a distance. Feed back has been given and a new format together with new sample printing paper of 20 labels per A4 page is being worked on. He will carry sample papers on follow up visits are scheduled in November 2017.



- We agreed on a 1D bar code for the seed lots, laboratory tubes, humidity chamber containers and nursery house plants. This was due to the size of the containers and tubes that would hold the barcode label.

## Way forward

- Not all genotypes have ITC codes. As a feedback from us users, Guillaume should make room for other names, or even pollination codes to be accommodated. This will help in situations where accessions/hybrids have been allocated new easier codes in place of their pollination codes as is the case of the mapping populations at Sendusu. This fix will also cater for the easy identification of pedigrees.
- There is need to feed the common names with pictures of the accessions. For example we have a *Pisang sipulu*, and now with the pictures available, Rony can definitely state that this is NOT a *Pisang sipulu*. So pictures are also needed to be sure of what we are dealing with. Also for example Zebrina GF is NOT a true *Musa acuminata* ssp. *zebrina*. Again pictures can show that. The correct identification is key for breeding for taste. We have now at IITA large files with minimal descriptors with the standard pictures of most landraces in Arusha, Sendusu and Ibadan. They are ready to be fed into MusaBase once MusaBase agrees on the right format of uploading such large files by the BTI team.
- The ontology on the live site was still undergoing an upgrade. Guillaume will notify us once it is up and running and then we can fully utilize the data base to create trait files for the different running trials and to upload phenotypic data.
- We plan to have trial entries created for all existent trials and upload phenotypic data for trials that have data already available by end of this year.
- Henry ( NARO) and Violet (IITA) have planned to generate bar codes for at least one trial each at Sendusu and Kawanda to test plot/plant level barcodes labels using the sample print paper we brought with us. This will enable us to give feedback to the BTI team about the efficiency of the current Musa bar code format, design and size of the labels by the end of October. Alex Ogbonna from BTI will be visiting the three sites in November to check on the progress of bar code use roll- out.
- We will keep generating trial templates for field book from MusaBase using the data base trait codes and continue to collect data using field book on the tablets as well as continue conforming the remaining existing trials to the MusaBase format and uploading them to the data base.
- There is a need to understand the integration of MusaBase interphase with the IBP interphase. We need to understand where each of them starts and stops to avoid overlap. We hope that Margaret and Trushar can visit Sendusu when the team from BTI comes over to check the progress of the barcode labeling.
- With formatting to MusaBase format and uploading data, the training was mainly on dealing with phenotypic data. There is need to learn on how to get the genomic/genetic data on the database. This



discussion and dialogue has already been opened up with Brigitte after Lukas, Guillaume and Violet had a brief meeting about the issue.

- We did not learn data analysis using the data base. We would very much benefit from this if we get further training on this and the use of the “ANALYZE” data base tool to do create a selection index, especially for the EET and PYT selection exercise.

### **Acknowledgement**

We are very grateful to our supervisors Dr. Brigitte Uwimana, Dr. Allan Brown, Dr. Robooni Tumuhimbise and Prof. Rony Swennen for nominating and letting us get this training. Many thanks to Guillaume for being an enthusiastic and patient instructor, taking our endless questions and going over things again and again for us to grasp every detail of the data base operations. Our appreciation goes to Dr. Lukas Mueller of the Boyce Thompson Institute for supporting the training in terms of human resource of the trainers, funding our travel and stay at BTI, Cornell University. Thank you to the entire BTI team especially the Mueller Lab team that made sure we have a pleasant stay and training at Cornell. We are confident that the skills acquired will help make the banana breeding work easier and efficient for all parties involved.

## 7. Student Progress

### 7.1 PhD Research Progress Report (2016-2017)

**Name:** Ivan Kabiita Arinaitwe

**Title:** Genetic Analysis of Resistance Against *Fusarium oxysporum* f. sp. *cabense* (Foc) in Selected Banana Populations Using Molecular Markers and Linkage Mapping Approaches

**Supervisors:** Rofina Yasmin Othman  
Jennifer Ann Harikrishna  
Chee How Teo  
Fatimah Kayat  
Robooni Tumuhimbise

**Timeline:** 2016-2020

**University:** University of Malaya

#### Research Objectives

List the individual topics of study – objectives or study areas

1. To identify sources of genetic variability to *Foc* race 1 and race 4 in diploid bananas for use in genetic studies,
2. Develop and phenotype at least two unrelated diploid mapping populations for *Foc* race 1 and race 4 resistances,
3. Assess the genetics of *Foc* race 1 and race 4 resistance in diploid bananas,
4. Evaluate different molecular markers (SNP, IRAP, REMAP, SSR and ISSR) for *Foc* race 1 and race 4, and
5. Develop two SNP-based linkage maps for *Foc* race 1 and race 4 indicating the location of *Foc* resistance QTL

#### Achievements

Highlight significant achievements – e.g. in bullets

1. Sources of genetic variability to *Foc* race 1 already identified; Calcutta 4 and Monyet are resistant whereas Mshale and Kokopo are susceptible to *Foc* race 1
  - Parents for *Foc* race 4 are under rescreening to identify contrasting parents for use to generate population
2. Two unrelated diploid mapping populations for *Foc* race 1 resistances developed. One is phenotyped (60%) and another is ready to be weaned in the nursery for planting
3. Different molecular markers IRAP, SSR and ISSR evaluated on the parents contrasting for *Foc* race 1. Markers that are showing polymorphism with contrasting for *Foc* race 1 parents have been identified.

#### Background/introduction

## Brief background

Bananas and plantains (*Musa* spp.) are a major staple food for many millions of people in the tropics and subtropics. In Uganda, 13 million people with 66% of the country's urban population depend on the crop for food. However, banana production is constrained by low soil fertility, high perishability, pests and diseases. Among the key diseases is Fusarium wilt. Fusarium wilt is a destructive fungal disease of banana and plantain, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Fusarium wilt is a soil-borne disease, reproduced by spores, survives in the soil for decades and has four races that are separated based on host susceptibility. It causes an annual yield loss of 60 to 90% in many countries (Bhuvanendra et al., 2010).

Efforts to manage banana Fusarium wilt using biological, chemical and cultural control measures have not been effective. Long-term survival of Foc in soil and ability to evolve into variants that can affect different varieties has made control very difficult. Host plant resistance seems to be the best alternative to control Fusarium wilt: durable, environmentally friendly, cheap for the poor resource farmers.

Diploid banana segregating populations can enable the study of inheritance and understand the resistance mechanisms of Foc race 1 and 4. Also, to shorten the banana breeding cycle, there is a need to apply Markers/ (MAS, MAB) in banana improvement. Markers/ (MAS, MAB) increase the effectiveness in breeding and significantly shorten the selection time of plants, which is useful additional tool in plant breeding (Bueren et al., 2010).

### Objective / Study 1. Identification of sources of genetic variability to Foc race 1 and race 4 in diploid bananas for use in genetic studies

Several diploids available at the banana breeding programmes of both NARO-Uganda and IITA, Sendusu-Uganda were screened for Foc race 1 resistance. Whereas, open pollinated *malaccensis* banana diploids are under screening for Foc race 4 at University of Malaya. This is to identify parent diploids contrasting for Foc race 1 and race 4 for use in generating segregating diploid populations.

Table1. Banana diploids screened for resistance to Fusarium wilt.

|             | Diploid parents (Race 1) | F2 diploid banana plants (Race 1) | OP- <i>malaccensis</i> (Race 4)                               |
|-------------|--------------------------|-----------------------------------|---|
| 1           | TMB2X614-1               | 123 F2 diploid banana plants      | 45 plants from an open pollinated bunch of <i>malaccensis</i> |
| 2           | Pahang                   |                                   |   |
| 3           | Kokopo                   |                                   |   |
| 4           | Long tavoy               |                                   |   |
| 5           | Calcuta 4                |                                   |   |
| 6           | Zebrina                  |                                   |   |
| 7           | Kasaska                  |                                   |   |
| 8           | Borneo                   |                                   |   |
| 9           | PisangLilin              |                                   |   |
| 10          | Monyet                   |                                   |   |
| 11          | Mwitu Pemba              |                                   |   |
| Resistant   | TMB2X8075                | Mpologoma                         |   |
| Susceptible | Mshale                   | Kayinja                           |   |

## Screening procedure

Three months old TC plantlets were screened for Foc resistance in a pot experiment using colonized millet grain inoculum. Yellowing was scored every after 2 weeks to determine Leaf symptom index (LSI). Two months after inoculation, the plants were uprooted and assessed for corm discolouration index (RDI). Experimental design was Randomised Complete Block Design (RCBD) and Data was analysed using GenStat 14th edition.

Table 2. Scale for scoring different parameters for Fusarium disease resistance

Table 3. Interpretation of LSI and RDI (Muhammed et al., 1999), DSI (Sutanto et al., 2011).

| Scale LSI |  | wilting                 | stem splitting  | RDI                                     |
|-----------|--|-------------------------|-----------------|---|
| 1         | No yellowing                             | No wilting              | No cracking     | No discoloration                        |
| 2         | Slight yellowing of the leaves           | Slight wilting          | Slight cracking | Discoloration at root and corm junction |
| 3         | Yellowing of the lower leaves (Advanced) | Advanced (50%)          | Advanced        | Discoloration of 5% stellar region      |
| 4         | Yellowing of all the leaves (extensive)  | Extensive (90%)         |                 | 6-20% stellar region discoloration      |
| 5         | Entire foliage is brown (dead plant)     | Entire foliage is brown |                 | 21-50% discoloration                    |
| 6         |  |                         |                 | More than 50% discoloration             |

| DSI (RDI) | DSI (LSI) | Translation        |
|-----------|-----------|--------------------|
| 1         | 1         | Resistant          |
| 1.1-3     | 1.1-2     | Partial resistance |
| 3.1-5     | 2.1-3     | Susceptible        |
| 5.1-6     | 3.1-4     | Highly Susceptible |

## Results

Table 4. Analysis of variance for LSI and RDI for screened banana diploids

| Source of variation | Diploid parents |         |         | F2 diploid banana plants |        |        | OP-malaccensis |        |                   |
|---------------------|-----------------|---------|---------|--------------------------|--------|--------|----------------|--------|-------------------|
|                     | df              | RDI     | LSI     | d.f                      | RDI    | LSI    | d.f            | RDI    | LSI               |
| Total               | 77              | 2.7     | 0.6     | 530                      | 4.2    | 0.4    | 77             | 2.6    | 0.3               |
| Rep                 | 5               | 2.9     | 0.2     | 4                        | 3.4    | 1.1    | 1              | 19.8   | 0.3               |
| Genotype            | 12              | 7.73*** | 2.13*** | 124                      | 9.3*** | 0.9*** | 44             | 3.8*** | 0.3 <sup>ns</sup> |
| Residual            | 60              | 1.6     | 0.4     | 402                      | 3.1    | 0.3    | 32             | 1.3    | 0.2               |

\*\*\* P>0.001, ns= non-significant

Table 5. Categorisation of the genotypes within germplasm using DSI (RDI)

**Conclusion**

| Diploid parents    |                          |  |                 | F2 diploid banana plants |   |   |  | OP-malaccensis     |   |    |   |    |
|--------------------|--------------------------|--|-----------------|--------------------------|---|---|--|--------------------|---|----|---|----|
|                    | R                        | PR   | S               | HS                       | R   | PR  | S  | HS                 | R | PR | S | HS |
|                    | Long<br>tavoy            | TMB2X614-<br>1, Mwituu<br>Pemba,<br>Monyet,<br>Pisang<br>Lilin,<br>Borneo,<br>Kasaska,<br>Zebrina,<br>Pahang | Kokopo<br>(3.5) |                          | 55,<br>62,<br>80,<br>82,<br>120,<br>109,<br>234 | 2, 3, 4, 7,<br>8, 11, 13,<br>14, 16, 17,<br>19, 25, 26<br>, 30, 35,<br>37, 39, 41,<br>42, 49, 51,<br>52, 54, 59,<br>61, 63, 64,<br>65, 67, 69,<br>74, 77, 79,<br>81, 83, 84,<br>85, 87, 91,<br>94, 96,<br>110, 113,<br>117, 120,<br>128, 131,<br>132,<br>135, 137,<br>138, 141,<br>142, 143,<br>144, 146,<br>151, 153,<br>159, 160,<br>161, 165,<br>171, 174,<br>178, 184,<br>196, 204,<br>205, 215,<br>216, 217,<br>218, 219,<br>221, 222,<br>227, 229 | 1, 5, 10,<br>15, 18,<br>20, 33,<br>38, 43,<br>51, 56,<br>66, 90,<br>102,<br>112,<br>114,<br>121,<br>125,<br>134<br>135,<br>139,<br>143, 169<br>179,<br>205,<br>211,<br>223,<br>230 | 68,<br>162,<br>164 |   |    |   |    |
| <b>TOTAL</b>       | 1                        | 9  | 1               |                          | 7   | 81  | 32   | 3                  |   |    |   |    |
| <b>Resistant</b>   | <b>TMB2x8075 (DSI=1)</b> |  |                 |                          | <b>Mpologoma (DSI=1.2)</b>                      |   |  |                    |   |    |   |    |
| <b>Susceptible</b> | <b>Mshale (DSI=4.2)</b>  |  |                 |                          | <b>Kayinja (DSI=5.4)</b>                        |   |  |                    |   |    |   |    |

- Germplasm screened showed variability to Foc race 1 and 4 and grouping into resistant and susceptible.
- More parents are being screened in the on-going experiments
- Identified contrasting diploid banana parents can be used for crossing to generate a Foc segregating population for studying genetics of resistance to Foc race 1 and 4 and identifying markers for Foc race 1 and 4 resistance and Linkage map construction and Identifying QTL for Foc race 1 and 4.

## Objective / Study 2. Development and phenotyping of at least two unrelated diploid mapping populations for Foc race 1 and race 4 resistance

Developing two populations for Foc race 1.

1. A resistant Monyet was crossed with a susceptible Kokopo banana plant to generate an F<sub>1</sub> population of 180 genotypes.

The 133 F<sub>1</sub> genotypes were screened with Foc race 1 in a pot experiment as described in objective one.

### Results

Table 6. DSI screening results for Kokopo X Monyet F<sub>1</sub> population (133/200)

|                              | Resistant | Partially resistant | Susceptible | Highly susceptible |
|------------------------------|-----------|---------------------|-------------|--------------------|
| <b>Rhizome Discoloration</b> | 42        | 60                  | 19          | 12                 |
| <b>Leaf severity index</b>   | 3         | 87                  | 35          | 8                  |
| <b>Stem Splitting</b>        | 114       | 15                  | 4           |                    |

Table 7. Summary of screening

|                           |            |
|---------------------------|------------|
| <b>Genotypes screened</b> | <b>133</b> |
| To be Screened            | 85         |
| To be rescreened          | 70         |

Batch one with 70 genotypes is to be rescreened as advised.

2. A resistant Calcutta 4 was crossed with a susceptible Mshale banana plant to generate an F<sub>1</sub> population of 135 genotypes.

The population is on rooting media in tissue culture laboratory and to be weaned in October 2017.

### Conclusion / next steps

The table 8 summarises the plan for screening remaining Kokopo X Monyet F<sub>1</sub> hybrids, Calcutta 4 x Mshale F<sub>1</sub> hybrids and screening open pollinated malaccensis plants to identify parents varying for Foc race 4.

Table 8. Timelines for screening the remaining genotypes and populations

| <b>Kokopo x Monyet</b>       | <b>Copies to be initiated ASAP</b> | <b>Copies in TC</b> | <b>Rooting</b> | <b>Weaning</b>                                       | <b>Planting</b>                      | <b>Screening</b>   | <b>Termination</b>                                   |
|------------------------------|------------------------------------|---------------------|----------------|--|--------------------------------------|--|--|
|                              | Approx. 70                         | 70 genotypes        | 70 genotypes   | 70 genotypes with 7 copies weaned in Sept 2017       | No planting in field                 | 70 Genotypes to be inoculated in November (6 copies per genotypes) | 70 genotypes (Feb-18)<br>70 genotypes (June 2018)    |
|                              |                                    |                     |                |  |                                      | 50 genotypes in 6 reps under screening                             | October 2017   |
| <b>Mshale x Calcutta 4</b>   |                                    | <b>Copies in TC</b> | <b>Rooting</b> | <b>Weaning</b>                                       | <b>Planting</b>                      | <b>Screening</b>   | <b>Termination</b>                                   |
|                              |                                    | 135 genotypes       | 135 genotypes  | 135 genotypes with all copies weaned in October 2017 | 135 genotypes to be planted in field | 50 genotypes to be inoculated in November 2017.                    | 80 genotypes (Feb-2018)<br>Remaining (December 2018) |
| <b>OP-<i>malaccensis</i></b> |                                    |                     |                |  |                                      | Parents at UM for screening with Foc 4                             |  |

### Objective / Study 3. Assessing the genetics of Foc race 1 and race 4 resistance in diploid bananas

This objective depends on objective two. For a population that will be segregating for Fusarium, the data generated will be used to assess the genetics of resistance as below:

i. Nature of inheritance

Nature of inheritance will be determined using frequency histograms.

ii. Determining broad sense heritability (H)

$$H = VG/VP$$

iii. Genetic ratios

Using Chi-square test of goodness of fit to determine number of genes involved in each trait

**Objective / Study 4. To evaluate different molecular markers (SNP, IRAP, REMAP, SSR and ISSR) for Foc race 1 and race 4**

- DNA were extracted from Cigar leaves of *Foc* segregating populations + parents (Min CTAB)
  - DNA qualification by electrophoresis using a 1% agarose gel
  - DNA quantification using Nanodrop
- PCR was run for parent DNA (Monyet + Kokopo and Calcutta 4 + Mshale) against **3 IRAP and 40 ISSR** markers to identify markers showing polymorphism for the contrasting parents.
- Gradient PCR was run for **37 SSR** markers against parent DNA (Monyet + Kokopo and Calcutta 4 + Mshale) to determine the best annealing temperature at which the primers amplify the DNA. Then the primers that showed polymorphism at those temperatures were selected.
- Data was analysed by scoring presence or absence of bands

**Results**

**1. Monyet and Kokopo**

Table 9. IRAP and ISSR markers that showed polymorphism between the contrasting parents of Monyet and Kokopo

| Category | Primer Name | Annealing Temps. | Sequence                        |
|----------|-------------|------------------|---------------------------------|
| IRAP     | 1 GyLTRev   | 62°C             | 5'CTTAGGCAAACCAGCTAAGTCCG<br>3' |
|          |             |                  |                                 |
| ISSR     | 1 CTC6T     | 50°C             | 5'CTCCTC CTCCTC CTCCTCT3'       |
|          | 2 AC10T     |                  | 5'ACACACAC ACACACACACACT3'      |
|          | 3 CA10G     |                  | 5'CACA CACA CACACACA CACAG3'    |
|          | 4 AC10G     |                  | 5'ACAC ACAC ACACACAC ACACG3'    |
|          | 5 CTC6G     |                  | 5'CTCCTC CTCCTC CTCCTCG3'       |
|          | 6 TG10G     |                  | 5'TGTG TGTG TGTGTGTG TGTGG3'    |
|          | 7 GTG6T     |                  | 5'GTGGTG GTGGTG GTGGTGT3'       |
|          | 8 TC10A     |                  | 5'TCTC TCTC TCTCTCTC TCTCA'     |
|          | 9 GTG6A     |                  | 5'GTGGTG GTGGTG GTGGTGA3'       |
|          | 10 CAC6T    |                  | 5'CACCAC CACCAC CACCACA3'       |



Table 10. SSR markers that showed polymorphism between the contrasting parents of Monyet and Kokopo

| Category |    | Primer Name |         | Sequence                       | Annealing Temp |
|----------|----|-------------|---------|--------------------------------|----------------|
| SSR      | 1  | AGMI189     | Forward | 5'AACACCGTACAGGGAGTCAC3'       | 49.9           |
|          |    | AGMI190     | Reverse | 5'GTGAGATAAACAATTACTAGGG3'     |                |
|          | 2  | AGMI129     | Forward | 5'GGAGGCCCAACATAGGAAGAGGAAT3'  | 54             |
|          |    | AGMI130     | Reverse | 5'CACAACCACACACAGCCAATCTTTC3'  |                |
|          | 3  | AGMI197     | Forward | 5'CTTTTGGAGATTATTGCCTACA3'     | 55             |
|          |    | AGMI198     | Reverse | 5'AGTAATCTTTTGTCTTCAGCT3'      |                |
|          | 4  | AGMI199     | Forward | 5'TATCCATCGACGTGATCCC3'        | 55             |
|          |    | AGMI200     | Reverse | 5'TACGATATTGGAATCTCCG3'        |                |
|          | 5  | AGMI127     | Forward | 5'AAGTTAGGTCAAGATAGTGGGATTT3'  | 55             |
|          |    | AGMI128     | Reverse | 5'GTCCCTCGATTGGTTCCAAGC3'      |                |
|          | 6  | AGMI187     | Forward | 5'GCAACTTTGGCAGCATTTT3'        | 55             |
|          |    | AGMI188     | Reverse | 5'TGAGATATAGAGGAAAATAATGTTA3'  |                |
|          | 7  | AGMI131     | Forward | 5'ATCTTTTCTTATCCTTCTAACG3'     | 55             |
|          |    | AGMI132     | Reverse | 5'CGCTTTAGATTCTGTTTAAG3'       |                |
|          | 8  | AGMI145     | Forward | 5'AGCTATTACTTGTTTTTATCTTGAA3'  | 55             |
|          |    | AGMI146     | Reverse | 5'AAGGACANAAAAGACAGGA3'        |                |
|          | 9  | AGMI139     | Forward | 5'GGGGAACAGCACGGTCACAT3'       | 55             |
|          |    | AGMI140     | Reverse | 5'ACGATGACAACCATTACTAC3'       |                |
|          | 10 | AGMI141     | Forward | 5'TACAAAGAGAAAAGTGCAGGGGAATA3' | 55             |
|          |    | AGMI142     | Reverse | 5'CNGCTATAAAGACCACCAGCTTCAT3'  |                |
|          | 11 | AGMI137     | Forward | 5'CTTCCTTCTGTCTTTTGGATTGTA3'   | 56             |
|          |    | AGMI138     | Reverse | 5'GCAAGTCCTTCTGAATCTTAT3'      |                |
|          | 12 | AGMI159     | Forward | 5'GTTTGGTTGATCCTCCCTTTA3'      | 56             |
|          |    | AGMI160     | Reverse | 5'GAAAACAAGAGAGAGAGAGAGAG3'    |                |
|          | 13 | AGMI203     | Forward | 5'TGCTGCCTTCATCGCTACTA3'       | 56             |
|          |    | AGMI204     | Reverse | 5'GGAACATCGCCCCGCCAC3'         |                |
|          | 15 | AGMI147     | Forward | 5'CTGCAGCAACCCAAATTTATTTTC3'   | 56             |
|          |    | AGMI148     | Reverse | 5'AAATAAGCTCATATGGGTACAGTCA3'  |                |
|          | 16 | AGMI143     | Forward | 5'TCAAGAGCAATGAAGACCTCAA3'     | 56             |
|          |    | AGMI144     | Reverse | 5'TTTTACATGTACAAGGTCAAGCAAT3'  |                |

## 2. Calcutta 4 and Mshale

Table 11. IRAP and ISSR markers that showed polymorphism between the contrasting parents of Calcutta 4 and Mshale

| Category | Primer Name | Annealing Temps. | Sequence                        |
|----------|-------------|------------------|---------------------------------|
| IRAP     | 1 GyLTRev   | 62°C             | 5'CTTAGGCAAACCAGCTAAGTCCG<br>3' |
|          |             |                  |                                 |
| ISSR     | 1 CTC6T     | 50°C             | 5'CTCCTC CTCCTC CTCCTCT3'       |
|          | 2 AC10T     |                  | 5'ACACACAC ACACACACACACT3'      |
|          | 3 AC10G     |                  | 5'ACAC ACAC ACACACAC ACACG3'    |
|          | 4 CTC6G     |                  | 5'CTCCTC CTCCTC CTCCTCG3'       |
|          | 5 TC10A     |                  | 5'TCTC TCTC TCTCTCTC TCTCA'     |
|          | 6 GTG6A     |                  | 5'GTGGTG GTGGTG GTGGTGA3'       |
|          | 7 CAC6T     |                  | 5'CACCAC CACCAC CACCACA3'       |
|          | 8 CT10G     |                  | 5'CTCT CTCT CTCTCTCT CTCTG3'    |
|          | 9 TCG6G     |                  | 5'TCGTCG TCGTCG TCGTCGG3'       |
|          | 10 TCG6A    |                  | 5'TCGTCG TCGTCG TCGTCGA3'       |
|          | 11 CTC6A    |                  | 5'CTCCTC CTCCTC CTCCTCA3'       |
|          | 12 ACC6T    |                  | 5'ACCACC ACCACC ACCACCT3'       |
|          | 13 AC10C    |                  | 5'ACAC ACAC ACACACAC ACACC3'    |
|          | 14 ACC6G    |                  | 5'ACCACC ACCACC ACCACCG3'       |

Table 12. SSR markers that showed polymorphism between the contrasting parents of Calcutta 4 and Mshale

| Category | Primer Name | Sequence        | Annealing Temp |                                |      |
|----------|-------------|-----------------|----------------|--------------------------------|------|
| SSR      | 1           | AGMI189         | Forward        | 5'AACACCGTACAGGGAGTCAC3'       | 47   |
|          |             | AGMI190         | Reverse        | 5'GTGAGATAAACAATTACTAGGG3'     |      |
|          | 2           | AGMI133         | Forward        | 5'GTGGTTTGGCAGTGGAAATGGAA3'    | 47   |
|          |             | AGMI134         | Reverse        | 5'GTATGGCTCAGCTGTATCCATC3'     |      |
|          | 3           | AGMI155         | Forward        | 5'CGAAACCTGCTGGACGAGT3'        | 50   |
|          |             | AGMI156         | Reverse        | 5'CGGGACCCAAGGAGGAGG3'         |      |
|          | 4           | AGMI187         | Forward        | 5'GCAACTTTGGCAGCATT3'          | 52   |
|          |             | AGMI188         | Reverse        | 5'TGAGATATAGAGGAAAATAATGTTA3'  |      |
|          | 5           | AGMI131         | Forward        | 5'ATCTTTTCTTATCCTTCTAACG3'     | 52   |
|          |             | AGMI132         | Reverse        | 5'CGCTTTAGATTCTGTTTAAG3'       |      |
|          | 6           | AGMI201         | Forward        | TGGTTGAGTAGATCTTCTTGTGTTT      | 52   |
|          |             | AGMI202         | Reverse        | CAAGAAAATGATAATACCATAATGA      |      |
|          | 7           | AGMI145         | Forward        | 5'AGCTATTACTTGTTTTTATCTTGAA3'  | 54   |
|          |             | AGMI146         | Reverse        | 5'AAGGACANAAAAGACAGGA3'        |      |
|          | 8           | AGMI129         | Forward        | 5'GGAGGCCCAACATAGGAAGAGGAAT3'  | 54   |
|          |             | AGMI130         | Reverse        | 5'CACAACCACACACAGCCAATCTTTC3'  |      |
|          | 9           | AGMI147         | Forward        | 5'CTGCAGCAACCCAAATTTATTTTC3'   | 55.2 |
|          |             | AGMI148         | Reverse        | 5'AAATAAGCTCATATGGGTACAGTCA3'  |      |
|          | 10          | AGMI139         | Forward        | 5'GGGGAACAGCACGGTCACAT3'       | 56   |
|          |             | AGMI140         | Reverse        | 5'ACGATGACAACCATTACTAC3'       |      |
|          | 11          | AGMI137         | Forward        | 5'CTTCCTTCTGTCTTTTTGATTGTA3'   | 56   |
|          |             | AGMI138         | Reverse        | 5'GCAAGTCCTTCTGAATCTTAT3'      |      |
|          | 12          | MusaBAG1_SSR1_F | Forward        | 5'GACTCTGGAGCATCTTGCCAT3'      | 56   |
|          |             | MusaBAG1_SSR1_R | Reverse        | 5'CTTTATCTTCGCCAACCCCTAACGG3'  |      |
|          | 13          | AGMI203         | Forward        | 5'TGCTGCCTTCATCGCTACTA3'       | 58   |
|          |             | AGMI204         | Reverse        | 5'GGAACATCGCCCCGCCAC3'         |      |
|          | 14          | AGMI143         | Forward        | 5'TCAAGAGCAATGAAGACCTCAAA3'    | 58   |
|          |             | AGMI144         | Reverse        | 5'TTTTACATGTACAAGGTCAAGCAAT3'  |      |
|          | 15          | MusaBAG1_SSR3_F | Forward        | 5'GGATGGAATTCTCCTCCATCTC3'     | 58   |
|          |             | MusaBAG1_SSR3_R | Reverse        | 5'GGAAGGAGAAGGATGCATGAAACAGG3' |      |

### Conclusion / next steps

The selected primers are being run with the respective parents and their F<sub>1</sub> hybrids. This will help to determine markers that segregate in the F<sub>1</sub> hybrids and show correlation with respective degrees of resistance.

**Objective / Study 5. Developing two SNP-based linkage maps for Foc race 1 and race 4 indicating the location of Foc resistance QTL**

- Genotyping + Sequencing (to be outsourced)
  - Cleaning up of the SNP data
  - Allele calling
- Combination of phenotypic data, and genotypic data
- Construction of linkage maps (JoinMap 4)
- QTL linkage analysis using MapQTL and GenStat

**Conclusion / next steps**

Waiting for SNP chip to screen the populations

## PhD Research Progress Report (2016-2017)

**Name:** Michael Batte

**Title:** Increasing efficiency of the East African highland banana breeding pipeline

**Supervisor:** Prof. Rodomiro Ortiz (SLU), Dr. Brigitte Uwimana (IITA), Dr. Allan Brown, Prof. Rony Swennen, Dr. Mulatu Geleta Dida, Dr. Helena Persson

**Timeline:** 1<sup>st</sup> August 2015 to 31<sup>st</sup> July 2019

**University:** Swedish University of Agricultural Sciences (SLU)

### Research Objectives

List the individual topics of study – objectives or study areas

- Assessing the suitability of available banana descriptors for characterizing East African Highland Bananas.
- Review of breeding East African highland bananas for the first twenty years.
- Mapping resistance to banana nematodes (*Radopholus similis*)
- Mapping resistance to banana weevils
- Determining grandparent heterobeltiosis of NARITA hybrids
- Identifying traits for banana ideotype

### Achievements

Highlight significant achievements – e.g. in bullets

- The research article<sup>8</sup> “Suitability of existing *Musa* morphological descriptors to characterize the East African highland ‘Matooke’ bananas” was published online on 18<sup>th</sup> September 2017 in the Genetic Resources and Crop Evolution Journal. <http://rdcu.be/vYbs>
  - From this study, it was discovered that the available minimum descriptor list for bananas is not suitable to characterize properly the East African highland bananas.
- Most female fertile triploid and tetraploid parents identified according to available records.
- Phenotyping of two populations (Kasaka × Borneo) and (Calcutta 4 × Zebrina GF) for resistance to *Radopholus similis* is going on.
- Phenotyping of population (Monyet × Kokopo) is going on 62 genotypes have been established in pot screening trial.
- Data recorded from field: about 90% for cycle 1, 75% for cycle 2, and 45% for cycle 3.

### Background/introduction

The East and Central Africa (ECA) region has over 50% of its cropping area under banana cultivation, which represents around half of the total area under banana cultivation across Africa. Banana production in ECA has stagnated at least 11 times lower than their yield potential. Pests and diseases have been a substantial component of the problem and pose a particularly great threat to the future sustainability of banana production, with the potential of further destabilizing both food security and household incomes across this region. This project will have a major focus on mapping host plant resistance to banana weevil and burrowing nematode in diploid banana germplasm with the aim of increasing the pace and efficiency of breeding by identifying DNA markers for early selection of priority traits such as host plant resistance. This PhD research will combine

<sup>8</sup> Batte, M., A. Mukiibi, R. Swennen, B. Uwimana, H. Persson, M. Geleta, R. Ortiz. 2017. Suitability of *Musa* morphological descriptors to characterize East African highland ‘Matooke’ bananas. *Genetic Resources and Crop Evolution* DOI: 10.1007/s10722-017-0562-9

association genetics and genomic research on pre-existing segregating populations for mapping sources of resistance to both target pests. The International Institute of Tropical Agriculture (IITA) and Uganda's National Agricultural Research Organisation (NARO) provide the mapping populations. NARO and IITA have released the first ever hybrid cultivars of the East Africa highland banana (NARITA) for food and juice. The secondary triploids NARITA performed better than the local check matooke cultivars for all traits evaluated, e.g., 96% of NARITA had a bunch weight greater than that of the local matooke check. This PhD research will study the underpinning of best (grand-)parent heterosis (known also as heterobeltiosis) using plant crop and ratoon trials, following a rectangular lattice design, including NARITAs, their parents, grandparents and local matooke cultivars as checks. The research will also measure genetic diversity by DNA markers to determine if it correlates with heterosis for evaluated traits in NARITA. Literature review research coupled with breeding records from IITA East African banana breeding program will allow analysing retrospectively its efficiency and determine the combining ability of banana germplasm used as male and female parents. Likewise, path analysis will facilitate noting what traits are to be included in an ideotype to guide East African highland banana breeding. A set of reference cultivars will be characterized using available descriptor list and the analysis of diversity using multi-variate stats will assist on identifying the most discriminating descriptors to distinguish matooke cultivars.

### Objective / Study 1. Assessing the suitability of available banana descriptors for characterizing East African Highland Bananas.

Morphological traits are commonly used for characterizing plant genetic resources. Germplasm characterization should be based on distinctly identifiable, stable and heritable traits that are expressed consistently and are easy to distinguish by the human eye. Characterization and documentation of a representative sample of East African highland bananas (Lujugira–Mutika subgroup) was carried out following an internationally accepted standard protocol for bananas. Eleven cultivars were characterized using an existing set of minimum descriptors (31 qualitative and quantitative traits) with the aim of determining stable descriptors and the ability of these descriptors to distinguish among East African highland banana cultivars. There was variation in stability of these descriptors within cultivars and across the 11 cultivars. Only 10 (32%) out of 31 descriptors studied were stable in the 11 cultivars. However, they had similar scores and therefore are not suitable to distinguish between cultivars within this group. Nonetheless, these 10 descriptors may be useful for distinguishing the East African highland bananas as a group from other groups of bananas. A few descriptors were unique to the cultivar ‘Tereza’ and may be used to distinguish this cultivar from other ‘matooke’ cultivars. None of the quantitative descriptors were stable.

The manuscript “suitability of existing *Musa* morphological descriptors to characterize the East African highland ‘Matooke’ bananas ‘ was published online on 18<sup>th</sup> September 2017 in the journal *Genetic Resources and Crop Evolution* (open access at <http://rdocu.be/vYbs>).

### Objective / Study 2. Review of breeding East African highland bananas for the first twenty years.

The drafting of the manuscript about the review of breeding East African highland bananas for the first two decades, with a working title “Breeding bananas, a tricky business: 20 years of genetic enhancement in East Africa” is ongoing. Some preliminary results (Table 1 and Table 2) show the most female fertile triploids and tetraploids according to the available records in IITA’s breeding program. They should be given high priority in further crossing blocks for getting primary tetraploids, and producing secondary triploids, respectively.

**Table 1. Female fertility in East African highland banana cultivars**

| Clone set       | Cultivar     | No. of bunches pollinated | Total no. of seeds | No. of bunches without seed | Highest no. of seed per bunch | Average no. of seed per bunch | Pollination success (%) |
|-----------------|--------------|---------------------------|--------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------|
| <b>Mbidde</b>   | Endirira     | 26                        | 0                  | 26                          | 0                             | 0                             | 0                       |
|                 | Kabula       | 19                        | 0                  | 19                          | 0                             | 0                             | 0                       |
|                 | Nalukila     | 27                        | 0                  | 27                          | 0                             | 0                             | 0                       |
|                 | Nsowe        | 20                        | 0                  | 20                          | 0                             | 0                             | 0                       |
| <b>Musakala</b> | Mayovu       | 9                         | 0                  | 9                           | 0                             | 0                             | 0                       |
|                 | Mukazialanda | 21                        | 0                  | 21                          | 0                             | 0                             | 0                       |
|                 | Murure       | 17                        | 0                  | 17                          | 0                             | 0                             | 0                       |
|                 | Musakala     | 18                        | 0                  | 18                          | 0                             | 0                             | 0                       |
|                 | Muvubo       | 41                        | 0                  | 41                          | 0                             | 0                             | 0                       |

| Clone set         | Cultivar         | No. of bunches pollinated | Total no. of seeds | No. of bunches without seed | Highest no. of seed per bunch | Average no. of seed per bunch | Pollination success (%) |
|-------------------|------------------|---------------------------|--------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------|
|                   | Nakibizzi        | 10                        | 0                  | 10                          | 0                             | 0                             | 0                       |
|                   | Namunwe          | 22                        | 1                  | 21                          | 1                             | 0.1                           | 4.6                     |
| <b>Nakabululu</b> | Bukumu           | 27                        | 1                  | 26                          | 1                             | 0.04                          | 3.7                     |
|                   | Kazirakwe        | 1043                      | 599                | 891                         | 30                            | 0.6                           | 14.6                    |
|                   | Kibuzi           | 44                        | 3                  | 43                          | 3                             | 0.1                           | 2.3                     |
|                   | Mukubakkonde     | 18                        | 0                  | 18                          | 0                             | 0                             | 0.0                     |
|                   | Nakabululu       | 35                        | 54                 | 23                          | 10                            | 1.5                           | 34.3                    |
|                   | Nakasabira       | 1567                      | 1875               | 1245                        | 305                           | 1.2                           | 20.6                    |
|                   | Nakayonga        | 924                       | 542                | 781                         | 28                            | 0.6                           | 15.5                    |
|                   | Nakyatengu       | 777                       | 957                | 654                         | 147                           | 1.2                           | 15.8                    |
|                   | Salalugazi       | 18                        | 0                  | 18                          | 0                             | 0                             | 0.0                     |
|                   | <b>Nakitembe</b> | Mbwazirume                | 29                 | 0                           | 29                            | 0                             | 0                       |
| Nakitembe         |                  | 479                       | 16                 | 473                         | 9                             | 0.03                          | 1.3                     |
| Nandigobe         |                  | 33                        | 3                  | 31                          | 2                             | 0.1                           | 6.1                     |
| Nyamwihogora      |                  | 14                        | 5                  | 12                          | 3                             | 0.4                           | 14.3                    |
| <b>Nfuuka</b>     | Bitambi          | 183                       | 21                 | 174                         | 6                             | 0.1                           | 4.9                     |
|                   | Entukura         | 2264                      | 1169               | 2011                        | 47                            | 0.5                           | 11.2                    |
|                   | Enyeru           | 1651                      | 491                | 1524                        | 36                            | 0.3                           | 7.7                     |
|                   | Enzirabahima     | 2277                      | 1537               | 2015                        | 102                           | 0.7                           | 11.5                    |
|                   | Kabucuragye      | 1405                      | 300                | 1310                        | 23                            | 0.2                           | 6.8                     |
|                   | Kibalawo         | 30                        | 16                 | 24                          | 4                             | 0.5                           | 20.0                    |
|                   | Kulwoni          | 29                        | 0                  | 29                          | 0                             | 0                             | 0.0                     |



| Clone set | Cultivar         | No. of bunches pollinated | Total no. of seeds | No. of bunches without seed | Highest no. of seed per bunch | Average no. of seed per bunch | Pollination success (%) |
|-----------|------------------|---------------------------|--------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------|
|           | Nabusa           | 661                       | 115                | 634                         | 21                            | 0.2                           | 4.1                     |
|           | Nakawere         | 38                        | 53                 | 26                          | 13                            | 1.4                           | 31.6                    |
|           | Nakinyika        | 33                        | 9                  | 31                          | 6                             | 0.3                           | 6.1                     |
|           | Namwezi          | 1117                      | 401                | 1012                        | 29                            | 0.4                           | 9.4                     |
|           | Nante            | 234                       | 10                 | 229                         | 4                             | 0.04                          | 2.1                     |
|           | Nassaba          | 30                        | 0                  | 30                          | 0                             | 0                             | 0                       |
|           | Ndibwabalan gira | 41                        | 20                 | 36                          | 8                             | 0.5                           | 12.2                    |
|           | Nfuuka           | 1088                      | 339                | 995                         | 23                            | 0.3                           | 8.6                     |
|           | Siira            | 31                        | 0                  | 31                          | 0                             | 0                             | 0                       |
|           | Tereza           | 2609                      | 2107               | 2239                        | 201                           | 0.8                           | 14.2                    |

**Table 2. Female fertility and hybridization success among tetraploid bred hybrids in Uganda**

| Genotype       | No. of bunches pollinated | Total no. of seeds | No. of bunches without seed | Highest no. of seed per bunch | Average no. of seed per bunch | Pollination success (%) |
|----------------|---------------------------|--------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------|
| <b>1201K-1</b> | 1656                      | 48012              | 854                         | 722                           | 29.0                          | 48.4                    |
| <b>917K-2</b>  | 2372                      | 93032              | 1229                        | 2279                          | 39.2                          | 48.2                    |
| <b>660K-1</b>  | 1746                      | 25776              | 987                         | 454                           | 14.8                          | 43.3                    |
| <b>222K-1</b>  | 472                       | 7818               | 279                         | 450                           | 16.6                          | 40.9                    |
| <b>1438K-1</b> | 1591                      | 30706              | 989                         | 885                           | 19.3                          | 37.8                    |
| <b>365K-1</b>  | 670                       | 7687               | 455                         | 284                           | 11.5                          | 32.1                    |
| <b>376K-7</b>  | 736                       | 7161               | 543                         | 467                           | 9.7                           | 26.2                    |
| <b>401K-1</b>  | 723                       | 4787               | 535                         | 348                           | 6.6                           | 26.0                    |
| <b>199K-4</b>  | 293                       | 64                 | 280                         | 12                            | 0.2                           | 4.4                     |

**Objective/ Study 3. Mapping resistance to banana nematodes (*Radopholus similis*)**

Phenotyping of two populations (Kasaka × Borneo) and (Calcutta 4 × Zebrina GF) is going on using the cup method. These two populations are being phenotyped in the screen house in a randomized complete block design with 3 replications. The experiments are being run in series of 33 plants, per experiment including the

parents, and the susceptible (Valery) and resistant (Km5) controls. Four to six roots are inoculated with 50 nematodes 8 weeks after planting. Each experiment is terminated 8 weeks after inoculation. The phenotypic data recorded are percentage root necrosis and total nematode count per inoculated root. Tables 3 and Table 4 show preliminary results for data from the two populations analyzed using SAS software, where genotypes were compared with resistant and susceptible checks using Dunnet's test.

Seven experiments have been established for the Kasaska × Borneo F<sub>1</sub> segregating population. Each experiment handles 29 test genotype (29 × 7 = 203 genotypes out of 242 genotypes). Preliminary results from 6 experiments of 137 genotypes with complete data sets are given in Table 3.

Six experiments have been established for Calcutta 4 × Zebrina GF F<sub>1</sub> segregating population. Each experiment handles 29 test genotypes (29 × 6 = 174 genotypes out of 200 genotypes). Preliminary results from the 3 experiments harvested, of 61 genotypes which had complete data sets are given in Table 4.

**Table 3. Statistical comparison of total nematode counting for genotypes from (Kasaska × Borneo) F<sub>1</sub> segregating population with the resistant (R) and susceptible (S) controls using Dunnet's test**

| Statistical comparison with Valery (S) | Comparison with Km5 (R)     | Host Response            | Number of genotypes |
|--|-----------------------------|--------------------------|---------------------|
| <b>Significantly different</b>         | Not significantly different | Resistant (R)            | 28                  |
| <b>Significantly different</b>         | Significantly different     | Partially resistant (PR) | 7                   |
| <b>Not significantly different</b>     | Not significantly different | Inconclusive             | 75                  |
| <b>Not significantly different</b>     | Significantly different     | Susceptible (S)          | 27                  |
| <b>Total</b>                           |                             |                          | 137                 |

**Table 4. Statistical comparison of total nematode counting for genotypes from (Calcutta 4 × Zebrina GF) F<sub>1</sub> segregating population with the resistant (R) and susceptible (S) controls using Dunnet's test**

| Comparison with Valery (S)         | Comparison with Km5 (R)     | Host Response       | Number of genotypes |
|------------------------------------|-----------------------------|---------------------|---------------------|
| <b>Significantly different</b>     | Not significantly different | Resistant           | 31                  |
| <b>Significantly different</b>     | Significantly different     | Partially resistant | 0                   |
| <b>Not significantly different</b> | Not significantly different | Inconclusive        | 20                  |
| <b>Not significantly different</b> | Significantly different     | Susceptible         | 10                  |
| <b>Total</b>                       |                             |                     | 61                  |

#### Objective / Study 4. Mapping resistance to banana weevils

A segregating population for banana weevil resistance (Monyet × Kokopo) is being phenotyped using a short screening protocol according to Sadik *et al.* 2010,<sup>9</sup> with a few modifications. Suckers after undergoing hot water treatment are used in this experiment. Three suckers per test genotype are planted in a completely randomised design replicated twice. Parents and resistant checks (Calcutta 4, Km5) and susceptible checks (Nakyatengu, Kabucuragye) were also included in the experiment. 62 test genotypes out of 208 genotypes have been established in this trial and screening is on-going.

#### Objective / Study 5. Studying heterobeltiosis of NARITA hybrids

<sup>9</sup> Sadik, K., M. Nyine, M. Pillay. 2010. A screening method for banana weevil (*Cosmopolites sordidus* Germar) resistance using reference genotypes. *African Journal of Biotechnology* 9, 4725–4730

NARITA hybrids, their parents, grandparents and local 'Matooke' cultivar checks were planted in the field following a rectangular lattice design with two replications. Agronomic data are being collected at flowering and harvest. About 90% of data for cycle 1, 75% for cycle 2 and 45% for cycle 3 have been collected. Table 5 shows some preliminary results from bunch weight data for 13 NARITA hybrids that had some data for three cycles, which were used to calculate grandparent heterobeltiosis using the formula:

$$\text{Heterobeltiosis} = \frac{\text{NARITA hybrid} - \text{Grandparent}}{\text{Grandparent}} \times 100$$

**Table 5. Mean bunch weight  $\pm$  standard error and grandparent heterobeltiosis for 13 NARITA hybrids**

| Genotype  | Female parent | Male parent | Grandparent  | Bunch weight (kg) | Grandparent heterobeltiosis (%) |
|-----------|---------------|-------------|--------------|-------------------|---------------------------------|
| NARITA 2  | 401k-1        | 9128-3      | Entukura     | 17.8 $\pm$ 1.9    | 112.3                           |
| NARITA 4  | 660k-1        | 9128-3      | Enzirabahima | 17.7 $\pm$ 3.5    | 118.9                           |
| NARITA 6  | 222k-1        | 9128-3      | Nfuuka       | 18.2 $\pm$ 1.7    | 46.8                            |
| NARITA 9  | 917k-2        | SH3217      | Enzirabahima | 24.4 $\pm$ 3.5    | 201.6                           |
| NARITA 11 | 1201k-1       | 9128-3      | Nakawere     | 17.0 $\pm$ 2.1    | 58.6                            |
| NARITA 12 | 1201k-1       | 9128-3      | Nakawere     | 17.3 $\pm$ 1.4    | 62.0                            |
| NARITA 13 | 1201k-1       | SH3362      | Nakawere     | 22.3 $\pm$ 1.3    | 108.7                           |
| NARITA 14 | 917k-2        | 7197-2      | Enzirabahima | 22.1 $\pm$ 5.5    | 172.8                           |
| NARITA 15 | 660k-1        | 9128-3      | Enzirabahima | 15.2 $\pm$ 2.3    | 87.7                            |
| NARITA 16 | 917k-2        | SH3362      | Enzirabahima | 12.0 $\pm$ 2.5    | 47.7                            |
| NARITA 19 | 1201k-1       | 8075-7      | Nakawere     | 11.3 $\pm$ 1.0    | 5.3                             |
| NARITA 22 | 917k-2        | 9128-3      | Enzirabahima | 22.9 $\pm$ 2.4    | 183.1                           |
| NARITA 23 | Kazirakwe     | 7197-2      | Kazirakwe    | 27.6 $\pm$ 1.8    | 148.3                           |

#### Objective / Study 6. Identifying traits for banana ideotype

The agronomic data from heterobeltiosis trial will be used for path analysis to determine the traits for defining an ideotype for banana breeding.

#### Conclusion / next steps

- ❖ Conclude phenotyping of the populations for *Radopholus similis* resistance
- ❖ Conclude phenotyping of population for banana weevil resistance
- ❖ Genotype the above populations using a SNP chip which is being developed for this purpose
- ❖ Genetic map for the resistance to *Radopholus similis*
- ❖ Mapping quantitative trait loci accounting for resistance to banana weevil

## PhD Research Progress Report (2016-2017)

**Name:** Privat Ndayihanzamaso

**Title:** Evaluation of African bananas for resistance to *Fusarium oxysporum* f. sp. *cabense*

**Supervisor:** Professor Altus Viljoen

**Timeline of study:** 2015-2019

**University:** University of Stellenbosch

### Research Objectives

1. Develop molecular markers specific to Foc Lineage VI of *Fusarium oxysporum* f. sp. *cabense*.
2. Develop a rapid screening method of bananas for resistance to *Fusarium oxysporum* f. sp. *cabense* (Foc).
3. Evaluate Mchare and NARITA for resistance to Foc Lineage VI.

### Achievements

1. Develop molecular markers specific to Foc Lineage VI
  - Two primer pairs of markers specific to Foc Lineage VI were developed, tested and validated on a large population of fungal isolates from different regions of the world.
  - Markers were used to identify isolates collected from five screening sites selected for the East African Banana Breeding Project (EABBP).
  - An article is being reviewed for publication.
2. Develop a rapid screening method of bananas for resistance to Foc
  - The optimization of the inoculation methods, inoculum concentration and disease intensity evaluation has been completed.
  - The optimization of age of plantlets and use of biochemicals involved in banana defence mechanisms as indicators of resistance are in progress.
3. Evaluate Mchare and NARITA for resistance to Foc lineage VI
  - Mshare bananas have been evaluated for Foc race 1 resistance in the screenhouse.
  - Field evaluation of Mshare bananas against Foc race 1 is in progress.
  - Data is being collected on a monthly basis from five screening sites in Tanzania and Uganda to evaluate their susceptibility to Foc race 1.

## Background/Introduction

Banana production in eastern and central Africa (ECA) is dominated by the cultivation of East African Highland banana (EAHB), which are grown as cooking and beer bananas. Cultivars such as Pisang Awak, Bluggoe, Sukari Ndiizi (Kamaramasenge), Gros Michel, Cavendish and FHIA tetraploid bananas have also been introduced and adopted by farmers and are now grown in mixtures with EAHB. All EAHB cultivars are resistant to *Fusarium oxysporum* f sp. *ubense* (Foc) race 1, a soil-borne fungus responsible for Fusarium wilt of banana, but Pisang Awak, Sukari Ndiizi, Gros Michel and other local varieties grown in the region are susceptible. Foc race 1 also affects Mchare bananas, a cooking banana grown in some regions in Tanzania and Kenya. Foc race 1 is still spreading throughout the region because of the use of susceptible cultivars.

The only means to effectively control Foc is to prevent its introduction into disease-free areas, and to plant banana varieties resistant to Foc. Breeding bananas for resistance is a slow process, which requires many years of breeding, and field-testing of hybrids under different environmental conditions. Field-testing is labour intensive and expensive, and depends on the presence of Foc at high inoculum pressure for the tests to be of value. Rapid and standardized *in vitro* methods to screen local varieties and breeding materials against all Foc forms can speed up the process, but have to reflect field results.

The diversity of Foc pathogens in a target areas also needs to be known. Six vegetative compatibility groups (VCGs) within Foc race 1 have been identified in ECA. These are all phylogenetically related and group together in Foc Lineage VI. To detect and identify the fungus in ECA, a molecular-based diagnostic targeting Foc Lineage VI needs to be developed for rapid and accurate identification. Many strains of Foc, thus, need to be collected in ECA to ensure that breeding programmes target all variants of the fungus in the region.

### Objective 1. To develop molecular markers specific to Foc Lineage VI.

This study aimed at developing a molecular diagnostic marker for the detection of Foc strains associated with banana in ECA (Foc Lineage VI). The marker can be used to mitigate banana Fusarium wilt in ECA and wherever Foc race 1 and/or 2-susceptible cultivars are grown. The markers will be used to characterise Foc isolates collected at the five NARITA screening sites in Tanzania and Uganda.

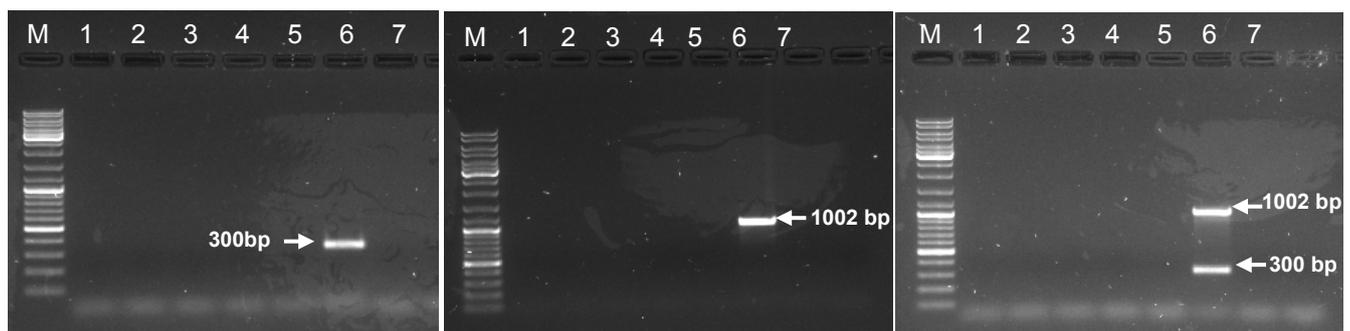
### Materials and Methods

A primer pair specific to Foc Lineage VI was developed from the DNA-directed RNA polymerase second largest subunit (RPB2) gene region, which is known to be very informative for phylogenetic analysis. The primer pair was tested on Foc isolates representing seven different Foc lineages. This primer set was then combined in a multiplex assay with primers designed in the translation elongation factor (TEF-1 $\alpha$ ). They were tested for specificity on 84 Foc isolates, including all 24 Foc VCGs, as well as other *formae speciales* and non-pathogenic strains of *F. oxysporum*. The two primer pairs were subsequently optimized for the *in vitro* and *in planta* detection of Foc Lineage VI isolates in ECA, and validated on a set of 693 Foc isolates and other *Fusarium* species collected from different parts of the world. Foc isolates from ECA that were not identified with the multiplex were subjected to VCG testing as well as morphological and molecular identification tools.

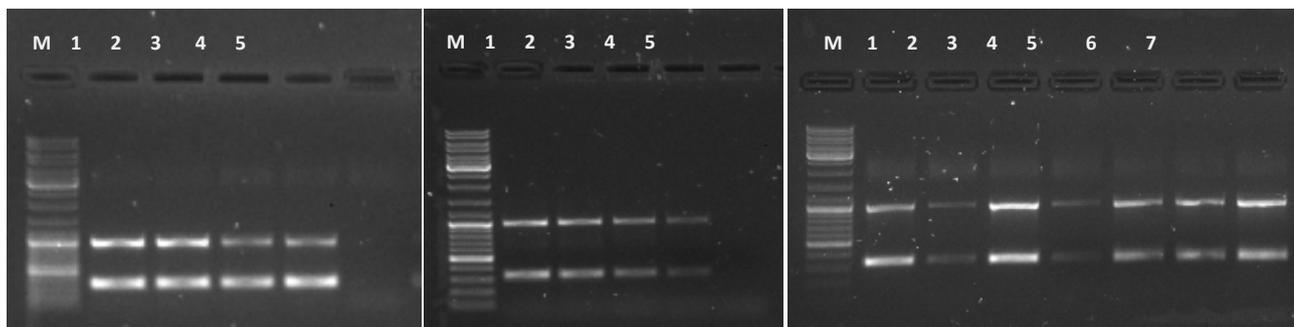
## Results

### PCR markers and multiplex assay

Two primer pairs were developed from the TEF and RPB2 gene regions which amplified 300- and 1002-bp DNA fragments in Foc Lineage VI isolates, but no other Foc lineages or non-pathogen *F. oxysporum* isolates (Fig.1). The primer pairs were successfully combined in a multiplex PCR reaction (Fig.1). When the specificity test for the primer set was extended to 84 isolates and later to a global collection of 693 fungal isolates they showed specificity and consistency by only amplifying Foc Lineage VI isolates. The primer pairs could detect pure fungal DNA as low as 0.1 ng/μl, as well as fungal DNA in presence of 50 ng of banana at a concentration of DNA 0.1 ng/μl (Fig. 2). Additionally, the primer pairs successfully amplified the two expected DNA fragments from infected planting materials (Fig. 2).



**Figure 1.** Specificity testing of two primer sets in individual and multiplex PCR assays for Lineage VI of *Fusarium oxysporum* f. sp. *cubense*. Left: A 300-bp fragment amplified by the FocLin6b-F/R primers, Middle: A 1002-bp fragment amplified by the FocL in VI-F/R primers, Right: Both 300-bp and 1002-bp fragments of Foc amplified by the two primer sets in a multiplex PCR assay. Lanes 1-7: Isolate CAV 980, 618, 789, 871, 968, 2260 and 317; representing Foc Lineage IV, III, V, VIII, VI and *F. oxysporum* f. sp. *melonis*, respectively.



**Figure 2.** Sensitivity testing of FocLin6-F/R and FocLinVI-F/R markers for specific detection of Lineage VI of *Fusarium oxysporum* f.sp. *cubense*. Left: Amplification of Foc DNA at decreasing concentrations (with lanes 1-5 corresponding to 5; 2; 1; 0.1 and 0.01 ng/μl DNA, respectively). Middle: Amplification of Foc DNA at decreasing concentrations in presence of banana DNA (lane 1-5 corresponding to 5; 2; 1; 0.1 and 0.01 ng/μl fungal DNA mixed with 50 ng of banana DNA). Right: Detection of Foc in infected planting materials with lanes 1-7 representing seven infected banana plants.

### Foc diversity across the five NARITA screening sites

To assess Foc diversity, samples were collected from bananas showing typical symptoms of Fusarium wilt at Kawanda and Mbarara in Uganda, and Arusha, Mbeya and Bukoba in Tanzania. The multiplex PCR was first used to identify Foc isolates associated with the Lineage VI. Samples that tested positive were then subjected to VCG analysis by pairing them with VCG testers in Foc Lineage VI. Of the 258 fungal cultures collected, 215 isolates (83%) were associated with the Foc Lin VI, five were heterokaryon self-incompatible (HSI) isolates of Foc Lineage VI, and 38 were *Fusarium* and non-*Fusarium* species. VCGs that were identified included VCGs 0124, 0125, 0128, 01212, 01220, 01222 and complexes thereof. VCGs 0124, 01222 and complex 0124/22 were found in five sites in Uganda and Tanzania, and represented 47.3% of all the Foc isolates collected. The complex 0124/22 was dominant in Mbarara, Kawanda, Kagera and Arusha, and VCG 01212 in Mbeya. VCG 01212 and 01220 were not identified in the screening sites in Uganda. VCG 01220 was the least represented, with only five isolates collected at the Kagera site. Other *Fusarium* species, such as non-pathogenic *F. oxysporum* and *F. sacchari*, were also isolated from banana at the five sites.

### Varieties affected by Foc across the five screening sites

Fusarium wilt affected various banana varieties across the five screening sites. Sukari Ndiizi and Pisang Awak were host to 78% of Foc isolates collected at the five sites. Foc isolates obtained from Mshare bananas represented 13% of the samples collected. The remaining 9% isolates were collected from various cultivars grown in the region or from banana collections, such as Khom, Safeti Velchi, Embu, Figue Pomme Geante, Kisubi, Kataraza, Kikonjwa, Gros Michel, Home, Igyinga and Kijoge. Fusarium wilt was not observed on East African Highland Bananas (EAHB) and Cavendish cultivars grown in mixture systems with EAHB.

## **Objective 2. Develop rapid screening method of bananas for resistance to Foc**

To rapidly assess resistance to Foc in banana varieties and breeding materials, greenhouse and laboratory testing methods of plants will be developed. For greenhouse testing, the effect of inoculum level, inoculation methods and age of plantlets will be investigated, and results compared to field evaluation of the same material. For laboratory testing, metabolites known to be associated with banana resistance following Foc infection will be determined, quantified and correlated to field resistance. These metabolites include phenolic compounds, phenylalanine ammonia lyase, peroxidases, polyphenol oxidases and chitinases.

## **Materials and Methods**

### Optimizing inoculum level and inoculation methods

The effect of inoculum level and inoculation method on banana Fusarium wilt development has been optimized. In this experiment, plantlets of Gros Michel cultivar were inoculated using three inoculation methods and different concentrations of the Foc. The inoculation methods included a Foc drenching technique, a Foc-colonised millet seed method, and a combination of dipping of plants in a Foc suspension followed by planting in soils with Foc-colonised millet seed. Plantlets were hardened off for 4 months to a height of 20-30 cm high. For the drenching method (M1), 50 ml of  $10^2$ ,  $10^4$  and  $10^6$  Foc spores/ml (T1, T2 and T3, respectively) were poured onto the surface of the potting soil. For the millet seed method (M2), bananas were planted in infested soil with millet seeds at concentrations of 1, 2, 5 and 10 g of inoculated millet seeds per 1 kg of soil (T4, T5, T6 and T7). The combined method (M3) consisted of dipping plantlets in  $10^2$ ,  $10^4$  and  $10^6$  spores/ml for 5 min before

replanting in sand infested with 2 g of millet seeds per kg sands (T8,T9 and T10). Three replications of eight plant each were randomized in a complete block design (RCBD). A rhizome discolouration index (RDI) with a rating scale ranging from 1 (healthy plant) to 6 (dead plant) was used to evaluate disease severity, 6 weeks after inoculation. Correspondence analysis, as well as ANOVA (XLSTAT, edition 2017), were used to compare the three methods and concentrations.

#### Comparing inoculation methods to distinguish cultivars

Three inoculation methods with the same concentrations mentioned above were used on four different Cavendish selections, namely Williams (susceptible), GCTCV-119 (resistant), Cavendish Aska (intermediate) and DPM-25 (intermediate). A leaf discolouration index (LDI), with a rating scale ranging from 1 (healthy plant) to 5 (dead plant) and a RDI were used to evaluate disease severity 6 weeks after inoculation. Multiple correspondence analysis (MCA) was used to compare the three methods and concentrations.

### **Results**

#### Optimizing inoculum level and inoculation methods

Plants showed typical external symptoms 3-4 weeks after inoculation. The drenching method using  $10^2$  and  $10^4$  Foc spores/ml (T1 and T2), and the millet seeds method at 1 g of millet seeds per kg of soil (T4), caused less or no symptoms of Fusarium wilt, which were not significantly different from the controls (Table 1). The drenching method at  $10^6$  Foc spores/ml and millet seeds method at 2, 5 and 10 g of millet seeds per 1 kg of soil (T3, T5, T6 and T7) were associated mostly with disease rating of 2 and 3. The combined inoculation method at all three concentrations (T8, T9 and T10) caused significantly more disease than other methods (Table 1). The millet seed inoculation method at all concentrations except 1 g/kg caused less symptoms of the rhizome compared to the combined method. There was no significant difference of the disease severity between the application of 2, 5 and 10 g of millet seeds per kg of soil.



**Table 1.** Effect of inoculation method and concentration of Foc on disease incidence and severity as scored by rhizome discolouration index (RDI).

| Inoculation method                        | Inoculum concentration              | Incidence (%) | RDI mean<br>( $P < 0,001$ ) |
|---|-------------------------------------|---------------|-----------------------------|
| <b>Dipping method + millet seeds (M3)</b> | 10 <sup>2</sup> spores/ml (T8)      | 96            | 4,41 ± 0,24 <sup>a</sup>    |
|   | 10 <sup>4</sup> spores/ml (T9)      | 100           | 4,20 ± 0,24 <sup>a</sup>    |
|   | 10 <sup>6</sup> spores/ml (T10)     | 92            | 4,50 ± 0,24 <sup>a</sup>    |
| <b>Millet seeds method (M2)</b>           | 1g/kg of soil (T4)                  | 17            | 1,29 ± 0,24 <sup>c</sup>    |
|   | 2g/kg of soil (T5)                  | 67            | 2,41 ± 0,24 <sup>b</sup>    |
|   | 5g/kg of soil (T6)                  | 67            | 2,91 ± 0,24 <sup>b</sup>    |
|   | 10g/kg of soil (T7)                 | 71            | 2,58 ± 0,24 <sup>b</sup>    |
| <b>Drenching method (M1)</b>              | 50 x 10 <sup>2</sup> spores/ml (T1) | 8             | 1,13 ± 0,24 <sup>c</sup>    |
|   | 50 x 10 <sup>4</sup> spores/ml (T2) | 4             | 1,04 ± 0,24 <sup>c</sup>    |
|   | 50 x 10 <sup>6</sup> spores/ml (T3) | 58            | 2,33 ± 0,24 <sup>b</sup>    |
| <b>Controls</b>                           | Control for M1 (C1)                 | 0             | 1,00 ± 0,24 <sup>c</sup>    |
|   | Control for M2 (C2)                 | 0             | 1,00 ± 0,25 <sup>c</sup>    |
|   | Control for M3 (C3)                 | 0             | 1,00 ± 0,24 <sup>c</sup>    |

#### Comparing inoculation methods to distinguish cultivars

The results indicate that the different inoculation methods clustered into three groups. The first group includes control treatments, soil drenching with 10<sup>2</sup> and 10<sup>4</sup> spores/ml (T1 and T2), and soil infestation with 1 g of millet seeds/kg of soil (T4). These inoculation methods inconsistently caused the disease and mostly rated 1 (no symptoms) or 2 (few internal spots) on a scale of 6. Soil drenching with 10<sup>6</sup> spores/ml (T3), soil infested with 2 and 5 g of inoculated millet seeds/kg of soil (T5 and T6) constitute the second group, and consistently developed symptoms that mostly rated 2 or 3. The third group includes the combined method at all concentrations (T8, T9 and T10), which mostly rated 4 to 6.

The two experiments have shown that some plants may escape the pathogen when inoculation by drenching was used. Millet inoculation and a combination of dipping and infested soil on the other hand caused consistent external and internal symptoms. The latter caused the disease to all cultivars with the highest ratings, irrespective of their susceptibility to Foc. The inoculum load is probably too much for the plant to deploy defence mechanisms and block the infection.

External symptoms were not always reliable to assess disease severity as they can be caused by different stresses. Some plants with high ratings for external symptoms developed no internal symptoms when the rhizome was cut. This means that the yellowing of leaves was not caused by Foc. On the other hand, plants with a healthy appearance sometimes showed internal symptoms.

Although the combination of root dipping and infested soil with millet seeds consistently caused the highest ratings to all cultivars, it cannot be used to rank cultivars. The intermediate cultivars were as severely infected as susceptible cultivars. The millet seed method seems to be the most appropriate, with consistent results that developed slowly and could distinguish between banana varieties.

### Objective 3. Evaluate Mshare diploids and NARITA hybrids for resistance to Foc Lineage VI

Mshare and NARITA banana varieties and breeding materials will be evaluated in the laboratory and greenhouse using the method developed above. The same materials will also be evaluated for resistance to Foc Lineage VI in fields in Tanzania and Uganda. Field results will be correlated with greenhouse results to determine the reliability of young plant resistance testing.

#### Materials and Methods

##### Evaluation of Mshare varieties in the screen house

Tissue culture plantlets of Mshare banana cultivars were produced at NARO-Kawanda and the IITA-Arusha station. Plantlets were multiplied in Arusha and Kawanda, hardened-off for 2-3 months, and then evaluated in a screen house and in the field. Mbwazirume (EAHB-AAA) and Sukari Ndiizi (AAB) were included as resistant and susceptible controls, respectively, at Kawanda, whereas Grande Naine (AAA) and Sukari Ndiizi served as controls at Arusha. The field and screen house trials were established in April and May 2017 at Kawanda and Arusha, respectively. For greenhouse trials, three replications of 10 plantlets each per cultivar were treated in RCBD, while three replications of 20 plantlets for each cultivar were planted in field trials.

The millet seed technique was used to inoculate plants in the screen house trials. Isolates from infected banana fields at Kawanda and Arusha were used to prepare inoculum in the two countries. The isolates were identified with a Lineage VI marker and by VCG analysis. The VCG identity of the Foc isolates used at Kawanda and Arusha were VCG complex 0124/5/8/22 (CAV 3856) and 0124/22 (CAV 3733), respectively. Disease incidence and severity were determined after 3 months using the RDI. Data were analysed with correspondence analysis, as well as ANOVA (XLSTAT, edition 2017).

#### Results

##### Evaluation of Mshare varieties in the screen house

The susceptible control and some Mshare cultivars developed typical symptoms of Fusarium wilt, which include the yellowing of leaves and brown discolouration of the rhizome. Disease development was slow at both sites, even for the susceptible control. The disease incidence and severity were also low (Table 2). The incidences for Gros Michel and Sukari Ndiizi, used as susceptible controls, were 33 and 57%, respectively. Disease severity mean was less than a rating of 2. Mbwazirume and Grande Nain plants, which were used controls, did not develop any symptoms of Fusarium wilt.

*Arusha:* All Mshare varieties, except Nshonowa, were infected by Foc race 1 with an incidence ranging from 4-21%. Disease severity was significantly lower than for the susceptible control. However, the disease severity of Mshare varieties was also not significantly different from Mbwazirume, the resistant control. Nshonowa did not develop any symptoms of Fusarium wilt. Correspondence analysis indicated that Mshare varieties were associated with lower ratings, and therefore clustered with the resistant control.

*Kawanda:* Mshare, Mshare Mlelembo and Nshonowa developed symptoms of Fusarium wilt at incidences of 23, 10 and 33%, respectively. The severity of Mshare and Mshare Mlelembo was lower and significantly different from the susceptible control. Nshonowa was severely infected, similar to Sukari Ndiizi, which was the susceptible control. Muraru, Kahuti, Kamunyila, Hutishamba and Njuru did not develop Fusarium wilt symptoms (Table 2).

*Comparison of results from the two sites:* It was not possible to compare the results of the Mshare varieties in the two countries because their names were different without a reference on synonyms. Mshare cultivars with similar names in the two countries showed that Nshonowa was susceptible at Kawanda but not at Arusha. Although the name is same, their real identities were uncertain, as there is no reference numbers to match the Mshare cultivars maintained at Kawanda and Arusha. This requires the harmonization of names to resolve discrepancies observed in banana germplasm maintained at different locations.

It is difficult to explain why *Fusarium* wilt took so long to develop in the screen houses in Kawanda and Arusha (3 months), as symptoms usually develop within 6 weeks. Nevertheless, a tentative ranking of the Mshare cultivars to *Fusarium* wilt was presented (Table 2). Those that developed symptoms but did not differ significantly from the susceptible control were considered susceptible, while infected cultivars that developed symptoms and grouped together with resistant control were considered as intermediate (Table 2).

Scoring disease severity on rhizome discolouration only is probably not good enough. The absence of symptoms for susceptible cultivars may be due to slow disease development or the absence of infection. Additional factors, such biochemical changes and fungal quantification *in planta*, are among potential indicators that can determine susceptibility.

## **Conclusion/next steps**

### Objective 1. Develop molecular markers specific to Foc Lineage VI

- The studies for this objective has been completed. An article on molecular markers is being reviewed for publication.

### Objective 2. Develop rapid screening method of bananas for resistance to Foc

There are three experiments that are still in preparation.

- Experiment 1: Optimising the age of plantlets for a rapid screening method, and the evaluation of phenolic compound and enzymes as early screening indicators of resistance to Foc. Plants are being multiplied at Stellenbosch University, and experiments are planned for January 2018 when the plants will be ready for inoculation.
- Experiment 2: Evaluate the use of *in vitro* banana plantlets for resistance to Foc
- Experiment 3: Validate the millet seeds technique and test any other method identified as a rapid screening technique for resistance to Foc.

### Objective 3. Evaluate Mshare and NARITA for resistance to Foc Lineage VI

- Collect field data on Mshare varieties and compare with the greenhouse results.
- Compile data and assess the resistance of NARITA hybrids to Foc.

**Table 2:** Disease severity of Mshare varieties to banana Fusarium wilt (Foc race 1) at Kawanda and Arusha.

| IITA-Arusha (Tanzania) |                 |          |               |                          |                             | NARO-Kawanda (Uganda) |                 |                      |               |                          |                                  |
|------------------------|-----------------|----------|---------------|--------------------------|-----------------------------|-----------------------|-----------------|----------------------|---------------|--------------------------|----------------------------------|
| No                     | Name            | ITC code | Incidence (%) | RDI* means               | Response to Foc VCG 0124/22 | No                    | Name            | NARO code collection | Incidence (%) | RDI means                | Response to Foc VCG 0124/8/20/22 |
| 1                      | Huti-white      |          | 21            | 1,35 ± 0,14 <sup>b</sup> | Intermediate                | 1                     | Nshonowa        | MMC 423              | 33            | 1,70 + 0,09 <sup>a</sup> | Susceptible                      |
| 2                      | Huti green bell | ITC1559  | 12,5          | 1,29 ± 0,14 <sup>b</sup> | Intermediate                | 2                     | Mshare          | MMC 501              | 23            | 1,37 + 0,09 <sup>b</sup> | Intermediate                     |
| 3                      | Mshare          |          | 17            | 1,21 ± 0,14 <sup>b</sup> | Intermediate                | 3                     | Mshare Mlelembo | MMC 453              | 10            | 1,10 + 0,09 <sup>c</sup> | Intermediate                     |
| 4                      | Ijihu Inkundu   | ITC1460  | 12,5          | 1,17 ± 0,14 <sup>b</sup> | Intermediate                | 4                     | Muraru          | MMC 421              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 5                      | Makyughu I      | ITC1454  | 12,5          | 1,14 ± 0,14 <sup>b</sup> | Intermediate                | 5                     | Kahuti          | MMC 483              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 6                      | Mshare Mlelembo | ITC1455  | 8             | 1,13 ± 0,14 <sup>b</sup> | Intermediate                | 6                     | Kamunyila       | MMC 479              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 7                      | Makyughu II     | ITC1446  | 4             | 1,09 ± 0,14 <sup>b</sup> | Intermediate                | 7                     | Hutishamba      | MMC 486              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 8                      | Akondro Mainty  | ITC0281  | 8             | 1,08 ± 0,14 <sup>b</sup> | Intermediate                | 8                     | Njuru           | MMC 418              | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 9                      | Nshonowa        |          | 0             | 1,00 ± 0,14 <sup>b</sup> | Resistant                   | 9                     | Sukari Ndiizi** |                      | 57            | 1,73 + 0,09 <sup>a</sup> | Susceptible                      |
| 10                     | Kahuti          | ITC1468  | -             | -                        | Not tested                  | 10                    | Mbwazirume***   |                      | 0             | 1,00 + 0,09 <sup>c</sup> | Resistant                        |
| 11                     | Gros Michel**   |          | 33            | 1,83 ± 0,14 <sup>a</sup> | Susceptible                 |                       |                 |                      |               |                          |                                  |
| 12                     | Grande Naine*** |          | 0             | 1,00 ± 0,14 <sup>b</sup> | Resistant                   |                       |                 |                      |               |                          |                                  |

\* RDI: rhizome discolouration index

\*\* Susceptible control

\*\*\* Resistant control

## Name of Student: Janet N. Kimunye

**Title:** Genetic diversity of *Pseudocercospora* spp. associated with banana Sigatoka in East Africa

**Supervisor:** Dr. Altus Viljoen and Dr. George Mahuku

**Timeline of study:** Oct 2015- Sept 2019

**University:** Stellenbosch University

### Research Objectives

1. Map the distribution, severity, genetic and pathogenic variability of Sigatoka pathogens in Uganda and Tanzania
2. Develop and validate a rapid method for screening banana germplasm for resistance to Sigatoka
3. Evaluate NARITAs and Mchare diploids for response to Sigatoka pathogens

### Achievements

- Distribution and severity of Sigatoka leaf spots in Uganda and Tanzania mapped
- Identification of pathogens recovered from surveys and preliminary characterisation done
- Trial for protocol validation established
- Response of Mchare diploids to Sigatoka determined.
- Evaluation of NARITA trials in all sites ongoing.

### Background/introduction

Banana production in African Great Lakes region is lower at <20 t Ha<sup>-1</sup>year<sup>-1</sup> (FAO, 2009) than the yield potential of >70 t Ha year (van Asten *et al.*, 2005). The significant difference between the actual yield and the potential has been attributed to pests and diseases (Gold *et al.*, 1999) among other abiotic constraints (Wairegi *et al.*, 2010; Wairegi & van Asten, 2011). Sigatoka is one of the most important banana diseases that reduces banana yields by as much as 50% (Akele *et al.*, 2000) and dramatically affects the quality of the fruit. *Pseudocercospora musae* and *P. fijiensis* have been reported in most banana growing regions both in Uganda and Tanzania but *P. eumusae* is yet to be documented in the region. The projected climate change scenario for the Great Lakes region is likely to see an increased incidence and severity of Sigatoka leaf diseases, as well as a shift in importance of the *Pseudocercospora* species associated with the diseases. For example, *P. fijiensis* was previously considered unimportant in the highlands but recent reports indicate that the pathogen is getting adapted to cooler climates and replacing *P. musae* to become the most important constraint to banana production (Arzanlou *et al.*, 2007; Zandjanakou-Tachin *et al.*, 2009). Co-existence of Sigatoka pathogens has also been observed in some banana growing regions (Zandjanakou-Tachin *et al.*, 2009).

Sexual reproduction occurs in *Pseudocercospora* species during the later stages of disease (Carlier *et al.*, 2000). Regular sexual reproduction occurs when the mating types are present in the ratio of 1:1 within a population which results in higher genetic diversity arising from new recombination. This leads to the emergence of new pathotypes that could be more virulent and capable of overcoming available resistance. Pathogens that maintain high genetic variation are hard to control because of high levels of natural selection towards any control measure i.e. chemical or host resistance. Considerable high levels of genetic diversity has been reported in *Pseudocercospora* populations all over the world. This plasticity has been implicated in resistance breakdown in cultivars with high Sigatoka resistance (Mouliom-Pefoura, 1999) and variable cultivar response across sites. This calls for a thorough evaluation of hybrids under different agro-ecologies where they are exposed to existing pathogen population before deployment. Earlier studies have reported a more or less homogeneous population structure in the African *Pseudocercospora* isolates (Carlier *et al.*, 2002;

Fahleson *et al.*, 2009). It is however important to monitor the current status of the population structure of the pathogen in the African great lakes region and use this to infer durability of developed host resistance.

Early selection of banana cultivars exhibiting resistance would greatly benefit breeding programs. Field evaluation is the most commonly used method but is expensive, lengthy and may suffer from inconsistencies in availability of natural inoculum and unpredictable weather patterns. As a result, development of screening techniques that are economical, and save on time and space is highly desirable. Several protocols using different pathogen inoculants like mycelial, conidial suspensions and culture filtrates have been developed (Foure, 1990; Harelimana *et al.*, 1997; Capó *et al.*, 2002; Donzelli & Churchill, 2007; Twizeyimana *et al.*, 2007). These methods however require optimization and validation for high throughput screening. Recently (Isaza *et al.*, 2016) elucidated on the possibility of using effector proteins to identify resistance sources in banana. If validated, use of effector proteins present a rapid, cheap, economical and objective assay void of evaluator bias associated with visual scale scores. The development of rapid and precise screening techniques for Sigatoka pathogens will accelerate the development of Sigatoka resistant banana varieties and contribute significantly to the economic development and food security of East African countries.

The specific hypotheses are:

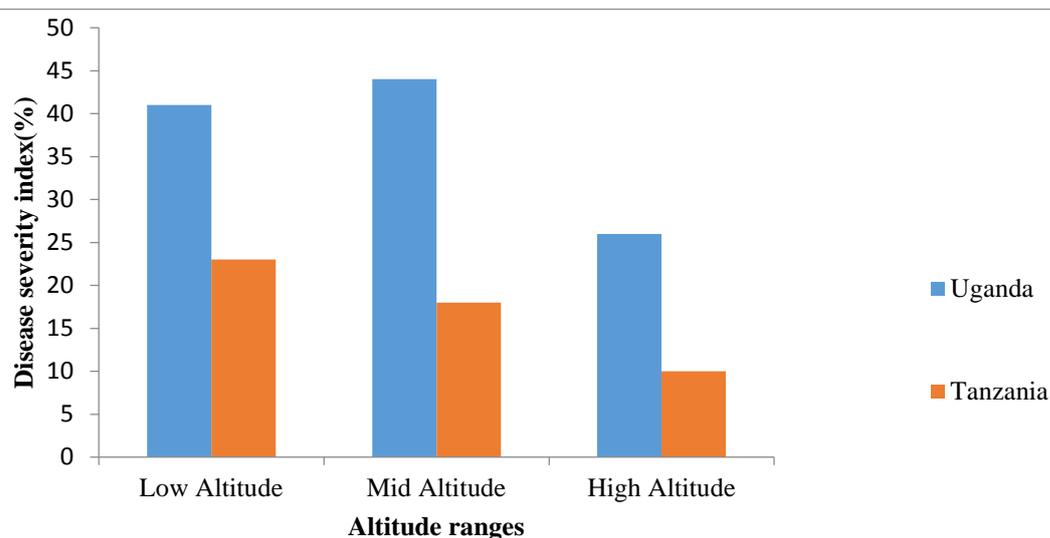
1. *Pseudocercospora* pathogens causing sigatoka in the African Great Lakes region are represented by limited genetic and pathogenic variability
2. Rapid screening methods are reliable in determining a genotype response to sigatoka infection
3. Effector proteins can be used for large scale identification of resistance sources in *Musa* spp.
4. The response of developed NARITA hybrids to Sigatoka infection is similar across the sites

This study will therefore determine the *Pseudocercospora* species distribution in Uganda and Tanzania, their genetic and pathogenic variability, evaluate response of NARITA hybrids under different environments and support breeding pipelines through development of rapid screening methods for early selection.

### **Objective 1: Map the distribution, severity, genetic and pathogenic variability of Sigatoka pathogens in Uganda and Tanzania**

Field surveys to determine Sigatoka leaf spot distribution and severity were conducted in Kilimanjaro, Mbeya, Bukoba (Tanzania), Luweero and Mbarara (Uganda). The survey sites were classified into low altitudes (<1200 m asl), mid altitudes (1201-1500 m asl) and high altitude (>1501 m asl). Banana growing farms were randomly selected for Sigatoka disease severity evaluation. Severity was determined on a 0-6 scale and disease severity index computed as  $DSI = \frac{\sum nb}{(N-1) T} * 100$ . Diseased samples were also collected for pathogen detection. DNA was extracted from lesions using CTAB method and amplified using species specific primers.

The majority of the sites visited in Mbeya, Bukoba and Luweero were in the low and mid altitude range, while sites in Arusha and Mbarara were in the mid and high altitude range. Generally, disease severity was significantly higher in Uganda with mean DSI 39.3% than Tanzania at 20.14%. At all sites, disease severity was significantly higher in the lower and mid altitude as compared to higher altitudes in Uganda and Tanzania. There was no significant difference in disease severity between low and mid altitudes (Figure 1).



**Figure 1:** Leaf spot disease severity indices at low altitude <1200 m a.s.l, mid altitude 1201-1500 and High altitude >1500 m a.s.l ranges in Uganda and Tanzania

*P. fijiensis* was detected in all sites and altitudes except Kilimanjaro. Earlier studies reported that *P. fijiensis* was restricted to low altitudes below 1500 m asl. However, in our studies, over 50 % of samples collected from sites above 1500 m asl tested positive for *P. fijiensis*. These results point to a shift in environmental suitability for survival of *P. fijiensis*. There is need to incorporate weather data in the study to determine if any changes in weather have occurred which could explain the expansion of the pathogen into higher altitudes. Identification of other species causing Sigatoka like symptoms are ongoing.

*Table 1: Summary of P. fijiensis positive samples from Uganda and Tanzania*

| District    | Altitude (m a.s.l.) | No farms surveyed | No. of samples tested | <i>P. fijiensis</i> positive samples |
|-------------|---------------------|-------------------|-----------------------|--------------------------------------|
| Mbarara     | 1411-1877           | 18                | 152                   | (67%)                                |
| Luweero     | 1077-1243           | 24                | 140                   | (77%)                                |
| Bukoba      | 1148-1394           | 24                | 140                   | (94%)                                |
| Mbeya       | 1064-1455           | 27                | 299                   | (34%)                                |
| Kilimanjaro | 1210-1530           | 17                | 159                   | 0                                    |

### Isolation, detection and mating type analysis

Recovery of pathogen on station and from survey areas was done using the ascospore ejection method to generate single spore isolates for characterisation. Pathogen detection was done by PCR using species specific primer pair MF137/R635. Mating type analysis was done by amplifying the mating type idiomorphs 1 and 2 using MAT 1 and MAT 2 genes primer (Conde – Ferraez et al., 2010). 200 single spores isolates of different morpho-types have been recovered and confirmed to be *P. fijiensis* from the expected 1000bp amplicon. They vary in color from pinkish, white and grey with regular or irregular edges (Figure 2). Mating type analysis of these isolates confirmed that both mating type are present in the pathogen population at 54% MAT 1 and 46% MAT 2. This suggests that sexual reproduction frequently occurs within the pathogen population which may lead to high genetic variability arising from recombination. This may in turn impact on pathogenic variability which has an implication on durability of introduced resistance. Further characterisation is in progress with molecular markers to determine extent of genetic variability and relate this to pathogenic variability.

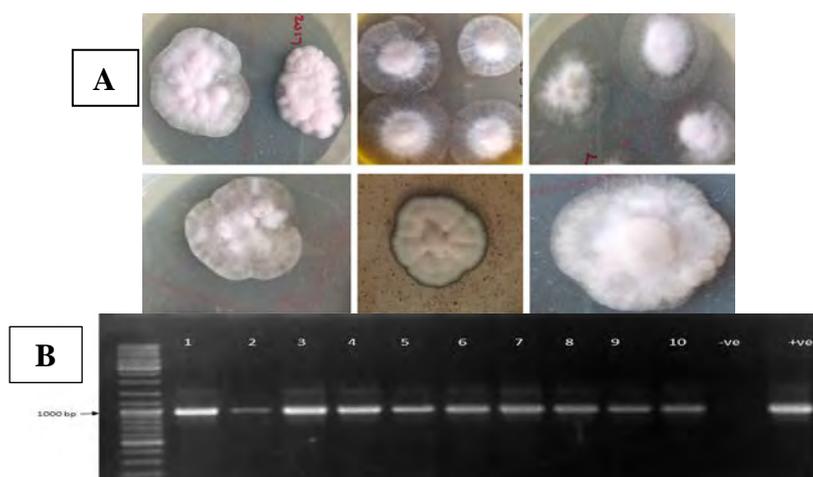


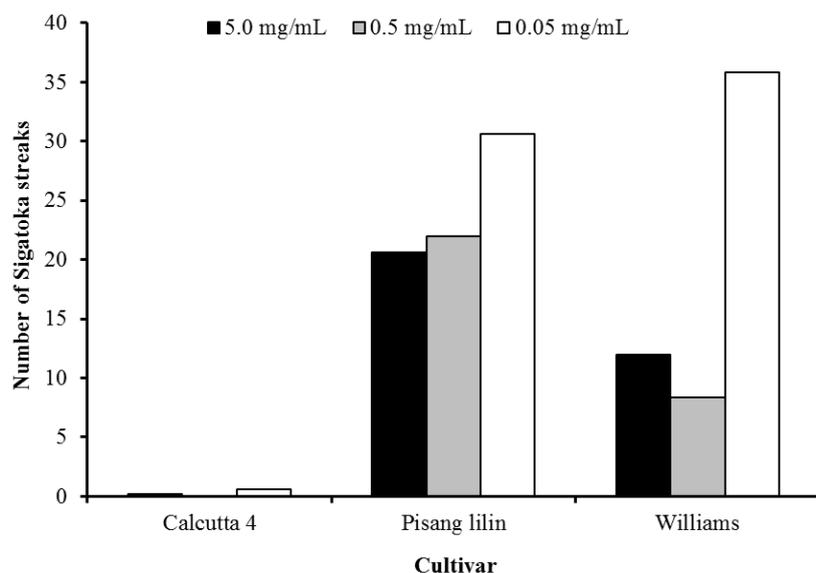
Figure 2: A) Different cultural morphology of isolates recovered on station and from screening sites and PCR; B) Detection of *P. fijiensis* using primer MF137/R635. The 1000bp amplicon indicates presence of *P. fijiensis* DNA and confirms the isolate identity as *P. fijiensis*.

## Objective 2: Develop and validate a rapid method for screening banana germplasm for resistance to Sigatoka

Optimisation of inoculation protocols and inoculant levels are ongoing. Different inoculants i.e. mycelial fragments, conidial suspensions and culture filtrates at different rates will be used to determine their suitability in screening for Sigatoka resistance. In addition, effector proteins will be produced, purified and infiltrated on genotypes varying in resistance levels to determine their utility as rapid screening tools. Detached leaf assays have been used previously for rapid screening for Sigatoka resistance (Twizeyimana et al., 2007). Detached leaves from Williams, Pisang liliin and Calcutta 4 were inoculated with different weights of *P. fijiensis* mycelial suspension to determine if they can reliably be used to discriminate the cultivars. Sigatoka streaks were counted weekly on both the abaxial and adaxial surfaces. Initial black Sigatoka symptoms were observed at 7 and 21 days post inoculation for both Pisang liliin and Williams, and Calcutta 4, respectively. Symptoms appeared as light brown streaks that later darkened and enlarged, only in Pisang liliin and Williams but not in Calcutta 4. Controls did not show any disease symptoms.

More disease streaks were observed on leaf discs inoculated with lower concentrations mycelial fragments at (Fig. 3 & 4). This observation is suspected to be a result of self-inhibition similar to spore germination inhibition observed with inoculations with high density of *P. fijiensis* spores (Balint-Kurti and Churchill, 2004). The experiment will be repeated to confirm this observation. Pisang liliin is more sensitive to inoculum concentration as compared to Williams. Consequently, disease progression was more rapid at the lower concentrations. Inoculation with mycelia at the rate of 0.05 mg/mL appears to be the most discriminating as early as 7 d.p.i (data not shown) Williams, the susceptible check, has higher disease compared to *P. liliin* and Calcutta 4.

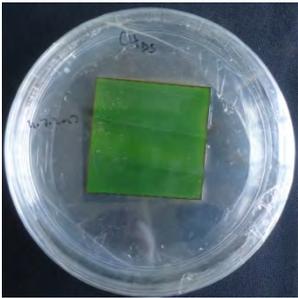
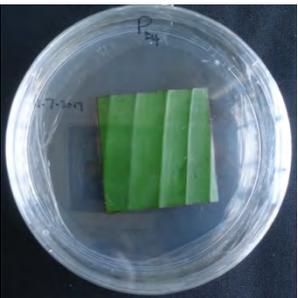
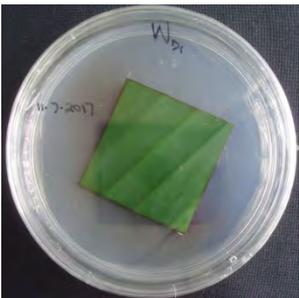




**Figure 3:** Effect of different concentrations of inoculum of *Pseudocercospora fijiensis* on disease severity on three *Musa* cultivars with different levels of susceptibility to black Sigatoka. Disease severity was assessed *in vitro* on leaf discs at 49 days after inoculation.

Experiments to induce profuse sporulation of the fungus for inoculation are in progress. Once optimum inoculum type and level is determined, small plants of different ages will be inoculated to determine the age at which artificial inoculation reflects a genotypes field response.

Sequences from three selected effector proteins present in *Cladosporium fulvum* were obtained from the gene bank. A blast search for the protein homologs in *P. fijiensis* genome was done to identify the gene sequences coding for these proteins and primers developed. These will be used to amplify the gene sequences for cloning and heterologous expression of the proteins in *Pichia pastoris*. Purified protein will be infiltrated onto different genotypes and asses their utility as rapid screening tool.

| Calcutta 4  | Pisang liliin   | Williams   | Treatment |
|---|---|--|-----------|
|  |  |  | Controls  |
|   |   |  | 5mg/mL    |

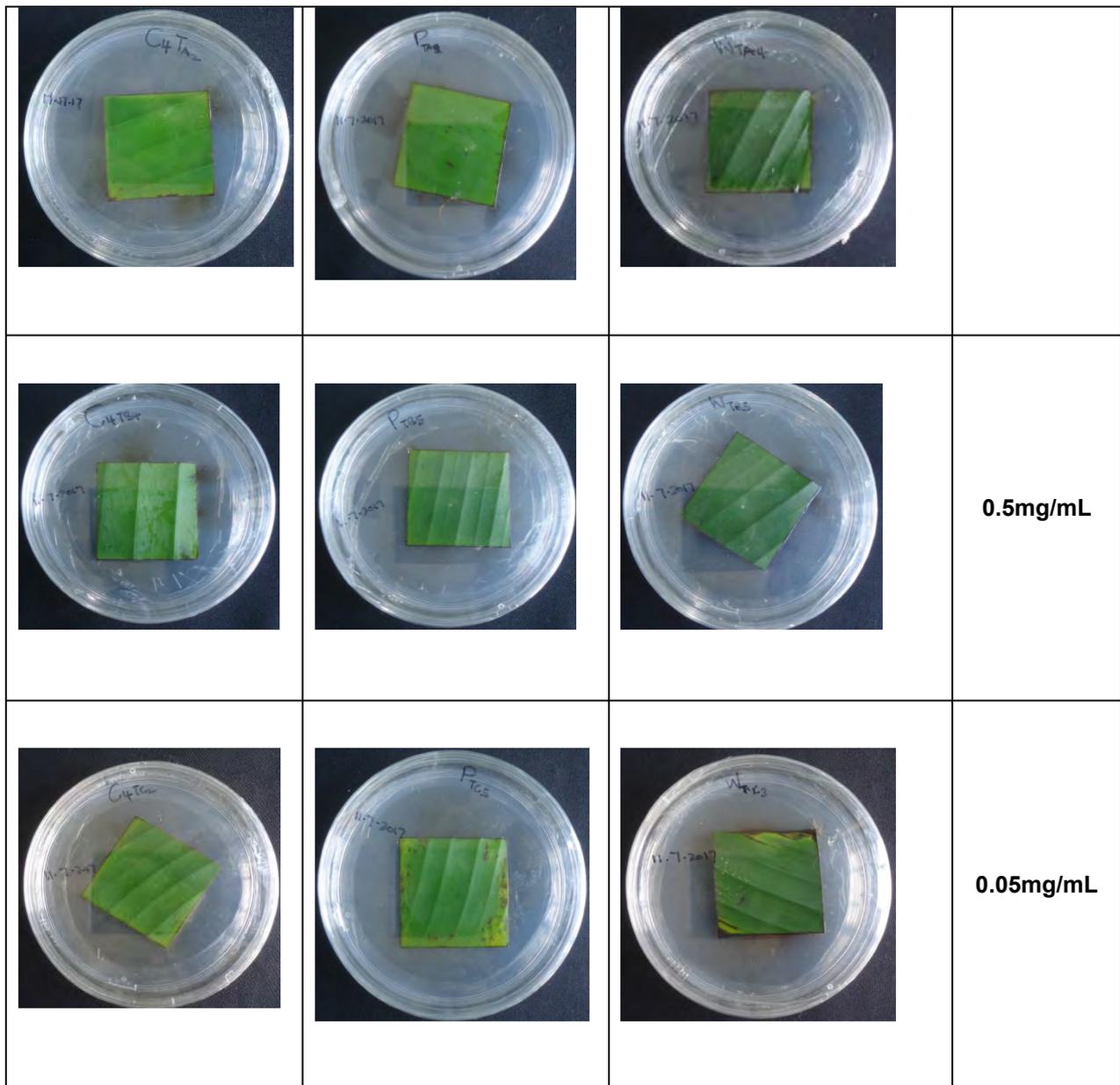


Figure 4: Images of leaf discs taken at 49 days post inoculation

A trial comprising of Sigatoka reference genotypes and germplasm used in the IITA breeding program was established in Sendusu. The results from these trial will be used to validate results of rapid screening methods. Data collection in the trial is in progress.

### Objective 3: Evaluate NARITAs and Mchare diploids for response to Sigatoka pathogens

Evaluation by the IITA group on the NARITAs is done on a quarterly basis across the testing sites. Three plants per genotype per replication are selected for disease severity studies. Disease severity is recorded according to modified Gauhl's 0-6 scale. Disease severity index is computed as

$$DSI = \left[ \frac{\sum nb}{(N-1) T} \right] * 100$$

Where:

n = number of leaves in each grade

b = grade

N = number of grades used in the scale (7)

T = total number of leaves scored

Disease was observed in all sites except Kilimanjaro where the disease is just setting in. There was a significant difference at  $p < 0.05$  in disease severity among the genotypes. Preliminary data indicates that sigatoka severity in the NARITAs is lower than the local checks EAHB (Matooke) and the susceptible check Williams. Response of cultivars across sites is comparable but this will be monitored over time to determine influence of environment on genotype response to Sigatoka.

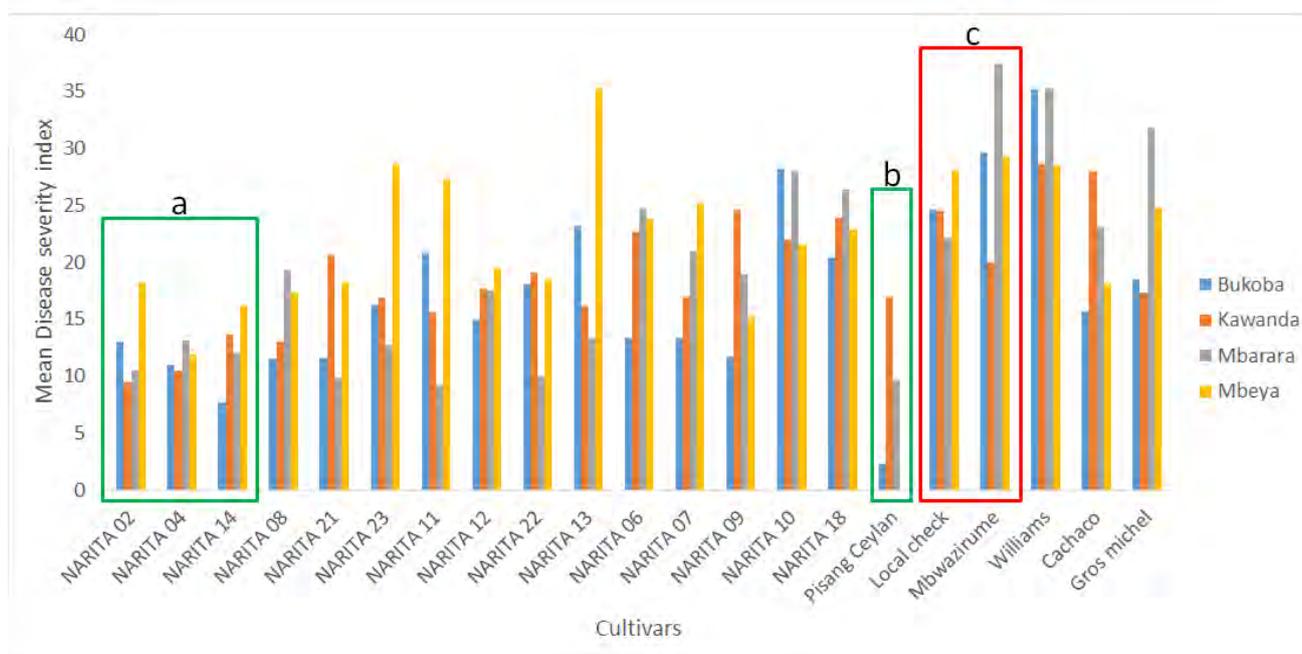


Figure 5: Disease severity index of selected NARITAs across screening sites; a) best performing NARITAs, b) Pisang ceylan is a Sigatoka resistant check; c) Local checks across sites and a common EAHB cultivar Mbwazirume

Evaluation of Mchare genotypes in Kawanda was done as above. There was no significant difference in cultivar response to Sigatoka over the evaluation period. The mean DSI was 39.4%. Significant differences in mean DSI  $P < 0.001$  were observed between the evaluation times. The highest severity was observed in July 2016 mean DSI 51.85% while the lowest was in March 2017 mean DSI 22.98% across the cultivars (Table 2). Our results suggest that Mchare diploids evaluated in Kawanda are susceptible to Sigatoka. The difference is probably due to weather conditions and/or management practises like de-trashing that removes old and diseased leaves.

Table 2: Mean DSI (%) of Mchare diploids at different evaluation times in 2016 and 2017

| Cultivar    | April 2016 | July 2016 | December 2016 | March 2017 |
|-------------|------------|-----------|---------------|------------|
| Huti shamba | 35.76      | 50.43     | 47.23         | 28.05      |
| Kahuti      | 28.54      | 42.92     | 37.65         | 21.38      |
| Kamunyila   | 29.23      | 56.18     | 41.39         | 14.95      |
| Mlelembo    | 38.37      | 51.49     | 66.32         | 30.5       |
| Mshale      | 32.16      | 48.35     | 51.94         | 27.99      |
| Muraru      | 32.29      | 61.41     | 47.27         | 26.02      |
| Njuru       | 32.28      | 51.03     | 64.31         | 20.77      |
| Nshonowa    | 31.26      | 50.81     | 43.82         | 13.77      |
| LSD         | 14.19      |           |               |            |
| CV (%)      | 37.7       |           |               |            |

### Conclusion / next steps

Several challenges including failure of published primers to amplify, difficulties in single spore isolation, inconsistency in sporulation and inability to get sufficient numbers of plants for experiments have delayed the work. However, most of these challenges are being addressed and it is expected that work will progress as per schedule.

## PhD Research Progress Report (2016-2017)

**Name:** Mohamed Hussein Mpina

**TITLE:** Genetic analysis of resistances to *Fusarium oxysporum f. sp. cubense* (Foc) race 1 in Banana (Musa sp.)

**Supervisor:** University supervisors/advisors: Prof. Altus Viljoen (Stellenbosch university)

IITA Supervisor: Allan Brown (IITA), Dr. George Mahuku (IITA)

**Timeline:** August 2015 – August 2019

**University:** Stellenbosch University, South Africa

### Research Objectives:

- To understand inheritance of banana resistance to Foc race 1
- To construct high-density genetic linkage map with a diploid population
- To map QTLs and identify SNPs markers associated with resistance to Foc race 1

List the individual topics of study – objectives or study areas

- Segregation test for Paliama x Borneo mapping population
- Phenotyping of Paliama x Borneo mapping population
- Phenotyping of malaccensis x malaccensis mapping population
- Genotyping (SNP calling and linkage mapping)
- Marker-trait association (QTL mapping)

**Achievements:** Highlight significant achievements – e.g. in bullets

- Paliama x Borneo genotypes differ/segregate in resistance to Foc race 1
- 67 genotypes (34%) are evaluated for resistance to Foc race 1
- 200 genotypes from the field are in the TC growth room
- TC work for malaccensis x malaccensis genotypes is progressing. All genotypes are at rooting stage

## Background/introduction

### Brief background

*Fusarium* wilt caused by *Fusarium oxysporum f. sp. cubense (Foc)* is a serious disease, affecting many banana cultivars grown by smallholder farmers in various regions including East Africa. Chemical, physical and cultural control measures are not promising, among other reasons being long persistence of *Foc* in the soil and challenges facing fungicides applications for *Foc* control. Resistance among banana cultivars is the most effective and sustainable management option. Most cultivated cultivars grown in east Africa including “Sukari Ndizi” (AAB) and “Mchare” (AA) are susceptible to *Foc* race 1 except East Africa highland bananas (EAHB). Therefore *Foc* race 1 is considered to be one of banana production constraints to Africa great lakes region.

Banana resistance to *Foc* race 1 has been reported in several wild diploid bananas *M. acuminata ssp. malaccensis* and *burmannica*. However, introgression of resistance in edible cultivars appears to be taking remarkable time due to sterility and length of banana life cycle. Therefore, development of genetic markers for early selection of resistance traits could be of success to speed up the banana breeding process for *Foc* and other important traits. However, development of genetic markers is currently hindered by the presence of relatively few banana mapping populations for specific traits and lack of denser genetic linkage maps. SNPs markers which are not yet established for banana are the most abundant in a genome and hence suitable for analysis on a wide range of genomic scopes.

The aim of this study is to elucidate the genetics of resistance in banana and identifying genetic markers associated with resistance to *Foc* race 1. This study has the following objectives:

- To understand inheritance of banana resistance to *Foc* race 1,
- To construct high-density genetic linkage map with a diploid banana population and
- To map QTLs and identify SNPs markers associated with *Foc* race 1 resistance.

The findings from this study could be of value for marker-assisted selection in banana breeding programs. This will consequently contribute to banana improvement in Africa Great Lakes region. This study will be conducted in IITA Arusha station and plant pathology facilities at Stellenbosch University.

## Objective / Study 1

### To understand inheritance of banana resistance to Foc race 1

- Evaluation of Paliama x Borneo genotypes for resistance to Foc race 1
- Method: Millet seed inoculation technique as described by Viljoen *et al.* (2017)
- Results:
  - Paliama x Borneo genotypes differ/segregate in resistance to Foc race 1
  - Sixty seven (67) out of 200 genotypes (34%) are evaluated, more genotypes to follow in batches.

When all required genotypes are evaluated data will be subjected to analysis of variance (ANOVA) and means will be separated with Fischer's protected test and Joint Segregation and heritability analysis will be performed.

## Objective / Study 2

### To construct high-density genetic linkage map with a diploid population

- Methods: SNPs calling and linkage analysis with Join map version 4.1
- Results: Linkage maps will be constructed.

## Objective / Study 3

### Mapping of QTLs and identifying SNPs markers associated with Foc race 1 resistance in banana

Method: QTL mapping will be done with Map QTL

Results: QTLs associated with Foc race 1 resistance will be mapped.

## Conclusion / next steps

- TC for the remained Paliama x Borneo genotypes is continuing (sub culturing and later on rooting)
- Phenotyping of Paliama x Borneo mapping population is continuing
- TC work for malaccensis x malaccensis genotypes is continuing
- Phenotyping of malaccensis x malaccensis genotypes will be starting with the present genotypes.

## PhD Research Progress Report (2016-2017)

**Name:** Moses Nyine

**Title:** Genomic selection to accelerate banana breeding: Genotyping by sequencing of banana hybrids

**Supervisor:** Prof. Jaroslav Doležel, Prof. Rony Swennen, Dr. Brigitte Uwinama and Dr. Allan Brown

**Timeline:** 2014-2017

**University:** Palacký University Olomouc, Czech Republic

### Research Objectives

1. To assess the variation and correlation of traits in the genomic selection training population with respect to crop cycles and field management.
2. To determine the genetic diversity of the genomic selection training population.
3. To compare the predictive ability of a set of six models with marker, pedigree and both pedigree and marker information for fifteen traits scored in the training population and select the best genomic prediction model for each trait, or a group of traits.
4. To determine the predictive ability of models with a training population grown under two different field management practices (Genotype × Environment interaction).
5. To determine the predictive ability of the best model for prediction of traits within and across crop cycle 1 / mother plants and crop cycle 2 / first ratoons/first suckers (Genotype × Cycle interaction)
6. To determine the effect of accounting for allelic dosage on the predictive ability of the best genomic prediction model for each trait.
7. To determine the effect of using genomic prediction models fitted with averaged environment data and allele dosage SNP markers in the prediction of genotype performance in particular environments.
8. To determine the accuracy of selection achieved based on GEBV relative to phenotypic data within the training population.

### Achievements

- Results from objective one and two have been published and can be accessed from the link: <https://doi.org/10.1371/journal.pone.0178734>
- Results from objectives three to eight were summarized into a manuscript and submitted to Theoretical and Applied Genetics in August 2017. I am still waiting for the editor's decision.
- PhD thesis has been written summarizing all the objectives. As soon as the second manuscript is accepted, the thesis will be submitted for external review before arranging the defence.
- R-script developed to account for allelic dosage in SNP markers can be accessed from the link: [http://olomouc.ueb.cas.cz/system/files/users/public/scripts/AlleleDosage\\_R\\_function.docx](http://olomouc.ueb.cas.cz/system/files/users/public/scripts/AlleleDosage_R_function.docx)



## **Background/introduction**

Improvement of banana against biotic and abiotic production constraints through conventional crossbreeding is a slow and labour-intensive process. Approaches that can reduce the selection cycle are being investigated so that breeding and selection efficiency is increased. Among these approaches is genomic selection, a form of marker assisted selection that utilizes predictive models to generate the genomic estimated breeding values (GEBV) of the genotypes. Superior genotypes that have not been phenotyped are selected on the basis of GEBV and advanced in the breeding process, which increases the genetic gain per unit time and cost. The predictive models were derived from both phenotypic and genotypic data collected from a panel of 307 genotypes of varying ploidy levels constituting the genomic selection training population. The first step was to understand the effect of crop cycle, field management and their interaction with genotype on trait expression. The next step was to provide the first empirical evidence on the performance of six genomic prediction models for 15 traits in a banana genomic selection training population based on single nucleotide polymorphism markers from genotyping by sequencing (GBS) approach. The prediction models tested were Bayesian ridge regression (BRR), Bayesian LASSO (BL), BayesA, BayesB, BayesC and reproducing kernel Hilbert space (RKHS).

## **Summary of the study**

Banana (*Musa* spp.) is an important crop in the African Great Lakes region in terms of income and food security, with the highest per capita consumption worldwide. Pests, pathogens and environmental stress hamper sustainable production of bananas. Effort is being made to improve the East African highland bananas (EAHB) through conventional crossbreeding, but the selection cycle is too long. Improving the efficiency of selection in conventional crossbreeding is a major priority in banana breeding. Marker assisted selection (MAS) has the potential to reduce the selection cycle and increase genetic gain. However, the application of molecular tools has been hampered by the limitations inherent with the classical MAS tools and nature of traits in banana. While genomic selection can address some of the limitations of classical MAS, no report about its utility in banana is available to date. This study provides the first empirical evidence on the performance of six genomic prediction models for 15 traits in a banana genomic selection training population based on genotyping by sequencing (GBS) data. The prediction models tested were Bayesian ridge regression (BRR), Bayesian LASSO (BL), BayesA, BayesB, BayesC and reproducing kernel Hilbert space (RKHS). The aim was to investigate the potential of genomic selection (GS) as a method of selection that could benefit breeding through increased genetic gain per unit time and cost. Trait variation, the correlation between traits and genetic diversity in the training population were analyzed as an essential first step in the development and selection of suitable genomic prediction models for banana traits. A training population of 307 genotypes consisting of EAHB breeding material and its progeny was phenotyped for more than 15 traits in two contrasting conditions for two crop cycles. The population was also genotyped by simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers. Clustering based on SSR markers revealed that the training population was genetically diverse, reflecting a complex pedigree background, which was mostly influenced by the male parents. A high level of correlation among vegetative and fruit bunch related traits was observed. Genotype response to crop cycle and field management practices varied greatly with respect to traits. Fruit bunch related traits accounted for 31–35 % of principal component variation under low and high input field management

conditions. The first two principal components accounted for 50 % of phenotypic variation that was observed in the training population. Resistance to black leaf streak (Black Sigatoka) was stable across crop cycles, but varied under different field management depending on the genotype. The best cross combination was 1201K-1 × SH3217 based on selection response (R) of hybrids. The predictive ability of genomic prediction models was evaluated for traits phenotyped over two crop cycles and under different cross validation strategies. The 15 traits were grouped into five categories that included plant stature, suckering behaviour, black leaf streak resistance, fruit bunch and fruit filling. Models that account for additive genetic effects provided better predictions with 12 out of 15 traits. The performance of BayesB model was superior to other models particularly for fruit filling and fruit bunch traits. Reproducing kernel Hilbert space model fitted with pedigree and marker data (RKHS\_PM) produced mixed results with the majority of traits showing a decrease in prediction accuracy. Although RKHS models account for dominance and epistasis, heterosis is another non-additive genetic factor that affects prediction accuracy in bananas. Models that included averaged environment data for crop cycle one and two were more robust in trait prediction even with reduced numbers of markers. Accounting for allelic dosage decreased the predictive ability of all models by 15 % on average, but the trend of correlation between predicted and observed values remained the same across traits and within trait categories as predicted by bi-allelic SNP markers. Since high correlation in prediction was observed within trait categories, only traits easy to phenotype should be considered for genomic predictions during the breeding phase. Although validation and more optimization of model parameters is still required, the high predictive values observed in this study confirmed the potential of genomic prediction in selection of best parents for further crossing and in the negative selection of triploid hybrids with inferior fruits to reduce the number of progenies to be evaluated in the field.

Outside my PhD study scope, a genome wide association study (GWAS) was conducted using the data from the genomic prediction study to detect loci containing SNP markers that have significant association with fruit circumference (FC), a fruit filling trait. The mixed linear model in the TASSEL v5 software detected significantly associated SNP markers on chromosome three of the double haploid reference genome. The same location and SNP markers were detected using fruit circumference best linear unbiased prediction data and FC mean data. Using Primer-Blast, primers are being designed from sequences in that region and screened to identify PCR-based markers that can help to distinguish genotypes with good fruit filling from those with poor fruit filling characteristics. Pollination of NARITA hybrids is ongoing to determine their fertility despite being triploid.

### **Conclusion / next steps**

Genomic prediction is possible in banana and it is expected to improve breeding efficiency if applied on breeding populations. It will allow selection of best hybrids for multiple traits simultaneously. The high prediction of fruit filling could be used in negative selection of triploid genotypes that are likely to bear inferior fruits and thus, reduce the number of progenies to be evaluated in the field. The prediction models should be validated before being deployed in banana breeding. Following the observations made from genomic predictions, the next step was to conduct a GWAS for fruit filling within the training population. GWAS revealed that significant single nucleotide polymorphism markers associated with fruit circumference were located on chromosome three of the banana reference genome. Specific PCR based markers are being developed for that region as an alternative

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to genomic prediction for in house analysis of fruit filling. Finally, it is important that the fertility of triploid hybrids with high GEBV is tested so that they are also selected as parents for further crossing.

## PhD Research Progress Report (2016-2017)

**Name: Allan Waniale**

**Title:** Floral Biology and Crossability Studies for Improving Matooke and Mchare Banana (*Musa* ssp.) Breeding in East Africa

**Supervisor:** Assoc. Prof. Settumba B. MUKASA (Makerere University) and Prof. Rony Swennen (IITA Supervisor)

**Timeline of study:** May 1, 2015 to April 30, 2018

**University:** Makerere University, Kampala

### Research Objectives

List the individual topics of study – objectives or study areas

1. Ascertain pollination barriers at different developmental stages of banana flowers and determine when the flowers are most receptive for successful controlled pollination
2. Develop suitable *in vivo* pollination techniques that can be adopted to improve controlled pollination of Matooke and Mchare bananas
3. Determine the efficacy of the best new *in vivo* pollination techniques for overcoming seasonality effects and male differential effects of banana seed set

### Achievements

Highlight significant achievements – e.g. in bullets

- Coursework completed between September 2015 and July 2016
- Proposal successfully defended in January 2017
- One doctoral committee meeting held on 2<sup>nd</sup> May, 2017
- Increased seed set by 65% in seed fertile matooke – Enzirabahima
- Determined that glucose is a better energy source for pollen viability test and *in vivo* germination

### Background/introduction

Brief background

East African highland bananas (Matooke and Mchare cultivars) play an important socio-economic role in the livelihoods of smallholder farmers of the great lakes region in East Africa. However, pests and diseases significantly reduce the crop yield thus, directly affecting the livelihoods of the communities involved in banana production. Improving the existing cultivars is a viable option. However, most of the popular cultivars are sterile and hardly set seed – which makes their improvement through conventional breeding difficult. The core aim of my research is to manipulate banana flowers in order to increase seed set and break sterility in seed fertile and seed sterile EAHBs bananas respectively. This will broaden the progeny base as well as parents used in breeding EAHBs. Ultimately, there will be an increase in breeding efficiency for better EAHBs for small holder farmers in the East African region.

### Objective / Study 1: Ascertain pollination barriers at different developmental stages of banana flowers and determine when the flowers are most receptive for successful controlled pollination

Study one is dealing with study of flowers to identify entry where banana flowers are most receptive for successful controlled pollination. A photographic catalogue is being finalized for all flower developmental stages including pre-emergence, post-emergence, anthesis and post anthesis. It has been observed that style length undergoes minimal changes during the final stages development but stigma shape and colour change at a fairly fast rate. Timelapse movies have also been made to determine the time of flower opening and factors that influence opening. A Nikon D810 camera was positioned to capture pictures of banana flowering at 5 minutes intervals starting from just before the first bract opened. Weather data was also simultaneously taken using an automated system at one hour intervals; this included solar radiation, precipitation, wind speed, temperature, and relative humidity. Pictures have been taken on Enzirabahima, Nakitembe, Mlelembo and Kamunyira. It was observed that Nakitembe which is a seed sterile matooke has a much slower rate of opening compared to others. It has also been noted that 1 – 3 bracts can open simultaneously on the same bunch and bract opening generally starts in the evening. For reliability of results, the procedure is going to be repeated at Sendusu in Namulonge.

Still in study one, I have been trying to find ways of germinating pollen much faster both *in vitro* and *in vivo*. Preliminary results (Table 1) have shown that glucose works better than sucrose in germination media and it can aid faster germination of pollen on stigmas. Pollen germination media was prepared by weighing;

- 0.01g H<sub>3</sub>BO<sub>3</sub> (Boric acid),
- 0.25g MgSO<sub>4</sub>.7H<sub>2</sub>O (Magnesium Sulphate),
- 0.25g KNO<sub>3</sub> (Potassium Nitrate) and,
- 0.4g Ca(NO<sub>3</sub>)<sub>2</sub> (Calcium Nitrate).

The compounds were mixed into a one litre stock solution using deionised water and varying sugar concentrations were made and used for pollen germination as described in table 1. Diluted Nectar was used as a control.

**Table 1.** Pollen germination using different media after 3 hours of incubation in a humid chamber

| Media                | % Germ. Calcutta 4 | % Germ. 8075 | Mean       |
|----------------------|--------------------|--------------|------------|
| Diluted Nectar (1:5) | 40                 | 35           | 38b        |
| <b>3% Glucose</b>    | <b>65</b>          | <b>85</b>    | <b>75a</b> |
| 3% Sucrose           | 10                 | 8            | 9c         |
| 10% Glucose          | 7                  | 8            | 8c         |
| 10% Sucrose          | 25                 | 20           | 23b        |
| 20% Glucose          | 8                  | 5            | 7c         |
| 20% Sucrose          | 13                 | 20           | 17c        |

### Objective / Study 2: Develop suitable *in vivo* pollination techniques that can be adopted to improve controlled pollination of Matooke and Mchare bananas

Study two involves been manipulating flowers with different pollination techniques to come up with the best *in vivo* pollination technique. Preliminary results show that pollination media on stigmas can increase seed set in bananas. The germination media used was 3% glucose only with the rationale that the other requirements would be obtained from the stigma surface (Figure 1) but seed increase was only obtained in the dry season (Figure 2). Early pollination (about a day before flower opening) and pollination in the evening has not had any increase in seed set (Table 2). Results show that complete germination media enable germination of pollen and fertilization of ovules in both seed fertile and seed sterile EAHBs but ovules abort after 2 weeks (Figure 3). I am currently using germination media in combination with growth regulators especially those that are directly or

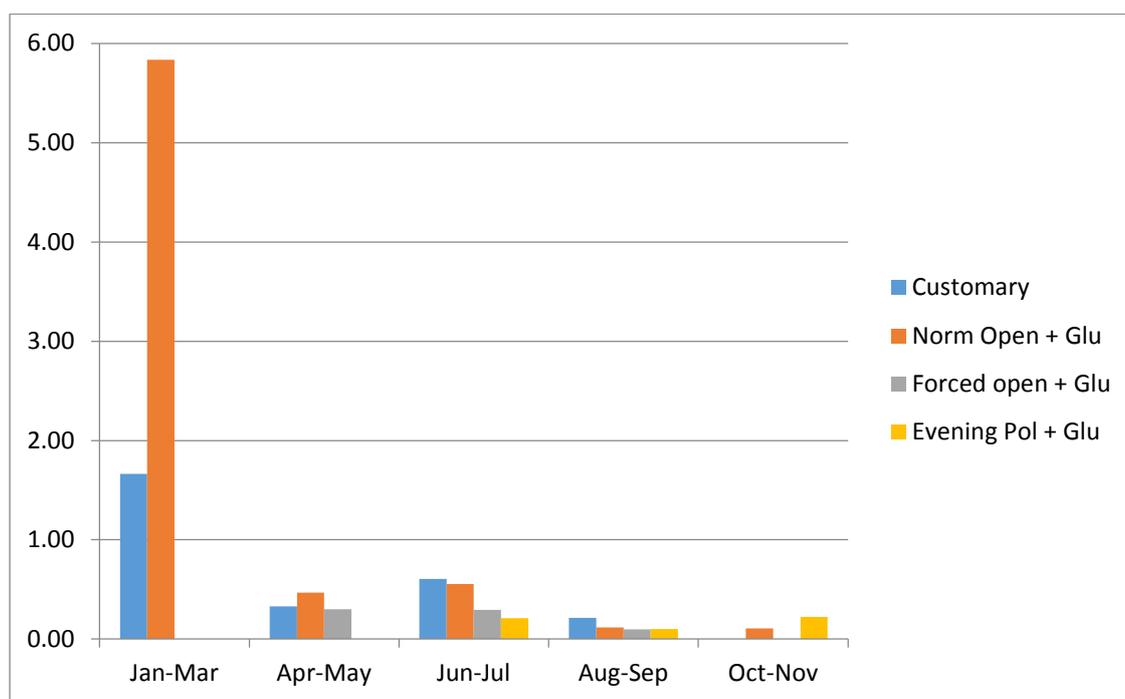
indirectly involved in seed development like auxins, salicylic acid, and abscisic acid (ABA). Preliminary results are showing that auxins are not working but salicylic acid and ABA are yet to be fully tested.



**Figure 1:** a photographic description of early pollination of bananas using a 3% glucose solution

**Table 2. Means of seed set per 10,000 ovules of EAHBs pollinated with Calcutta 4 between January and November 2016 using different pollination techniques**

| Pollination Technique              | No. Pollinated | No. with seed | Av seed per 10,000 ovules |
|------------------------------------|----------------|---------------|---------------------------|
| <b>Enzirabahima (seed fertile)</b> |                |               |                           |
| Customary                          | 43             | 14            | 0.62                      |
| Customary + 3% Glucose Solution    | 42             | 14            | 1.02                      |
| Evening + Glucose Solution         | 42             | 9             | 0.22                      |
| Early + Glucose Solution           | 27             | 4             | 0.15                      |
| <b>Nakitembe (seed sterile)</b>    |                |               |                           |
| Customary                          | 34             | 0             | -                         |
| Customary + 3% Glucose Solution    | 35             | 0             | -                         |
| Evening + Glucose Solution         | 18             | 0             | -                         |
| Early + Glucose Solution           | 23             | 0             | -                         |



**Figure 2:** Mean seed set per 10,000 ovules in EAHB – Enzirabahima pollinated with Calcutta 4 using various pollination techniques.



**Figure 3:** Smaller ovules from a bunch that was not pollinated compared to a few bigger aborted ovules from a pollinated matooke bunch

**Objective / Study 3: Determine the efficacy of the best new *in vivo* pollination techniques for overcoming seasonality effects and male differential effects of banana seed set**

Study three will involve the test of efficacy of the best new pollination technique for overcoming seasonality effects and different success levels when different male parents are used. But new *in vivo* pollination techniques are still being fine tuned and study three is yet to start. It will involve pollination of one seed sterile and one seed fertile EAHB with 8 selected male parents over a period of six months to evaluate consistence.

**Conclusion / next steps**

All bananas seem have a potential for producing seed and the secret lies in finding the right proceed to overcome pre- and post-fertilization barriers. Observations indicated that complete germination media can enable nearly complete fertilization of all ovules from ovaries (fruits) but they abort at about 2 weeks post pollination and fertilization. Hope lies in the use of hormones that are directly and indirectly involve in seed development. This is because fertilization is taking place but seed is not able to develop at early stages. There is a plan to develop an ovule culture procedure in bananas using wild types (Calcutta 4) and wild relatives (*Heliconia* and *Strelitzia*). In crops such as *Alstroemeria*, *Cyclamen*, *Lycopersicon*, *Nicotiana* and *Vitis*, ovule culture has been used and shown higher success than normal seed development. This may be the hope for improving seemingly sterile bananas.



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**Mid Term Written Report**

**PhD Research Progress Report (2016-2017)**

**Name:** Bert Stevens

**Title:** Modeling of the Banana cropping structure using the *AquaCrop* growth model

**Supervisor:** Prof. Rony Swennen, Prof. Jan Diels

**Timeline:** 1<sup>st</sup> August 2015 to 31<sup>st</sup> July 2019

**University:** KULeuven, Belgium

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## Summary

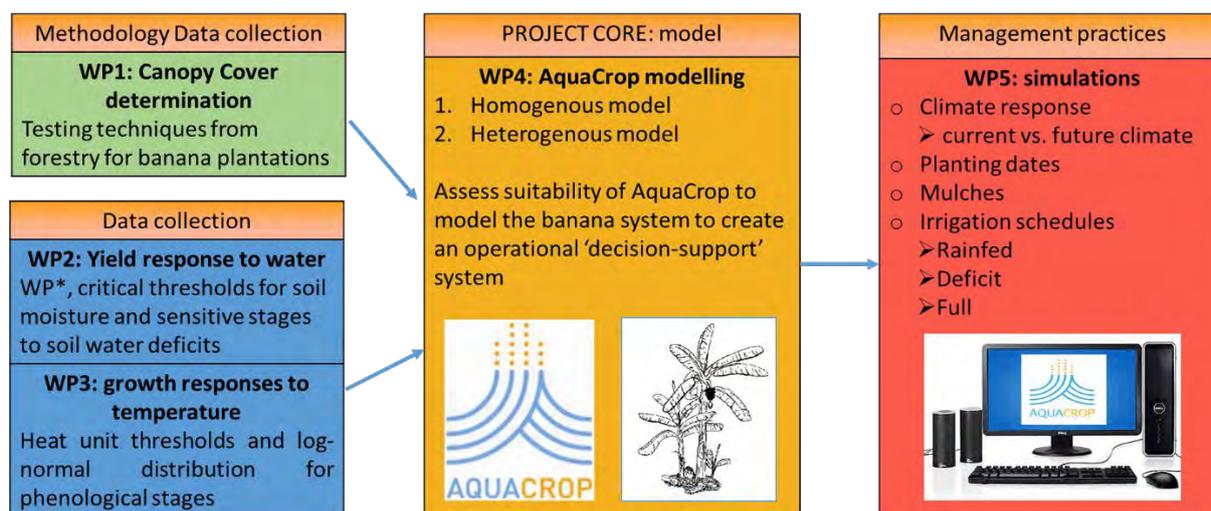
Currently, banana plantations are not optimally managed with water. Plants do not receive enough water when needed, or receive too much water when not needed, resulting in yield and water loss. To aid growers to address these problems we will adapt the *AquaCrop* computer simulation model for banana plantations. *AquaCrop* is a model that simulates how plants grow in response to water in the soil. The model exists for crops such as maize, wheat and barley where all individual plants in a field are of the same age and size. However, it does not exist for crops such as banana where plants of all ages and sizes grow together at the same time. We will be the first to include this 'heterogeneous' population structure in *AquaCrop*. With *AquaCrop* we can determine the effect of different management options and irrigation schedules on water use and yield of a banana plantation; we can simulate how a plant will grow under different conditions and observe yields and water uses for each management option we specify in the model. As such, we create a handy decision-support tool to guide water management in banana plantations.

In this report, a state of affairs is given regarding the research with a focus on the experiments required to get the necessary data for calibration purposes. We conducted 2 experiments: a greenhouse trial where plants were placed under 3 different soil moisture regimes to determine their sensitivity and growth under moisture stress and a field trial where plants are grown under optimal irrigation and deficit irrigation, which will act as the bulk of the database for modelling purposes.

## 1. State of the art

This PhD-thesis aims at increasing the resilience of banana production in drought stressed environments through adapting the *AquaCrop* model for banana. The model will serve as a decision-support system for implementing management and irrigation schedules in banana plantations to cope with drought.

Underlying schematic overview shows our research methodology to reach the abovementioned goal.



**Figure 2:** research methodology and flow of workpackages (WP)

This mid-term report will focus on the data collection part: WP1, WP2 and WP3. A thesis student (Casper Van Cleemput) is currently testing techniques to easily determine the canopy cover of banana plantations and his research fits in WP1. Another thesis student (Tonia El Hajj) is studying the irrigation structure in our plantations and her work fits in WP2.

I expect to have gathered all the data for *AquaCrop* modelling by the end of 2019. This includes growth measurements over 2 growing cycles in the field and more detailed greenhouse experiments to collect data necessary to calibrate *AquaCrop*, that is not easily determined in the field.

As this mid-term report focuses on the data collection part, an overview of experiments already carried out are given. The experiments are categorized in 2 categories: a greenhouse trial and a field trial.

### 1.1 Greenhouse trial

The **objective** of the greenhouse trial was to find several necessary parameters for the *AquaCrop* growth model for the banana plant. In this trial, we wished to determine 1) the normalized water productivity (WP\*) for our banana varieties and 2) pinpoint the soil water content (% of total available water) at which (a) the canopy expansion starts declining, and (b) at which transpiration starts to be reduced.

An additional objective of the greenhouse trial was to determine simple allometric relationships between the aboveground dry biomass and other easily measurable growth parameters of our banana plants.

The greenhouse trial was carried out between November 2016 and March 2017.

### Experimental setup

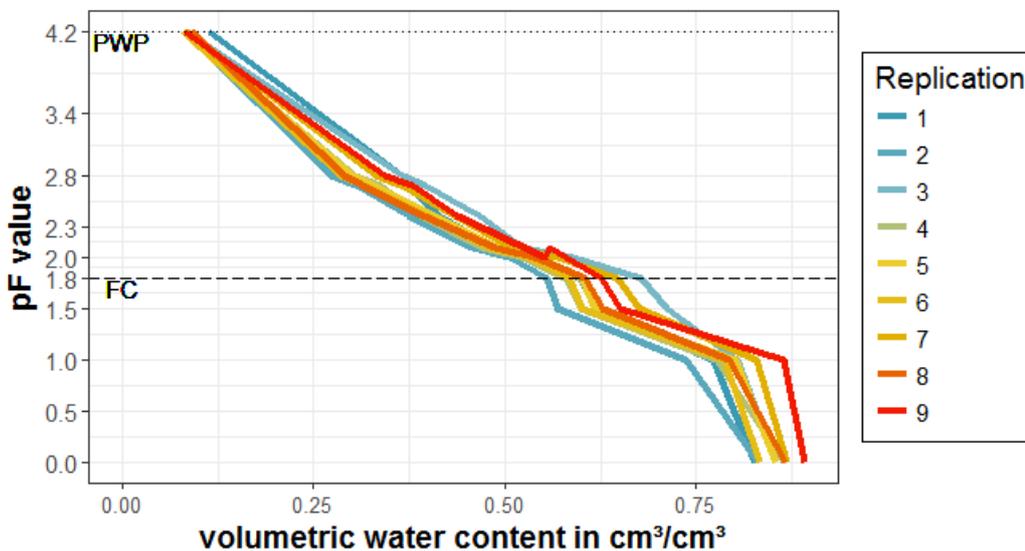
Banana seedlings were allocated to 3 different soil moisture levels and their growth was followed over the course of 7 weeks. During the first month, all plantlets were subjected to an optimal watering regime to allow optimal establishment. After 1 month at field capacity (growth phase), 72 seedlings of cultivar Mchare seedlings were assigned to the three irrigation treatments specified by a pF range as shown in Table 3 (experimental phase).

**Table 3:** irrigation/ soil moisture treatments

| Treatment | pF range | Volumetric water content lower threshold (m <sup>3</sup> /m <sup>3</sup> ) | Gravimetric Water content |
|-----------|----------|--|---------------------------|
|-----------|----------|--|---------------------------|

|                                    | (based on pF curve) |          | (kg water/kg soil) |
|------------------------------------|---------------------|----------|--------------------|
| <b>Irrigation</b>                  | 1.8 –<br>2.1        | 0.488416 | 3.1358             |
| <b>Deficit irrigation 1 (Def1)</b> | 2.1 – 2.4           | 0.409435 | 2.8284             |
| <b>Deficit irrigation 2 (Def2)</b> | 2.4 –<br>2.7        | 0.341471 | 2.1923             |

To keep the soil within its pF range, a soil-moisture retention curve was created for the potted soil, shown in Figure 3. Pots were covered with aluminium foil to exclude evaporation, and put on trays to exclude water loss from below. This enables to relate the pF values to volumetric water contents (m<sup>3</sup>water/m<sup>3</sup>soil), and these volumetric water contents to gravimetric water contents (kg water/kg soil) with the bulk density (BD) of water and soil. The BD of soil measures 0.155754 g/cm<sup>3</sup>, which is very low, but could be explained due to its high peat percentage.



**Figure 3:** pF curve potted soil. Permanent wilting point (PWP) has a pF value of 4.2 and Field Capacity (FC) has a pF value of 1.8.

For each of our pots, upper and lower target weights were determined to keep the pots within their specified soil moisture ranges. These target weights were calculated as follows:

$$\text{TARGET WEIGHT} = \text{kg water} + \text{kg dry soil} + \text{kg plant} + \text{kg pot} + \text{kg tray} + \text{kg cover}$$

Which then allows us to calculate the amount of water present in a pot:

$$\text{Kg water} = \text{Total} - \text{kg plant} - \text{kg dry soil} - \text{kg pot} - \text{kg tray} - \text{kg cover}$$

The pot, tray and cover weight were noted before the experiment started, and do not change over time. With the BD of the soil, and the volume of soil in our pots we determined the mass of dry soil present in our pots. The only weight changes that occur are those due to the growing of the plant (kg plant) or due to transpiration (and irrigation) (kg water). Every 2 weeks, the plant weight is updated when 18 plants (6 plants per irrigation treatment) are harvested destructively. Every plant is given a target weight corresponding to a certain amount of water (kg water) required to stay within a moisture regime and water is added daily to each pot to reach this target weight.

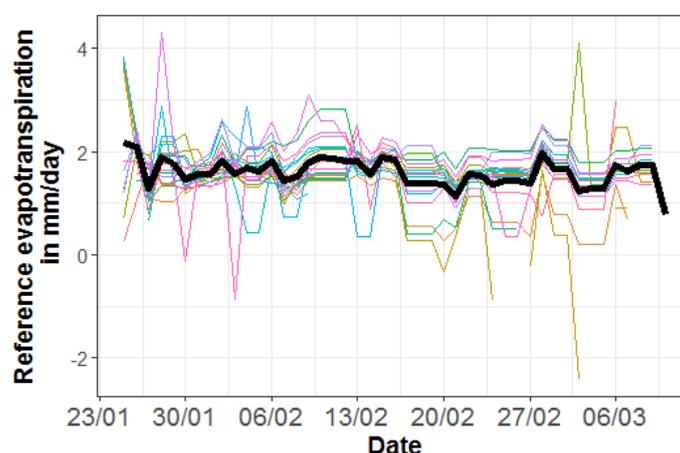
Following parameters were measured on a daily basis: transpiration of the seedlings and reference evapotranspiration; on a weekly basis: girth at base, height, leaf number, canopy cover and 3 plants per treatment were destructively harvested each week to measure data on biomass (fresh and dry) of the different components (pseudostem, roots, corm, leaves). For each of the seedlings, water loss (transpiration) was

measured by weighing the mass of the system on a daily basis early in the morning. The weight difference was then due to transpiration over the previous day. After weighing, we added water and noted the new weight as the starting weight for that day. This allows us to follow the daily transpiration of the seedlings and follow their weekly growth. As  $ET_0$  is approximately equal to the transpiration of an active growing reference grass of 11cm height without stress, we opted to grow ryegrass in the greenhouse compartment to ‘mimic’ the reference surface. Again we weighted our grass before and after giving water on a daily basis to determine the daily transpiration. The variety was English ryegrass, cv HUMBI 1, and this allowed us to determine the  $ET_0$  of the greenhouse compartment on a daily basis.

These measurements would allow us to see at which moisture level (Table 1) canopy cover expansion and transpiration of our banana plants would be reduced, and what would be the incorporated aboveground biomass per unit water transpired (WP).

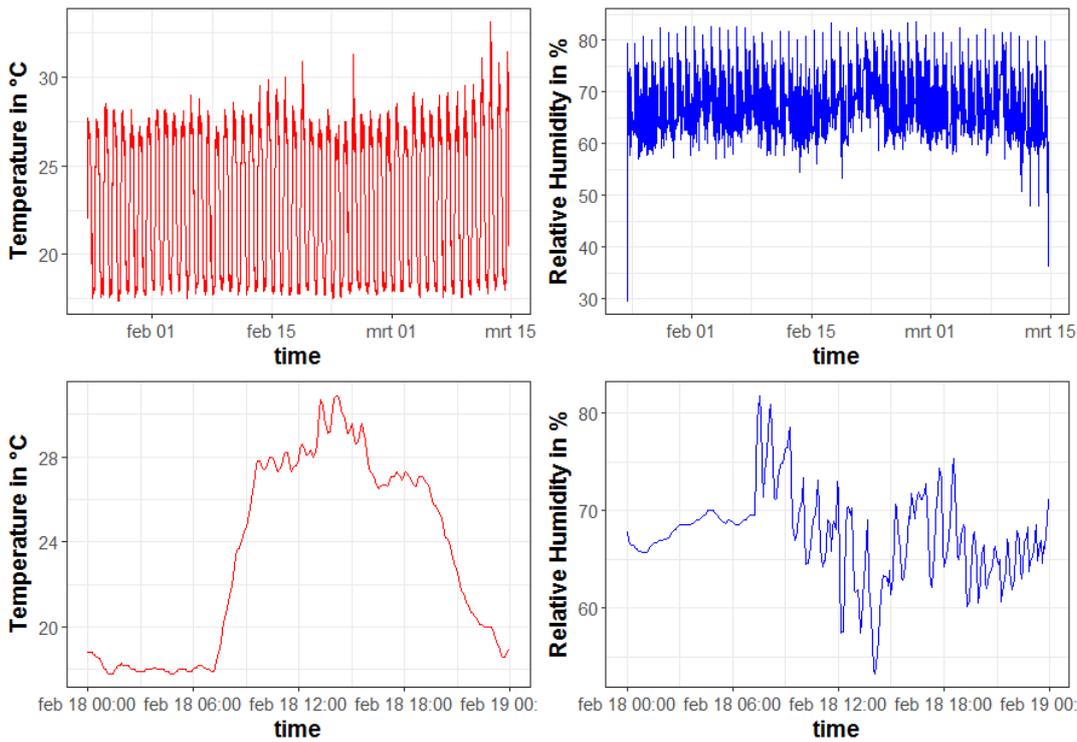
## **Results**

From the weight loss of the grass reference crop, we calculated the daily reference evapotranspiration. Figure 4 shows the reference evapotranspiration of the different grass trays. The dark line shows the mean  $ET_0$  in mm/day. This is about 1.65 mm/day over the entire experimental period, which took place between 24 January and 10 March. This seems to be a rather low value but could be due to the fact we performed the experiment in the winter in the greenhouse.



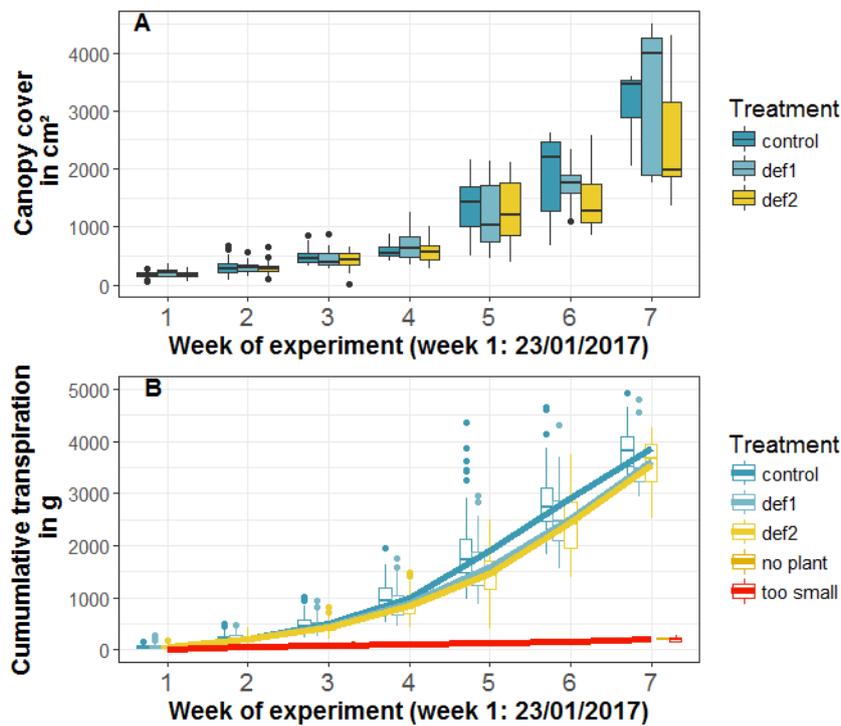
**Figure 4:** reference evapotranspiration ( $ET_0$ ) of the greenhouse crop as calculated by weight loss of our grass trays. Each color notes the  $ET_0$  of a different grass tray and the black bold line is the mean  $ET_0$  calculated from all the grass trays

The collected weather data are shown in figure 4.



**Figure 5:** climate data of the greenhouse compartment. Above: entire experimental period, below: 18 February to show the diurnal pattern.

Every week we determined the canopy cover of our banana plants to check at which moisture regime the canopy cover was affected. Figure 5 shows that the canopy cover of our different banana groups does not differ over the entire experimental period. Figure 5 also shows that the cumulative transpiration of the banana groups does not differ significantly over the entire experimental period. All plants showed severe drought stress symptoms as their leaves were hanging as shown in Figure 7, regardless of their moisture regime. In some cases the leaves were hanging so much that the petioles of our plants broke.



**Figure 6:** A) canopy cover of banana plants in the different treatment groups, B) cumulative transpiration of banana plants in the different treatment groups.



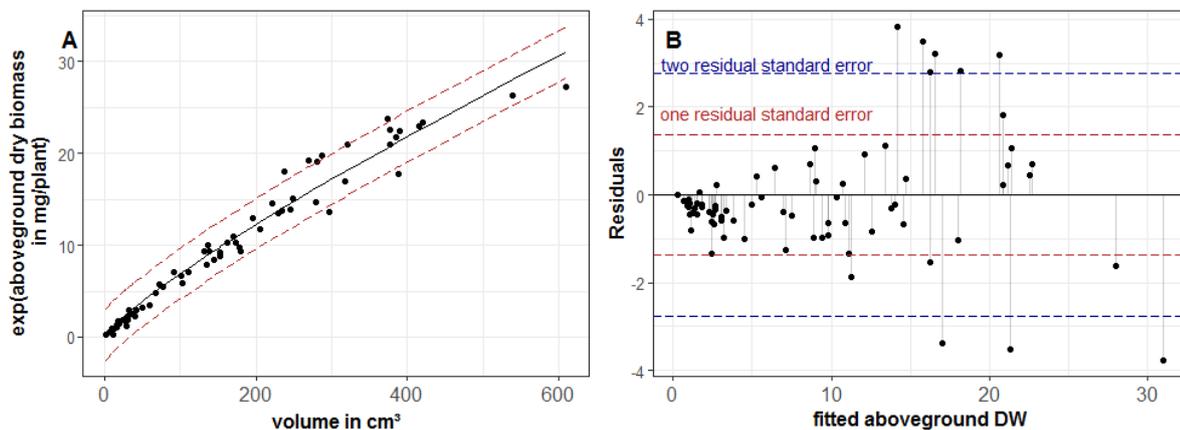


**Figure 7:** banana plantlet with hanging leaves. Healthy white roots however do not show signs of water surplus or deficiency.

An additional objective of this trial was to create allometric relationships for young banana plants to be used in our field trial. We want to determine a functional relationship to estimate our aboveground dry biomass (Dry weight, DW) as: Aboveground DW = f(collected growth data).

Our predictor variables were: Amount of leaves [amount] , height of plant [cm], girth at base [cm], canopy cover of plant [cm<sup>2</sup>], LAI-leaf area index at harvest [cm<sup>2</sup>], volume of pseudostem, [cm<sup>3</sup>] (determined by approximating the pseudostem as a cylinder) and the principal components of all this data. The best fit to our data was a power function with volume as explanatory variable. The function and residuals are shown in Figure 8. The mean deviation of the residuals is 1.381 from the mean, which is within 1 standard deviation from the mean.

$$\text{aboveground DW} = 0.15098 \times \text{volume}^{0.83026}$$



**Figure 8:** power function with Volume. A) black line is fitted line with functional equation. Red line is one standard error from the lines. B) residual plot.

### **Conclusion**

The normalized biomass water productivity (WP\*) and critical soil moisture threshold values for canopy expansion ( $p_{exp}$ ) and stomatal closure ( $p_{sto}$ ) were expected to be determined by comparing growth and transpiration under different soil moisture regimes in our pot-trial. However, the pot-trial revealed that there was no significant difference between transpiration and biomass incorporation of the different treatment groups. All plants showed symptoms of moisture stress regardless of the treatment. We were therefore unable to determine the soil moisture regime where canopy cover expansion and transpiration started to be reduced.

Possibly the calculations of our target weights were unsatisfactory and we needed to add more water but it was simply not possible to add more water, as the water would be lost due to overflowing trays and pots. Soil moisture contents were thus satisfactory in the pots. Possibly we gave too much water to our plants, but we had healthy white roots indicating no waterlogging or deficiency. This entails that the plants were not water stressed but were suffering from another stress. As we used a standard nutrient scheme of growing bananas with fertigation, and leaves were not showing any deficiency symptoms, we conclude that nutrient deficiency was also not a problem. The climate also does not seem to be a problem as temperatures during the experimental period ranged from 17.4 at night to 33.1 °C during the day and relative humidity ranged from 47.2 to 83.5 %.

Therefore, we believe there was something wrong with the plants themselves, or there was an external stress factor which was not moisture-stress, fertility-stress or climate influencing the plants. Therefore, we were unable to determine the critical soil moisture threshold values for canopy expansion and stomatal closure for our banana varieties. Given the nature and amount of our plant material it needs to be seen if our allometric relationships and WP\* can be used in our field trial and for other greenhouse tests.

The greenhouse trial therefore does not yield satisfactory results and needs to be repeated in the future with a new cultivar and a different method of water gift. Instead of repeating this test in the greenhouse, we are going to repeat this test under field circumstances.

## 1.2 Field trial

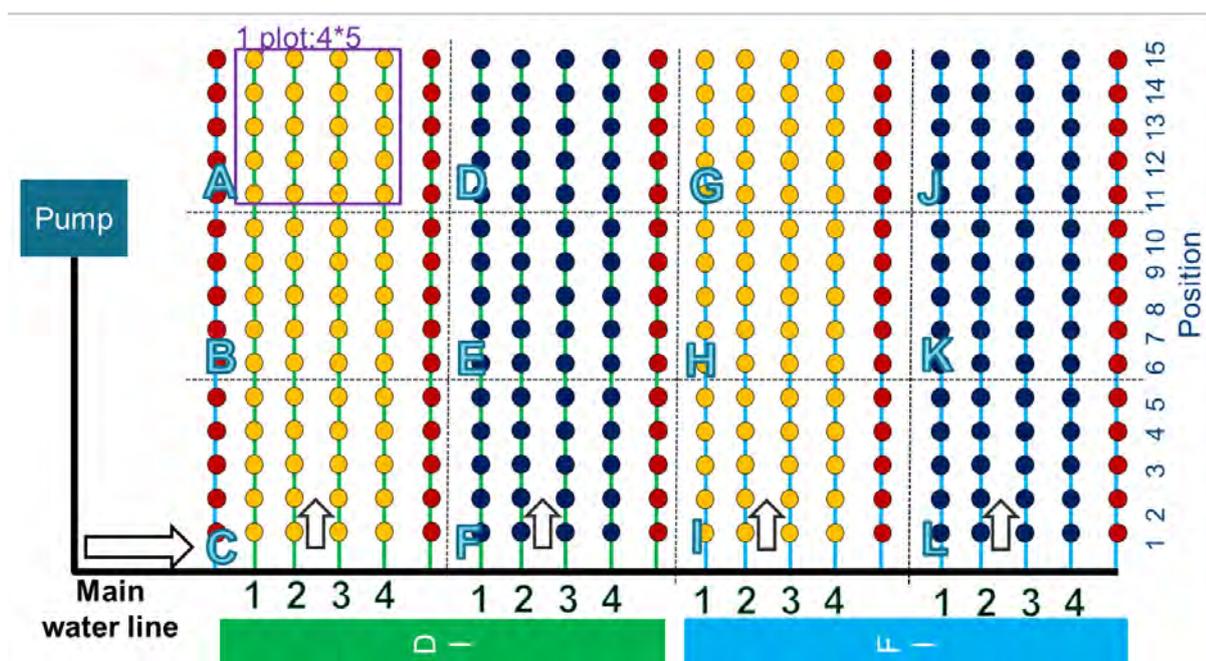
The **main objective** of the field trial is to create a database to calibrate *AquaCrop* for heterogeneous banana plantations. Currently the *AquaCrop* structure only allows to model homogeneous crops, and the modelling of heterogeneity will therefore constitute the main challenge of this thesis. Climate, soil, soil moisture, management and crop growth variables are collected throughout the 2 year field trial, with a special focus on the suckering behaviour of our banana plants, as this will determine the heterogeneity of our second cycle.

The banana plantation is drip irrigated, and we were the first to use this new irrigation system. Hence, an additional part of the fieldwork consisted in managing and studying this irrigation system. Tonia el Hajj, an MSc student from KU Leuven, conducted fieldwork on this topic in the summer of 2017.

Another thesis in the summer of 2017, carried out by Casper Van Cleemput an MSc student from KU Leuven, focused on a comparison of techniques to determine the canopy cover and leaf area index of banana plantations. *AquaCrop* needs data on the canopy cover of plantations.

### Experimental setup

Two highland banana cultivars 'Enshakara- Matoke' and 'Hutigreen-Mchare' tissue culture plantlets were planted on 3 May 2017 in a field trial in Arusha, Tengeru, Tanzania as shown in Figure 9. The field is equipped with a drip irrigation system and the cultivars are subjected to 2 different soil moisture regimes: 1 full irrigation 'FI' schedule and a deficit irrigation schedule 'DI' (only irrigation if more than 50% of plants start unfurling a cigar leaf before it fully exits the pseudostem). Irrigation is scheduled per row. Soil moisture is followed by time domain reflectometry (TDR) sensors installed at two depths (0-30 cm and 30-60cm) with one replication in each plot. Each morning TDR sensors are read out, and based on soil moisture irrigation occurs. During the first 4 months, soil moisture values were not allowed to be depleted to more than 25% total available water to ensure optimal growth of the seedlings. Afterwards, plants in the RF treatment were shut off from irrigation until 50% of plants in a plot were visibly affected (cigar starts unfurling inside the plant), and water is added to FC.



**Figure 9:** field layout of rows. Irrigation is specified per row. Orange notes “Matoke” and dark blue notes “Mshare”. the red dots note “Mbwazirume” borderrows rows. Number of Mshare:  $6 \times 4 \times 5 = 120$  and number of Matoke = 120. Arrows note the direction of waterflow.

To ensure optimal growth without nutrient stress, we apply fertilizers as shown in Table 4: fertilizer application rates

**Table 4:** fertilizer application rates in the field trial

| Fertilizer type              | Application rate (g/planting hole) |      |      |      |       |         | Total (g) |
|------------------------------|------------------------------------|------|------|------|-------|---------|-----------|
|                              | At planting                        | 2MAP | 3MAP | 5MAP | 6 MAP | 8-9 MAP |           |
| Urea (N)                     | 50                                 | 0    | 50   | 0    | 50    | 50      | 200       |
| MoP (K)                      | 62.5                               | 0    | 62.5 | 0    | 62.5  | 62.5    | 250       |
| MgSO <sub>4</sub> (S and Mg) | 30                                 | 0    | 30   | 0    | 30    | 30      | 120 g     |
| TSP (P and Ca)               | 0                                  | 50   | 0    | 50   | 0     | 0       | 100       |
| Animal manure/ cow dung      | 20L                                | 0    | 0    | 0    | 20l   |         | 30,000    |

In the first year (first cycle) the dry season will occur during the vegetative stage. Water deficits in the RF treatment will be relieved during the period of estimated floral initiation. In the second cycle the dry season will occur during and after floral initiation. This allows to compare the effect of drought stress at these stages on production.

During the trial, following parameters are measured:

- **Reference evapotranspiration (ET<sub>0</sub>)** is measured following the FAO 56 Penman-Monteith procedure, which serves as the standard for computing *ET<sub>0</sub>*. Necessary climatic data (rainfall, temperature, radiation, relative humidity, wind speed) are collected with a weather station on site (Decagon Services).
- **Management parameters: monthly weed cover and weeding frequency and fertilizer scheduling**
- **Soil moisture** is measured with TDR sensors which are installed at different depths (0-30 cm and 30-60 cm).
- **Banana growth parameters** are measured every 2 weeks on all plants per plot. Parameters include: length and width of each leaf, physiological active leaves, dead leaves, pseudostem height and girth at base and 1m height, number and position of suckers. At harvest, fresh bunch weight (FBW), number of hands and fingers,

middle finger girth and length, and dry bunch weight (DBW in kg) are measured. Plant biomass will be estimated from these collected data based on allometric relations determined by Nyombi et al. (2010) which are tested and adapted for our varieties through destructive sampling of border plants.

- **Rooting pattern** is determined through trenching methods for 6 plants per cultivar treatment combination at 4 months, 8 months and 12 months to determine maximum rooting depth and growth.

## Results

In Figure 10: weather data collected up to 13 July 2017. an overview of collected weather data necessary to calculate  $ET_0$  is given. After  $ET_0$  calculation we obtained lower  $ET_0$  values than could be expected in the Tengeru region as shown in Figure 11 and Figure 12. The solar radiation sensor appears to measure lower radiation values than what could be expected in the Tengeru region during this period. It appears the radiation as measured by the weather station gives lower values than expected.

It is also shown that temperature fluctuations are less during the rainy season than in June. Lower temperatures are reached which could give our plants a cold shock during this period.

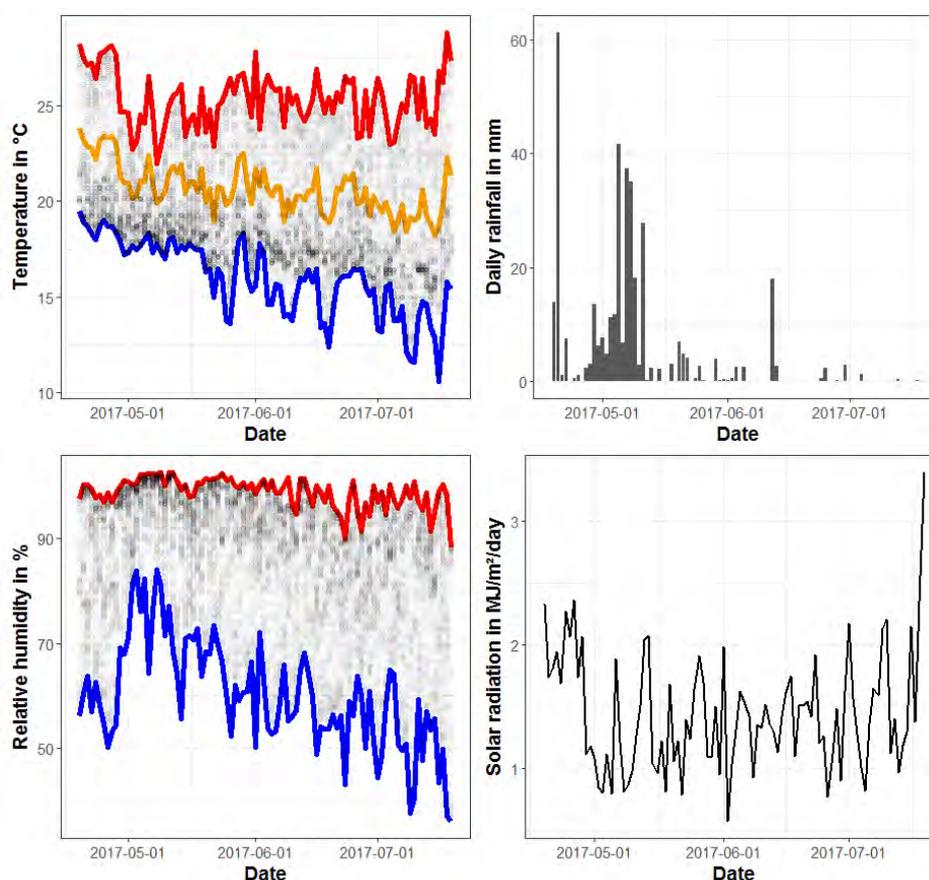
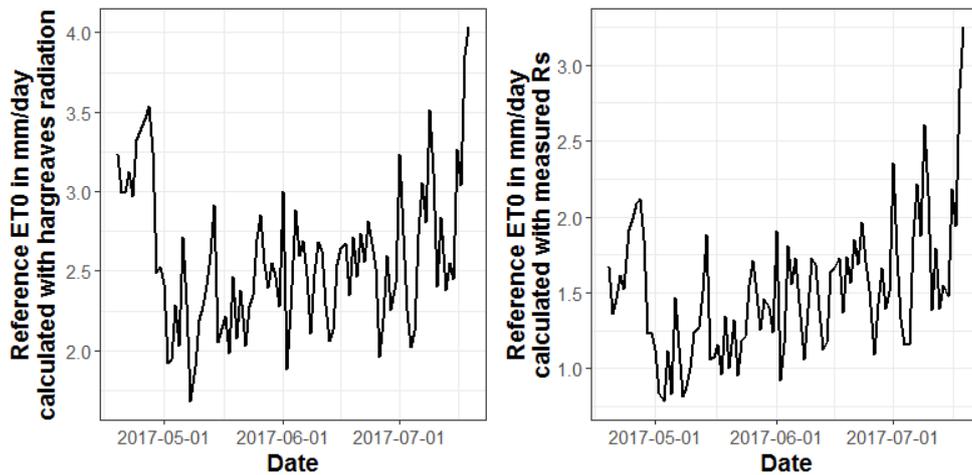
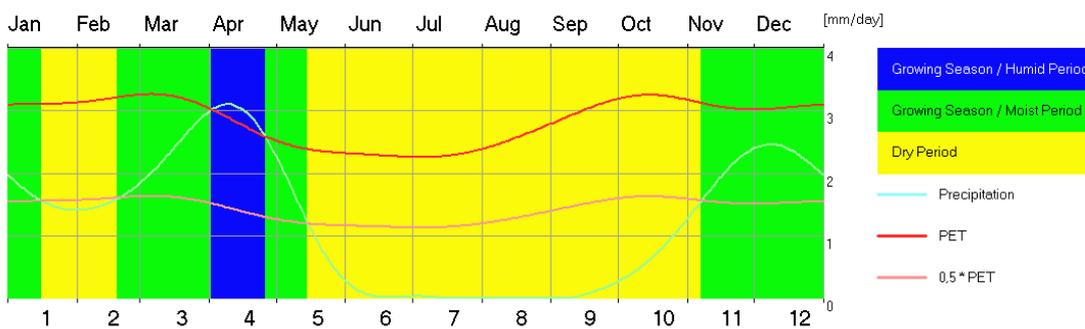


Figure 10: weather data collected up to 13 July 2017.

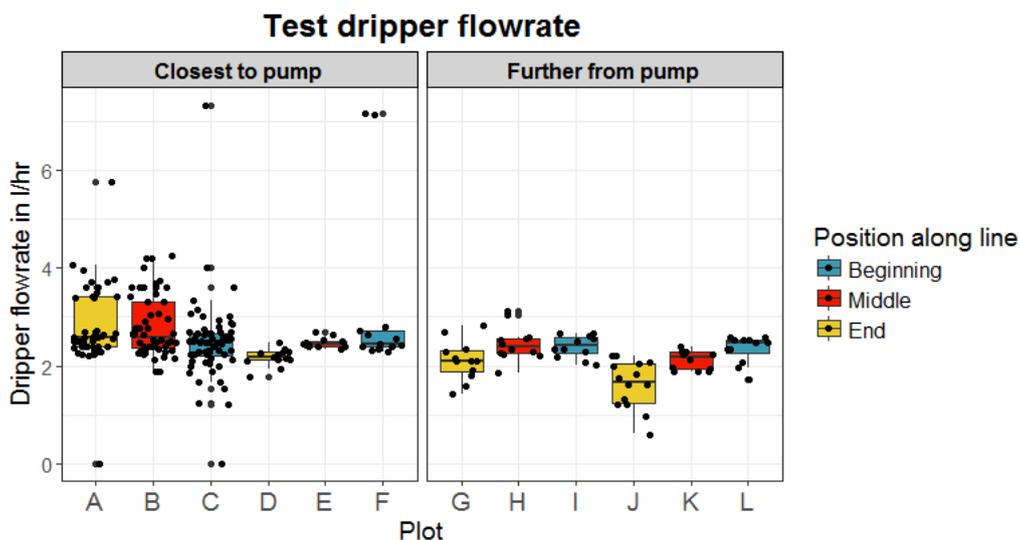


**Figure 11:** calculation of ET<sub>0</sub>. Left: ET<sub>0</sub> with missing radiation data, right: ET<sub>0</sub> with dubious ET<sub>0</sub> data



**Figure 12:**Vegetation period in Tenggeru. Monthly ET<sub>0</sub> is between 2 and 3 source: New LocClim database.

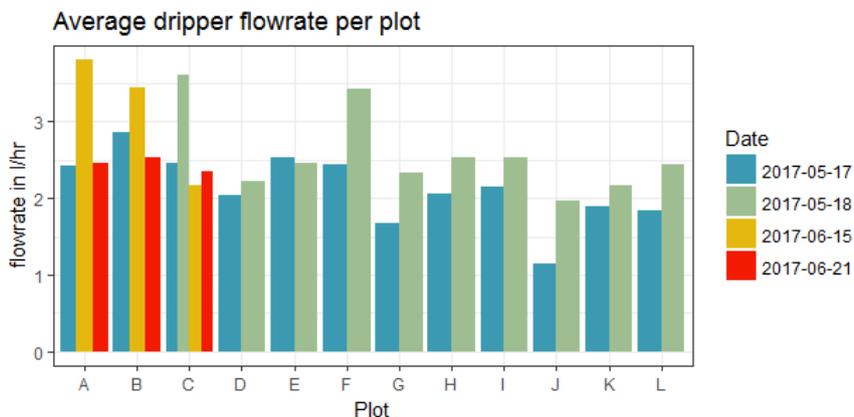
Studying the dripper flowrate, it appears not every dripper has a uniform flowrate. Plots located closer to the beginning of the secondary flowlines have a higher discharge in most cases as shown in Figure 13(F, I and L). However the difference between discharge of plots is only significant in the plots located furthest from the pump (J, K and L). This needed to be kept in mind during irrigating and determining the soil moisture balance of these plots.



**Figure 13:** dripper flow rate test with date from 21/06/2017. Significant difference between the different plots at significance level 0.05. (Kruskall Wallis).

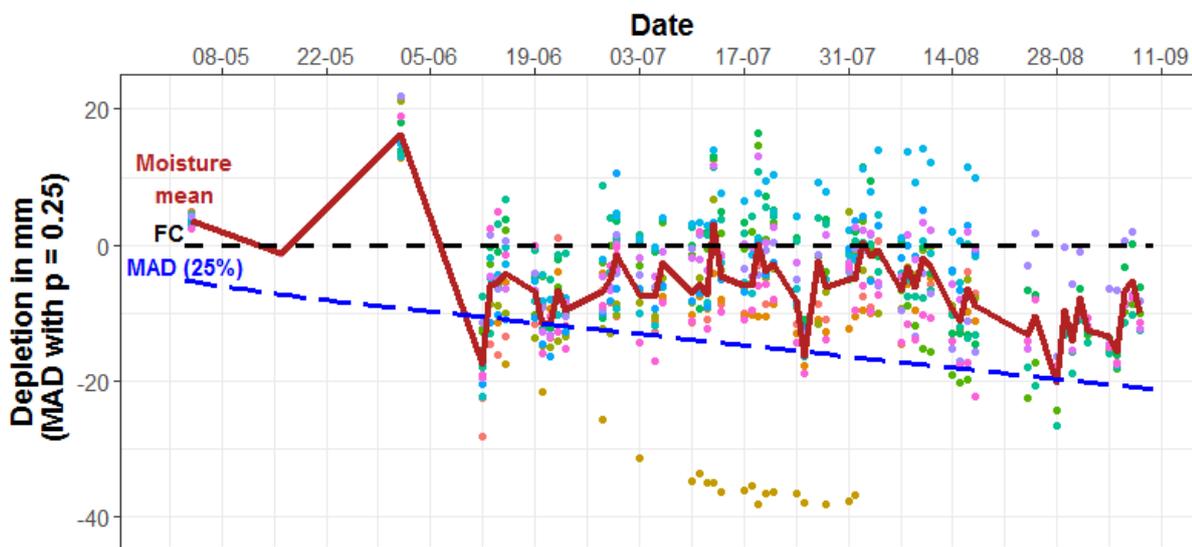
It also appears the dripper flowrates differ in time as shown in Figure 14. This is due to the fact one pump gives water to all fields in IITA and not solely to my plots. Depending on the amount of fields irrigated at the same time, the discharge changes. Since there are no pressure meters on the irrigation lines, we have to make sure

that the settings of the irrigation system are always the same. This means during irrigation, the irrigation infrastructure needs to be opened to the same amount of irrigation fields (= big compartments in the entire IITA campus) as the amount of big fields which are irrigated will have an effect on the pressure and hence the flowrate per dripper.



**Figure 14:** Difference in dripper flowrates depend on pressure in the irrigation lines.

Irrigation occurred based on soil moisture values since weather data was not present on a daily basis. TDR sensors were installed on 11 June, and used to measure soil moisture daily since then. Before, we measured moisture gravimetrically every 2 weeks. Figure 15 shows the soil moisture depletion levels in our soil from planting onwards and it shows moisture values are near FC and even higher in the rainy season. Between 02/06 and 14/06 the irrigation infrastructure was broken, and irrigation could not be applied by our drippers. We searched for a solution and were able to irrigate by hose on 08/06. However, in this period plants were slightly drought stressed.



**Figure 15:** soil moisture depletion levels in our field. different colored dots note moisture depletions for the different plots based on the TDR measurements and the red line notes the mean moisture depletion over all the plots. The black line notes a depletion of 0 indicating field capacity. The blue line notes the critical soil moisture depletion level (depletion of 25% total available water) below which the plants will be moisture stressed. MAD = management allowable depletion. P = fraction of total available water.

Every 2 weeks, growth parameters of the banana were measured and the data collection is still ongoing. A preliminary example of the height and girth evolution of the banana plants is given in Figure 16. It appears that the plants did not grow significantly during the first 6 weeks but after these weeks, plants started growing exponentially. At 12 weeks after planting our Matoke plants started growing abnormally and died in our fields as shown in Figure 17.

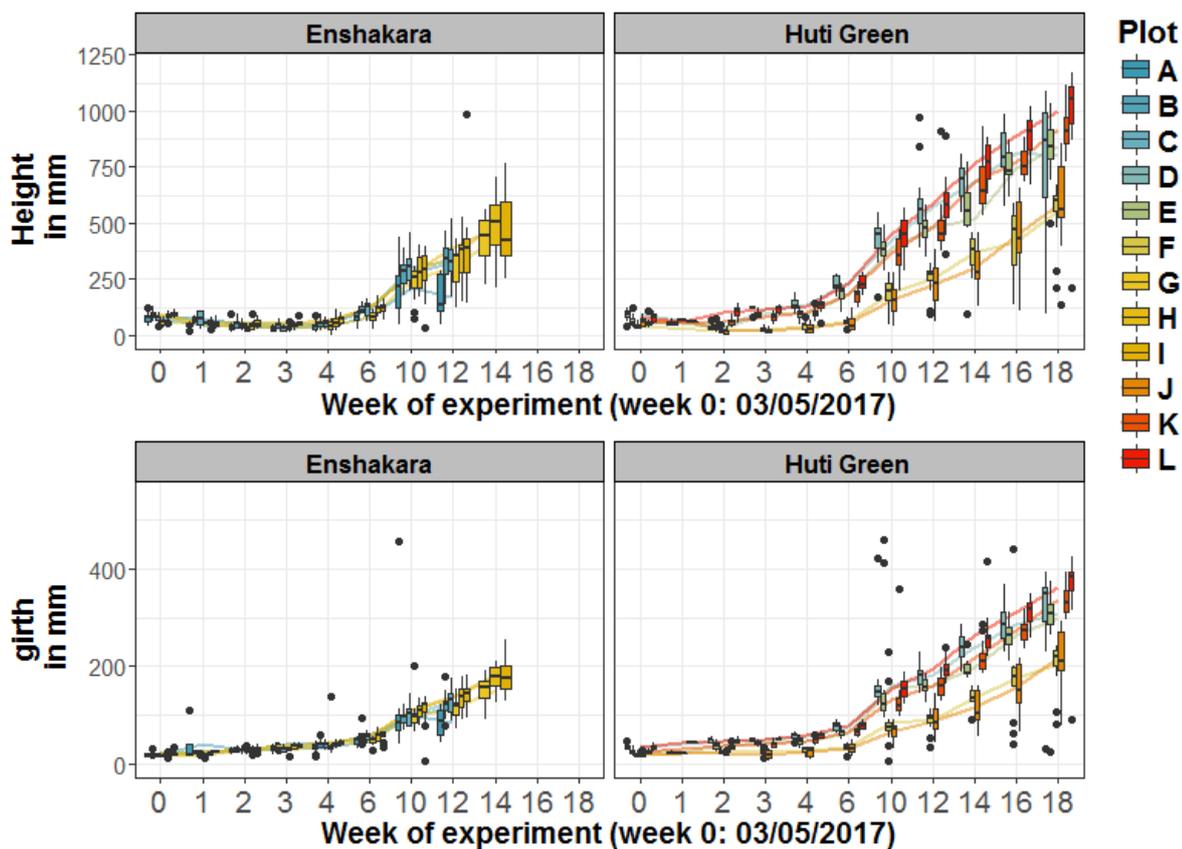


Figure 16: growth curves of bananas in trial. Other growth curves are similar



Figure 17: abnormal growth of Matoke cultivars. youngest leaves turn brown and die off, and the cigar leaf starts rotting from the inside

We also measured canopy cover of our plants over the growing season. When plants were small (the first 8 weeks) canopy cover was measured using the easy leaf area app. This resulted in a canopy growth curve as shown in Figure 18. After 8 weeks, plants grew too tall to simply take pictures from above the plant. A drone was purchased for this purpose, but the permit to fly the drone has not been given yet by the Tanzanian army. A mechanical structure was then built to take pictures from above a plantation as shown in Figure 19. The canopy cover pictures of this stand still need to be analyzed.

In addition, hemispherical pictures are taken from below every 2 months to provide a different technique to estimate canopy cover as shown in Figure 20. These pictures still need to be analyzed.

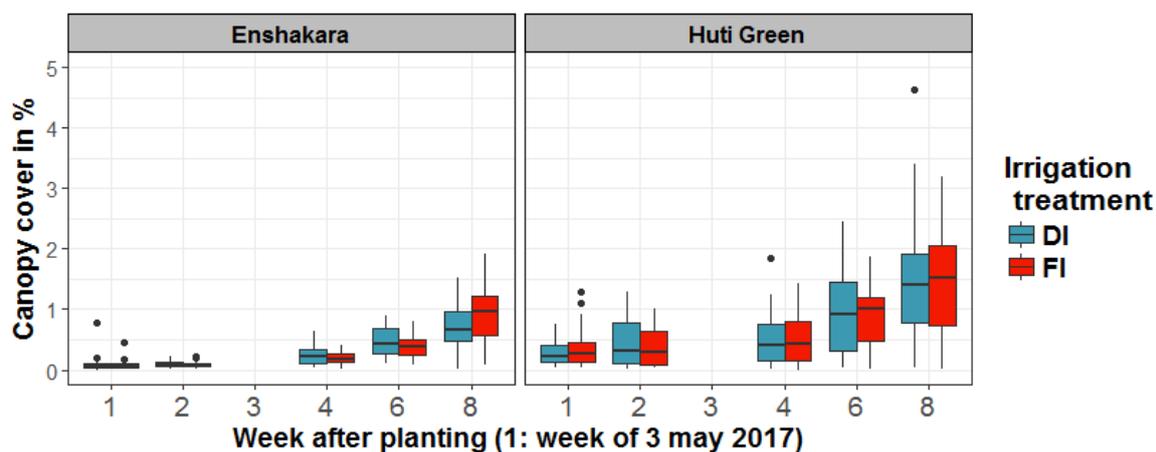


Figure 18: canopy cover evolution in the first 8 weeks



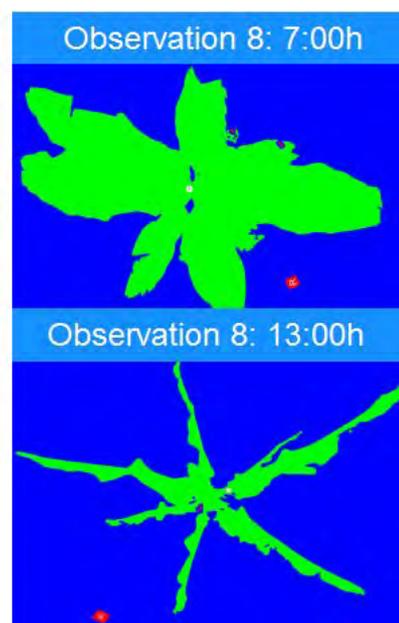
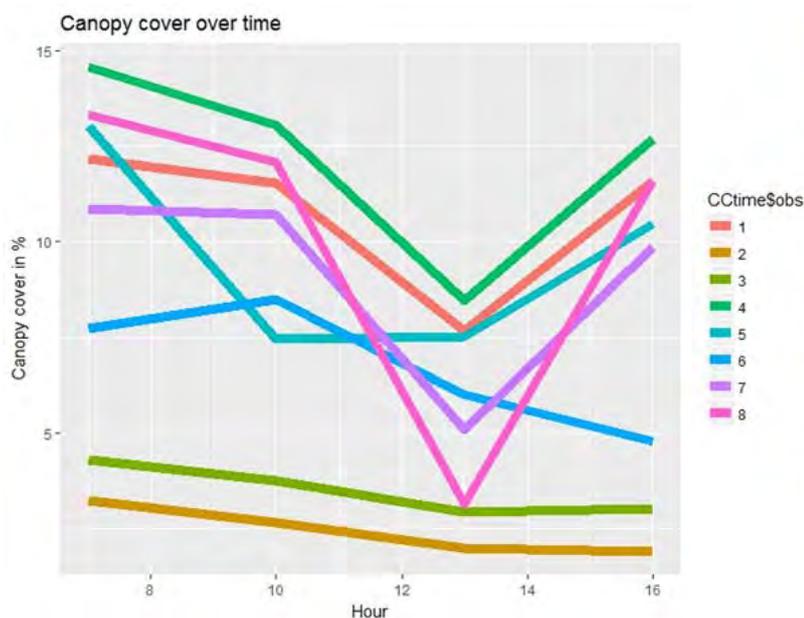
Figure 19: canopy cover stand and resulting picture



Figure 20: hemispherical pictures from below the plant

Previously, in IITA Uganda, a preliminary testing of techniques to study canopy cover was carried out in August 2016. Pictures of individual plants were taken by standing on a ladder and taking pictures from above the plant. It appears canopy cover changes significantly for juvenile plants (5 months) over the course of a day as they tend to fold their leaves due to sunlight (Figure 21). We wanted to test this in the field with a drone by flying over our plantations at different time points during the day over the course of a growing season, but given the lack of a drone permit and the time consuming (and dangerous) nature of taking pictures standing on a ladder or attaching our camera to the stand, this still needs to be carried out.





**Figure 21:** diurnal change of canopy cover

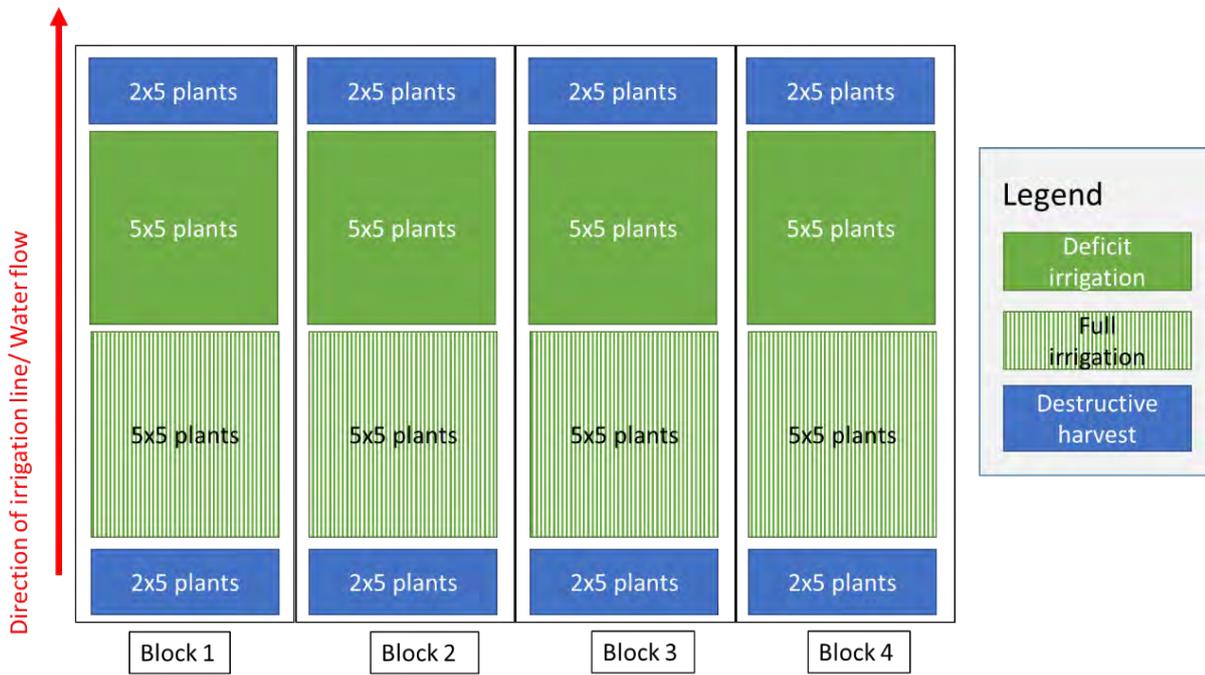
### **Conclusion**

Growth measurements are still ongoing and the methodology to determine canopy cover is still worked out in collaboration with Casper Van Cleemput. After growth cycle 1, we can start data analysis for *AquaCrop* calibration. Additionally, given the high plant mortality in our Matoke plots and the fact “Fusarium” was found in the IITA fields, we opted to order new plant material that is not susceptible to fusarium as the threat is too big for our surviving Mchare plots. We ordered 330 in vitro plants of the cultivar “Grande Naine” to replant our fields. The replanting will occur in October. This also gives us the opportunity to adapt our previous field trial.

Additionally to the new invitro plants, a new plot of sucker derived material will also be used for data collection. In a current “Nakitengwe” (Matoke) plot, a field of 14\*5 plants will be chosen where suckers of 30 cm height will be selected and the rest cut away. This will enable us to compare the growth curves of sucker derived material with in vitro derived material.

### **New experimental design**

We will adapt the original field experiment to ensure a better statistical layout: taking into account replications and blocking. The new experimental layout is given in Figure 22. By obstructing the irrigation lines in the middle, we are able to have always an irrigated and rainfed plot in each block. Each block consists of 5 rows of plants, so with 4 replications we are in need of at least 20 rows. The FI plots are always in the beginning of the line while the DI plots are always at the end of a dripline. This is due to the characteristics of the irrigation system. At the edges of the field, we have 2 destructive sampling plots (full and deficit irrigated) per block of 10 plants each. In total there are then 80 plants for destructive sampling.



**Figure 22:** Grand Naine plot. The first and last 2 rows are used for destructive sampling

## 2. Publication planning of research results

The planned outcome of the research will result in 1 PhDthesis manuscript and 2 to 3 peer-reviewed articles. So far, no research has been published.

**Publication 1:** PhD thesis - Combatting water stress in banana-based cropping systems: development of an *AquaCrop* computer simulation model for the banana plant as a decision-support tool for water management of a plantation.

**Publication 2:** Article in Peer reviewed journal - growth response of banana cultivars Mchare and Matoke to differential irrigation regimes in a running field trial in Arusha, Tanzania.

**Publication 3:** article in Peer reviewed journal – water productivity and diurnal transpiration characteristics of banana varieties under differential water regimes in greenhouse conditions.

**Publication 4:** Assessing the suitability of the *AquaCrop* model to simulate banana systems: using *AquaCrop* for modelling heterogeneous crops.

## 3. Educational supervision

### **Bachelor project supervision of 2 BSc Students: 2016-2017**

Birgit Skenazi: *Het effect van sweet priming op de droogteresistentie van Musa acuminata Colla*

Benjamin Crevits: *The importance of sweet priming in banana plants during drought stress*

### **Thesis supervision of 2 MSc students: 2017-2018**

Casper van Cleemput: *Assessing canopy cover and leaf area index values of banana plantations on the slope of Mt. Meru, Tanzania*

Tonia El Hajj: *Modelling soil water balances in banana plantations on the slope of Mt. Meru, Tanzania*

## 4. Formal course units

All courses of interest are shown in underlying table. The courses highlighted in blue are already taken up and followed. The others are pending.

Table 5: Formal course units. Followed (blue) and pending

| Name of the course   | Number of ECTS credit equivalent |
|--|----------------------------------|
| Networking for PhD researchers & postdocs                  | 1/3 ECTS                         |
| Assertive communication                                    | 1/3 ECTS                         |
| <i>AquaCrop</i> workshop Figaro (in Lisbon)                | 4/3 ECTS                         |
| Managing my PhD  | 1 ECTS                           |
| Statistical Software (B-KUL-G0A21A) 1 <sup>st</sup> term   | 3 ECTS                           |
| Experimental Design (B-KUL-G0B68A) 2nd term.               | 4 ECTS                           |
| Fundamental Concepts of Statistics (B-KUL-G0A17A) 1st term | 6 ECTS                           |

## 5. Time schedule

The underlying gant charts shows the planning of this PhD thesis. The original gant chart shows the planning of activities as proposed in September 2016, during the 9 months presentation. The updated gant chart shows how the planning changed over the course of the PhD, given the setback in the greenhouse trial and field trial.

Original Gant chart:

|  | Year  | Quartiles   | Previous year | First year  |    |    |    | Second year |    |    |    | Third year |    |    |    | Fourth year |    |    |    |
|--|---|-------------|---------------|---|----|----|----|-------------|----|----|----|------------|----|----|----|-------------|----|----|----|
|  |   |             |               | Q1  | Q2 | Q3 | Q4 | Q1          | Q2 | Q3 | Q4 | Q1         | Q2 | Q3 | Q4 | Q1          | Q2 | Q3 | Q4 |
|  |   | Dry season  |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  |   | On location |               | x   | x  | x  | x  | x           | x  | x  | x  | x          | x  | x  | x  | x           | x  | x  |    |
| LITERATURE STUDY                           |   |             |               | Throughout PhD  |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| WP   | Activity                                      |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| Canopy cover                               |   |             |               | Objective 1: CC methodology and CC-LAI relationship         |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| WP1  | 1.1 CC data collection                        |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 1.2 Image analysis                            |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 1.3 CC methodology                            |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| Plant water relations                      |   |             |               | Objective 2: yield response to water                        |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| WP2  | 2.1 Pottrial leuven                           |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 2.2 Field trial monitoring                    |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 2.3 normalized water productivity (WP*)       |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 2.4 Critical soil moisture values (p)         |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 2.5 Data analysis and manuscript plant -water |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| Cohort model: thresholds and distributions |   |             |               | Objective 3: phenological development                       |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| WP3  | 3.1 Phenologic development data collection    |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 3.2 Thresholds and log-normal distributions   |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 3.3 Cohort modelling and manuscript           |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| AquaCrop modelling                         |   |             |               | Objective 4: AquaCrop model                                 |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| WP4  | 4.1 AquaCrop calibration for homogenous model |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 4.2 Cohort implementation in AquaCrop         |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 4.3 Validation of AquaCrop and manuscript     |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| Scenario analyses with AquaCrop            |   |             |               | Objective 5: Scenario analyses to determine best management |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| WP5  | 5.1 Climate variability and variety selection |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 5.2 Management options                        |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
|  | 5.3 Thesis writing and presentation           |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| PUBLICATIONS                               |   |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |
| MILESTONES                                 |   |             |               |   |    |    |    |             |    |    |    |            |    |    |    |             |    |    |    |

Figure 23: Original Gant chart showing Work packages WP and timing of activities

**Milestone 1:** Methodology for canopy cover determination.

**Milestone 2:** Normalized water productivity (WP\*) and critical soil moisture thresholds (p) for canopy cover expansion and stomatal closure

**Milestone 3:** Heat unit thresholds and log-normal distributions for phenological stages

**Milestone 4:** Running *AquaCrop* model to model heterogeneous banana stands

**Milestone 5:** Management guidelines for multiple actual field conditions

Updated Gant chart: planning of activities from October 2017 onward

|   | Year  | Quartiles | Previously | 2017   |    |    |    | 2018        |    |    |    | 2019 |    |    |    | 2020 |    |    |    |
|---|---|-----------|------------|--|----|----|----|-------------|----|----|----|------|----|----|----|------|----|----|----|
|   |   |           |            | Q1   | Q2 | Q3 | Q4 | Q1          | Q2 | Q3 | Q4 | Q1   | Q2 | Q3 | Q4 | Q1   | Q2 | Q3 | Q4 |
|   |   |           |            | Dry season   |    |    |    | On location |    |    |    |      |    |    |    |      |    |    |    |
| <b>LITERATURE STUDY</b>                           |   |           |            | <i>Throughout PhD</i>  |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>WP</b>   | <b>Activity</b>                               |           |            | <b>Progress</b>  |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>Canopy cover</b>                               |   |           |            | <b>Objective 1: CC methodology and CC-LAI relationship</b>         |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>WP1</b>  | 1.1 CC data collection                        |           |            | [Gantt bars for 1.1]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 1.2 Image analysis                            |           |            | [Gantt bars for 1.2]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 1.3 CC methodology                            |           |            | [Gantt bars for 1.3]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>Plant water relations</b>                      |   |           |            | <b>Objective 2: yield response to water</b>                        |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>WP2</b>  | 2.1 Pottrial leuven/Arusha                    |           |            | [Gantt bars for 2.1]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 2.2 Field trial monitoring                    |           |            | [Gantt bars for 2.2]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 2.3 normalized water productivity (WP*)       |           |            | [Gantt bars for 2.3]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 2.4 Critical soil moisture values (p)         |           |            | [Gantt bars for 2.4]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 2.5 Data analysis and manuscript plant -water |           |            | [Gantt bars for 2.5]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>Cohort model: thresholds and distributions</b> |   |           |            | <b>Objective 3: phenological development</b>                       |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>WP3</b>  | 3.1 Phenologic development data collection    |           |            | [Gantt bars for 3.1]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 3.2 Thresholds and log-normal distributions   |           |            | [Gantt bars for 3.2]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 3.3 Cohort modelling and manuscript           |           |            | [Gantt bars for 3.3]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>AquaCrop modelling</b>                         |   |           |            | <b>Objective 4: AquaCrop model</b>                                 |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>WP4</b>  | 4.1 AquaCrop calibration for homogenous model |           |            | [Gantt bars for 4.1]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 4.2 Cohort implementation in AquaCrop         |           |            | [Gantt bars for 4.2]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 4.3 Validation of AquaCrop and manuscript     |           |            | [Gantt bars for 4.3]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>Scenario analyses with AquaCrop</b>            |   |           |            | <b>Objective 5: Scenario analyses to determine best management</b> |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>WP5</b>  | 5.1 Climate variability and variety selection |           |            | [Gantt bars for 5.1]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 5.2 Management options                        |           |            | [Gantt bars for 5.2]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
|   | 5.3 Thesis writing and presentation           |           |            | [Gantt bars for 5.3]   |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>PUBLICATIONS</b>                               |   |           |            | [Gantt bars for publications]                                      |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |
| <b>MILESTONES</b>                                 |   |           |            | [Gantt bars for milestones]  |    |    |    |             |    |    |    |      |    |    |    |      |    |    |    |

Figure 24: updated Gant chartshowing Work packages WP and timing of activities

## 7.2 MSc Research Progress Report (2016-2017)

**Name:** Jean Claude Habineza

**Title:** QTL mapping for resistance to nematodes (*Radopholus Similis*) in a diploid segregating banana population

**Supervisors:** Dr. Brigitte Uwimana (IITA),  
Dr. Coyne Danny (IITA)  
Dr. Richard Edema (Makerere University)

**Timeline of study:** 1<sup>st</sup> June 2017 up to 31<sup>st</sup> May 2018 (12 months)

**University:** Makerere University

### General objectives

To contribute to the enhancement of breeding for nematode resistant banana lines

### Specific objectives

- To understand the inheritance patterns of nematode resistance in a banana F<sub>1</sub> diploid population
- To identify and map QTLs associated with traits for *R. similis* resistance

### Summary of activities

- Phenotyping
- Genotyping
- Data analysis

### Study progress

#### Activity I: Phenotyping

This activity is related to the first objective entitled “**To understand the inheritance patterns of nematode resistance in a banana F1 diploid population**” and it includes the following:

- Screening of at least 116 genotypes from a cross between Zebrina GF and Calcutta 4 (F<sub>1</sub> diploid population) in 5 experiments
- RCBD design with three replications with 33 genotypes including 29 hybrids, 2 parents and 2 checks (KM5 &Valery)
- Parents and checks are tested for the effect of the experiment across time

### Achievements

| Activity         | EXP1     | EXP2     | EXP3     | EXP4     | EXP5     |
|------------------|----------|----------|----------|----------|----------|
| Planting date    | 04-04-17 | 29-06-17 | 03-08-17 | 26-09-17 | 24-10-17 |
| Inoculation date | 30-05-17 | 24-08-17 | 28-09-17 | 21-11-17 | 19-12-17 |
| Termination date | 25-07-17 | 19-10-17 | 23-11-17 | 16-01-18 | 13-02-18 |

According to this table, the first experiment has been terminated and the last experiment is not yet established while the other remaining three are still in the screen house with two already inoculated and waiting for termination and one waiting for inoculation.

### **Activity II: Genotyping**

This activity is related to the second objective **entitled “To identify and map QTLs associated with traits for *R. similis* resistance”** and it involves the following:

- Leaf samples will be collected for each genotype for DNA extraction
- DNA will be extracted and sent to Illumina for genotyping using a SNP Chip

### **Achievements**

This activity is not yet started but is planned soon

### **Activity III: Data analysis**

This activity combines both previous activities.

### **Phenotyping**

Data from experiment one have been subjected to  $\log(x+1)$  transformation for normalization and analysed with R software for variances and mean separation using Dunnett test. Among 12 genotypes from this experiment, the preliminary results showed that 2 were resistant, 1 inconclusive and 9 susceptible. Below is the summary table:

| Comparison with Valery             | Comparison with KM5         | Host response     | Genotype |
|------------------------------------|-----------------------------|-------------------|----------|
| <b>Significantly different</b>     | Not significantly different | Resistant         | 2        |
| <b>Not significantly different</b> | Significantly different     | Susceptible       | 9        |
| <b>Significantly different</b>     | Significantly different     | Partial resistant | 0        |
| <b>Not significantly different</b> | Not significantly different | Inconclusive      | 1        |

### **Genotyping**

The following analysis will be carried out, however they are not yet started.

- A linkage map will be constructed using JoinMap@4.
- Mark-traits association will be analysed using winQTL cartographer/MapQTL/GenStat

## MSc RESEARCH PROGRESS REPORT

### Name of MSc Student: Juliet Kemigisa

|                           |   |
|---------------------------|---|
| <b>Field of study:</b>    | MSc in Botany (Microbiology and Plant pathology)  |
| <b>University:</b>        | Makerere University   |
| <b>Timeline of study:</b> | 1st August 2016 to 31 <sup>st</sup> July 2018   |
| <b>Research title:</b>    | Evaluation of selected diploid banana genotypes for resistance to weevils in Uganda                 |
| <b>Supervisors:</b>       | Dr. Robooni Tumuhimbise (NARO), Dr. Jerome Kubiriba (NARO), Dr. Arthur Tugume (Makerere University) |

### Research Objectives

1. To assess the response of selected diploid banana genotypes for weevils infestation.
2. To develop a fast screening method for banana weevils.

### Achievements

1. Successfully completed year one (course work) at Makerere university. I undertook courses such as Advanced biostatistics, Advanced Remote Sensing and GIS, Natural Resources and Landscape processes, Project Planning and management, Communication Skills, Advanced Plant Pathology, Advanced Plant Virology, Systematics of Fungi and Bacteria, Physiology of Fungi and Bacteria, Food Microbiology, Applied microbiology, Compendium of diseases, seminar series.
2. Established a field screening trial of the selected diploid banana genotypes for weevils. Data collection is underway.
3. Short bioassays for the weevils are underway.

### Introduction

In Uganda, bananas are a staple and main source of income for many smallholder farmers that rely on the crop for their livelihoods. Banana production in Uganda achieves less than the expected potential yield of 70t/ha/yr due to pests, diseases and other abiotic factors such as declining soil fertility. Pests of major concern are the banana weevils with the estimated yield damages of 14 to 60% and have led to the disappearance of some popular local East African Highland cultivars. Attempt to control the weevil by using cultural, biological and chemical methods are feasible but not sustainable to resource limited farmers, since these methods are laborious, costly and harmful to the users and the environment.

Breeding for host resistance to weevils holds promise as the best control measure against weevils but has not been fully utilized because of the lack of appropriate sources of resistance to banana weevils. This is coupled with a long breeding cycle that is prolonged by field screening to identify and characterize resistance to weevils that takes over four years. The MSc study focuses on identifying natural sources of resistance to weevils from selected diploid banana genotypes and establishing a short screening method for the weevils. These two will be utilized by the banana breeding programmes in conventional breeding for resistance to weevils and early



selection of genotypes resistant to weevils. This will consequently benefit small scale banana farmers through growing improved weevil resistant varieties, thus reducing labour costs and chemical use. This will also result into increased and sustainable banana production and productivity.

**Objective 1; To assess the response of selected diploid banana genotypes for weevils invasion.**

The currently available and trusted methods for screening banana genotypes against weevils are planting field trials or using hardened tissue culture plantlets in screen houses. Field screening is a long term process that takes 3 to 5 years in collecting eligible data for the 1<sup>st</sup> to the 4<sup>th</sup> cycle since weevil effect is observed in older plantations than younger ones. A greenhouse screening method predicts plant resistance to weevils in 8 months. In this study, eleven banana genotypes were planted in a randomized complete block design in a field screening trial with five replicates. Eight of the genotypes are diploids obtained from International Transit Centre (ITC) and were selected based on their special agronomic traits yet their response to weevils is not known. Three are controls obtained from National Agricultural Research Laboratories (NARL). They were infested with 10 weevils (5 males and 5 females) at nine months after planting. Currently agronomic data at flowering and harvest for the first cycle is under way. Also weevil assessment on the corm is underway for the first cycle (only 5%). Since they are eleven different genotypes, their dates to flowering and maturity differ. This necessitated putting up a pot trial for the germplasm so as to easily estimate the weevil damage by the end of February 2018. Plants are already potted and are awaiting hardening and inoculation with weevils. The resistant genotypes will be recommended for breeders in generating new resistant hybrids to weevils.

**The Musa germplasm being evaluated for resistance to weevils**

| Ploidy | Study material    | Response to weevils |
|--------|-------------------|---------------------|
| AA     | Morongu Datu      | Not known           |
| AA     | Pisang Gigi buaya | Not known           |
| AA     | Pisang Tunjuk     | Not known           |
| AA     | SH-3142           | Not known           |
| AA     | Pisang Rotan      | Not known           |
| AA     | Gabah Gabah       | Not known           |
| AA     | Morong Princessa  | Not known           |
| AA     | Saing Hil         | Not known           |
| AA     | Calcutta 4        | Resistant           |
| AAB    | Kayinja           | Intermediate        |
| AAA    | Kibuzi            | Susceptible         |

## 2. To develop a short screening method for banana weevils

A laboratory screening protocol for weevils is necessary so as to reduce on the long breeding cycle, labour costs for maintaining field trials and reduce on the space that is required for massive numbers of breeders' material at early identification stages of breeding. In this MSc study, tissue culture plantlets are fed on weevils larvae for 10 days. Larvae survival, body size (length and weight) are recorded. It is a promising protocol that can predict resistance in 30 days, and does not occupy much space.

### Germplasm used in the short bioassay

| Cultivar      | Response to weevils |
|---------------|---------------------|
| Kayinja       | Intermediate        |
| Calcutta 4    | Resistant           |
| Yangambi KM 5 | Resistant           |
| Atwalira      | Susceptible         |
| Kisansa       | Susceptible         |
| M 9           | Intermediate        |
| Mbwazirume    | susceptible         |

### Conclusion

- Collect all the necessary data for the field trial, pot experiment and bioassay by March 2018
- Thesis write up and submission by May 2018

**Name of Student: Hassan Shaban Mduma**

**Title:** Role of plant parasitic nematodes on incidence and severity of Fusarium wilt disease of banana in Tanzania.

**Supervisor:** Dr. Allan Brown, Prof. Rony Swennen, Prof. Patrick Ndakidemi, Dr. Ernest Mbega.

**Timeline:** Nov 2015 – Nov 2017

**University:** The Nelson Mandela African Institution of Science and Technology

**Research Objectives**

- I. To determine the influence of lesion nematode (*Pratylenchus goodeyi*) on induction, and intensity of Fusarium wilt disease on banana
- II. To assess the response of selected Fusarium wilt resistant cultivars to single and co-inoculation with *Pratylenchus goodeyi* and *Fusarium oxysporum* f.sp. cubense (Foc)
- III. To establish the response of East African Highland Bananas (EAHB) to single and co-inoculation with *Pratylenchus goodeyi* and Foc

**Achievements**

- A pot culture experiment was established under screen house conditions.
- 9 cultivars of banana were sequentially and co inoculated with Foc and or nematodes
- External symptoms and growth parameters were weekly evaluated for 12 weeks after inoculation.

**Background/introduction**

In the Great Lakes region of Africa, bananas especially the East African highland bananas, such as Matooke (AAA-EA), the Illalyi (AAA), and Mchare (AA) are important staple as well as cash crops. The region has remained to be the largest producer and consumer of bananas in Africa where per capita consumption of banana ranges from 230 to 450 kg person-1 year-1. Tanzania produces about 3.7 million MT annually on 403,000 hectares. Kilimanjaro and Kagera are the most famous banana growing regions, which together produce about 2.5 million MT annually. Apart from Kilimanjaro and Kagera, other regions which also grow Highland bananas are: Kigoma, Mbeya, Kilimanjaro, Arusha, Tanga, Tarime district in Mara region and some parts of Morogoro region.

Despite their importance, banana yields in East Africa particularly in Tanzania are declining. Such declines in production has been associated to various abiotic and biotic factors including soil fertility problems, drought, pests and diseases. Of the recorded constraints, plant parasitic nematodes are among the destructive pests of banana in a variety of environments. They feed, migrate and multiply inside banana roots and corms causing root-tissue necrosis and root system reduction causing damage to plants, impaired transport and uptake of water and nutrients resulting in reduced plant growth and yield. In addition the anchorage function of the root system is adversely affected resulting in plant toppling.

The wounds resulting from nematode attack can also provide avenues for entry and infestation by soil-borne fungal organisms such as *Fusarium oxysporum* f. sp. cubense (Foc) causal agent of Fusarium wilt of banana. This disease is also known as Panama disease, as it first became epidemic in Panama in 1890 and proceeded to devastate the Central American and Caribbean banana industries that were based on the 'Gros Michel' (AAA) variety in the 1950s and 1960s. Fusarium wilt disease is in the same rank with some most devastating plant diseases of other crops such as wheat rust and potato blight in terms of crop destruction and has been reported from all banana growing regions including East Africa. Once Foc is present in the soil, it cannot be eliminated. It disrupts the plant's water conducting vessels, leaves become yellow (progressing from older to younger leaves) and wilted. Inside the pseudostem, brown, red or yellow lines are visible in vertical section which appear as rings in cross-section. Later, all leaves turn yellow and die and internal rotting becomes extensive. There

are four recognised races of the pathogen which are separated based on host susceptibility, but race 1 is important in East Africa region as common bananas including Mchare and Sukari ndizi are susceptible.

It is difficult to manage Fusarium wilt disease with most of the methods. The only hope remained was breeding for resistant varieties. However these resistant varieties do not always remain resistant if preceded with nematode infection. Complex interrelationship between nematode and Foc in bananas is believed to produce a combined effect which is greater than the sum of their separate effects.

While the interaction between parasitic nematodes and Foc is clear from studies carried outside East Africa, this has not been elucidated in Tanzania. Further, the agro-ecologies and banana germplasm in Tanzania are different. It is therefore important to clearly understand well the interaction between plant parasitic nematodes and Foc in the Tanzanian banana germplasm. Therefore, the aim of this study was to assess the effects of the lesion nematode (*Pratylenchus goodeyi*) on the incidence and severity of Fusarium wilt disease caused by *Fusarium oxysporum* f.sp. cubense on banana so that recommendations on developing management options can be made to small scale farmers in Tanzania.

### **Objective / Study 1, 2& 3**

9 genotypes were selected for single and co-inoculation with nematode and or Foc based on their resistance or susceptibility to both Foc and nematode. 6 treatments were assigned randomly to experimental plants replicated three times in a split plot design. Treatments were: 1. Foc, 2. Nematode, 3. Nematode + Foc, 4. Foc followed by nematode (14 days later), 5. Nematode followed by Foc (14 days later), 5. Control (no inoculation). Weekly record of the growth parameters (height, girth, number of leaves) were collected for twelve weeks starting seven days after inoculation.

Severity of Fusarium wilt disease were assessed as rated by Viljoen *et al.* (2016) where a scale of 1 to 5 for external symptom were used. The scale descriptions: 1; No visual leaf symptoms, 2; 0-33% of older banana leaves turning yellow, 3; 33-66% of older leaves turning yellow with some hanging down the pseudostem, 4; 76-100% of the leaves turning yellow and necrotic with leaves hanging down the pseudostem, 5; plant dead with brown leaves hanging down the pseudostem.

### **Preliminary results;**

- Foc severity is faster with co-inoculation
- No signs of Foc in resistant cultivars with single and co-inoculation
- Reduced plant growth with co-inoculation compared to single and control

### **Conclusion / next steps**

- Assessment of internal symptoms based on the corm rot, a scale of 1-6 adapted from Viljoen *et al.* (2016) will be used.
- Assess nematode damage by looking on the root necrosis using a scale of 1-5 as adapted from Speijer and De Waele (1997).
- Do nematode count by counting the female, male and juvenile nematodes from portion of each sampled plant.

## MSc Student Research Progress Report (2016-2017)

**Name:** Yusuph Mohamed

**Title:** Problem and infestation assessment of banana weevils (*Cosmopolites sordidus* Germar) in different banana farming systems

**Supervisors:** Prof. Ndakidemi, Patrick A (NM-AIST), Prof. Rony Swennen (IITA) and Dr. Mbega, Ernest (NM-AIST)

**Timeline:** Nov 2015-Dec 2017

**University:** Nelson Mandela African Institution of Science and Technology (NM-AIST)

### Research Objectives

- i. To assess presence of banana weevils in different banana farming systems
- ii. To assess banana weevil damage levels in different banana farming systems
- iii. To assess farmer's understanding on banana weevils in different farming systems

### Achievements

Highlight significant achievements – e.g. in bullets

- Review article titled 'Current control strategies and their potential application against banana weevils (*Cosmopolite sordidus* Germar) in Tanzania' finalized for publication
- Complete field survey in 20 different banana farming systems in Arusha and Kilimanjaro regions of Tanzania from June 05-25, 2017 and from August 28 to September 16, 2017 respectively.

## 1. INTRODUCTION

Banana weevil (*Cosmopolites sordidus* Germar 1824) is an important insect pest (Coleoptera: Curculionidae) of banana crops (*Musa* spp.) in most banana growing regions worldwide. It is believed to have originated from the Indo-Malaysian region with its current geographical distribution to Asia, Australia and Pacific Islands, America and Africa. In Africa, the banana weevil is a serious pest in Benin, Burundi, Cameroon, Comoros, Democratic Republic of Congo, Gabon, Ghana, Guinea, Kenya, Madagascar, Malawi, Mali, Nigeria, Rwanda, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Tanzania and Uganda.

In Tanzania, the banana weevil has been reported to be present in Arusha, Kagera, Kilimanjaro, Mbeya, Morogoro and Pwani regions.

Banana weevils attack all banana varieties in all phenological stages. Its infestation can cause yield loss up to 100%, crop failure due to snapping and toppling at the base of the plant during windstorms under heavy infestations as well as farm rejection. Adult weevils feed on banana debris, residues, rotting tissues and some time on young suckers but are less destructive and their yield loss is insignificant. Despite the importance of this pest in banana in Tanzania, information of infestation in different banana farming systems and farmer's understanding of the problem are lacking. Therefore this work was conducted to address this gap in Tanzania.

## MATERIALS AND METHODS

The study was conducted at villages of Nkoarangaa, Mbuguni and Ngurdoto (Arumeru District, Arusha region) and Uduru, Uraa and Mbosho (Hai District, Kilimanjaro region).

Experimental design used was split-split-plot block design (SSBD) with three replications. The main factors, sub-factors and sub-sub-factors being locations, banana farming systems and banana weevils respectively.

### 1. Presence of banana weevil in different banana farming systems

This was assessed by three banana pseudostem traps (representing three replications) with 25-30 cm length made and set according to Swennen, (1990) by cutting fresh pseudostems longitudinally in half. With cut surfaces facing the soil, pseudostem pieces were placed close to the bases of three randomly selected banana plants in each banana farm. Weevil adults were captured in five consecutive days followed by manual counting and recording. The varieties of banana and GPS were recorded.

**2. Weevil damage levels in different banana farming systems** This was done by using the coefficient of infestation method according to Oliveira et al. (2017) involving destructive random sampling. Three random selected banana rhizomes in each banana farming system were cut cross-sectionally at their maximum diameter to expose weevil galleries. Finally, square grid of 2025 cm<sup>2</sup>, with cells of 2.25 cm<sup>2</sup> was placed over their cut surfaces followed by counting cells (symptoms of necrotic or dark tissue). Through number of cells, coefficient of infestation were then established according to a damage scale of 0 (no galleries), 5 (traces of galleries), 10 (between 5 and 20 galleries), 20 (galleries in approximately 25% of the rhizome), 30 (galleries in approximately 20%-40% of the rhizome), 40 (galleries in approximately 50% of the rhizome), 50 (galleries in approximately 75% of the rhizome) and 100 (galleries in the entire rhizome).

### **3. Farmer's understanding on banana weevil in different farming systems**

This was done according to Wachira et al. (2013) with modifications involving semi structured questionnaire and standard interviewing to a total of 48 respondents interviewed in which two banana farmers randomly selected from each banana farming systems.

The following is the questionnaire used during the survey.

#### **Conclusion / next steps**

- ❖ Data analysis
- ❖ Publishing review article
- ❖ Preparing manuscript for publication

## Questionnaire

Region: \_\_\_\_\_ District: \_\_\_\_\_ Ward/village: \_\_\_\_\_

Questionnaire number: \_\_\_\_\_ Date: \_\_\_\_\_

GPS coordinates: \_\_\_\_\_

### SECTION I: Banana farmer personal information

Name: \_\_\_\_\_ Gender: ( ) Phone number: \_\_\_\_\_

Age in years: ( )

Marital Status: Single ( ) Married ( ) Divorced ( ) Widowed ( )

Educational level: Adult education ( ) Primary ( ) Secondary ( ) College ( ) others ( )

Family head: Male ( ) Female ( )

Occupation: Housewife ( ) Peasant ( ) Government ( ) Private company ( ) others ( )

### SECTION II: Banana production and banana weevil

1. How many years have you been in banana production activities? ( )
2. What are your banana yield in past three years ago in terms of bunches?  
First year ( ) Second year ( ) Third year ( )
3. What affects your banana yield?  
Diseases ( ) Insects ( ) Nematodes ( ) Climate change ( ) Fusarium ( ) Sigatoka ( )  
Others ( )
4. What are the major insect pests that cause great damage to the banana (*Rank in 1, 2, 3...*)  
Banana aphids ( ) Banana white flies ( ) Banana weevils ( ) Banana thrips ( ) Banana spider mites ( ) others ( )
5. Do you know banana weevil? Yes ( ) No ( ) Uncertain ( )
6. **If answer 5 is yes**, how did you know banana weevil?  
Fellow farmers ( ) observation ( ) extension service ( ) Agricultural exhibitions ( ) TV ( ) Radio ( ) Training ( )  
others ( )
7. Are weevil populations present throughout the year? Yes ( ) No ( )
8. Which season of the year weevil populations are said to be higher?  
Rainy seasons ( ) dry seasons ( ) others ( )

### SECTION III: Banana weevil infestation

9. Do you scout for insect pests in your banana farms? Yes ( ) No ( )
10. **If Question 9 answer is yes**, how frequently in a week? Once ( ) twice ( ) thrice ( ) all the week ( )
11. How many times you observe weevil infestation in your banana farms during scouting?  
Occasionally ( ) often ( ) always ( ) all the time ( )
12. At which banana plant stage, weevil damage is frequently observed during scouting? Young ( ) flowering ( )  
matured ( ) old ( )
13. Is the weevil infestation a problem to your banana production? Yes ( ) No ( )



14. If **Question 13 answer is yes**, what method(s) do you apply to control weevil infestations? Chemical ( )  
Biological ( ) Host plant resistance ( ) Cultural ( )

Specify:

.....  
.....

15. What are the symptoms of weevil infestation do you know? (*tick appropriate*)

1. Leaf chlorosis ( ) 2. Snatching ( ) 3. Toppling ( ) 4. Flowering delaying ( )

5. Weak plants (less vigour) ( ) 6. Others ( )

16. What are the results caused by high weevil infestations to your banana farm? (*Rank 1, 2*).

Yield loss ( ) farm rejection ( ) crop failure ( ) NIL ( ) others ( )

#### **Section IV: Banana farming systems**

17. Which of the banana farming systems are you practiced?

Monocropping ( ) Intercropping ( ) Mixed cropping ( )

Specify farming activity (ies): .....

18. Does different banana farming systems affects weevil infestation? Yes ( ) No ( )

19. If **Question 18 answer is yes**, then which of the following banana farming system reduce weevil infestations to banana crops?

Banana monocrop ( ) banana-beans ( ) banana-coffee ( ) banana-maize ( )

20. If **Question 18 answer is no**, then which of the following banana farming system favor weevil infestations to banana crops?

Banana monocrop ( ) banana-beans ( ) banana-coffee ( ) banana-maize ( )

#### **MSc RESEARCH PROGRESS REPORT**

## NAME: Mwanje Gerald

TITLE: QTL MAPPING FOR BANANA WEEVIL (*COSMOPOLITES SORDIDUS* GERMAR) RESISTANCE

SUPERVISOR: Dr. Brigitte Uwimana and Prof. Patrick Rubaihayo

TIMELINE OF RESEARCH: 1<sup>ST</sup> March 2017 to 28<sup>th</sup> February 2018

UNIVERSITY: Makerere University

### Research Objectives

1. To determine the inheritance of banana resistance to weevils
2. To identify and map QTLs associated with banana resistance traits to weevil

### Achievements

- Phenotyping of the F1 progenies of Monyet × Kokopo is ongoing in which 71 genotypes have been established in a pot screening experiment in two series (first series containing 42 genotypes and the second containing 29 genotypes).
- Phenotypic data for the first series (42 genotypes) have been collected.

### Background

Bananas and plantains are the fourth most important food crop in the world. They are staple foods in many developing countries, especially in Africa. Pest and diseases play an importance role in determining the level of crop losses incurred in banana production. Banana weevil infestation can result into yields losses of up to 50% - 100%. Therefore the focus of this research is to determine the inheritance of banana resistance to weevils as this will help in determining a banana weevil resistance breeding strategy. The research is also focusing on identifying QTLs specific locations in the banana genome which will aid in the development of markers for use for weevil resistance breeding.

#### Objective one: To determine the inheritance of banana resistance to weevils.

The F<sub>1</sub> genotypes from a cross between Kokopo which is the susceptible parent to banana weevil by Monyet which is the resistant parent to banana weevil are being phenotyped. The trial involves the use of Calcutta-4 and Yangambi km-5 which are the resistant checks and Nakyetegwa and Kabucuragye as susceptible checks. The experiment is set up in Randomized Complete Block Design replicated two times with three plants per genotype per replication.

The protocol according to Sadik *et al.* (2010) with a few modifications is being followed. So far 71 genotypes have been established among which phenotypic data of 42 genotypes have been collected whose preliminary results are shown in table 1 below.

The phenotypic data from the population will be used to determine the broad sense heritability (H) using the variance component formula:

$$H = \frac{\delta G}{\delta P} \text{ where } (\delta P = \delta e + \delta G) \text{ and } \delta e \text{ is Residual error mean square}$$

$$\delta e + R\delta G \text{ is Genotype expected mean square } \delta G(\text{Genetic variance component}) = \frac{Ms_{\text{Genotype}} - Ms_{\text{error}}}{R}$$

R = number of replications

**Table 1: Statistical comparison of percentage total corm damage for genotypes from (Monyet × Kokopo) population with the resistant and susceptible controls using Dunnet’s test**

| Statistical comparison with KM5 (Resistant check) | Statistical comparison with Nakyatengu (susceptible check) | Host response     | Number of genotypes |
|---|--|-------------------|---------------------|
| Significantly different                           | Not significantly different                                | Susceptible       | 7                   |
| Not significantly different                       | Significantly different                                    | Resistant         | 24                  |
| Not significantly different                       | Not significantly different                                | Inconclusive      | 11                  |
| Significantly different                           | Significantly different                                    | Partial resistant | 0                   |

**Objective two: To identify and map QTLs associated with banana resistance traits to weevil**

DNA will be extracted from immature unopened banana cigar leaves according to the CTAB (Cetyl trimethylammoniumbromide) procedure (Weising *et al.*, 1995), which was modified for *Musa* by Samarasinghe *et al.* (2001). The DNA will later be taken to Illumina for genotyping using the chip. The phenotypic data from the screening experiment and the genotypic data will be used to map the QTL.

**PENDING WORK**

- Phenotyping of the remaining genotypes for banana resistance to weevil in pot experiment.
- Phenotyping of genotypes for banana resistance to weevils using bioassay.
- Genotyping of the population.

<sup>i</sup> Sperling, Louise. 2008. When Disaster Strikes: A Guide to Assessing Seed System Security. Cali, Colombia: International Center for Tropical Agriculture. (read on: <http://seedssystem.org/>).

<sup>ii</sup> Kombo GR, Dansi A, Loko LY, Orkwor GC, Vodouhe R, Assogba P, Magema JM (2012). Diversity of cassava (*Manihot esculenta* Crantz) cultivars and its management in the department of Bouenza in the Republic of Congo, *Genet Resour Crop Evol*10: 1007-10722.

<sup>iii</sup> Otte, Evelien; Rousseau, Ronald (2002). "Social network analysis: a powerful strategy, also for the information sciences". *Journal of Information Science*. 28 (6): 441–453. doi:10.1177/016555150202800601