
# Breeding Better Bananas

## Annual Report 2018
## ANNEX 1

# Table of Contents

# 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 Agrosciences, USA
2. Hale Ann Tufan, Bioinformatics, Project Manager NextGen Cassava, Cornel 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
6. Klaus Koehler, NA Corn Breeding Operations and Logistics Lead, Corteva Agriscience™, Agriculture Division of DowDuPont™, USA

**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
Deputy: Robooni Tumuhimbise, NARO

WP2. Altus Viljoen, SU
Deputy: Diane Mostert, SU

WP3. Brigitte Uwimana, IITA
Deputy: Elizabeth Aitken, UQ

WP4. Inge van Den Bergh, Bioversity International
Deputy: Lewis Machida, Bioversity International

WP5. Lucas Mueller, BTI
Deputy: Guillaume Jean Bauchet, BTI

WP6. Danny Coyne, IITA
Deputy: Scola Ponera, IITA

## 1.2 Annual Project Meeting, Arusha 2018

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

### Project progress workshop

### Hosted by Nelson Mandela - African Institution of Science & Technology Arusha, Tanzania

### 23-25th April 2018

**Convenors:** *Danny Coyne* and *Rony Swennen* (IITA, Tanzania) and *Prof. Karoli Njau* (NM-AIST)
**Local Organizing Committee**: *Scola Ponera, Cornel Massawe* (ARI)
**Rapporteurs:** Each of the 5 work package leaders or their designate
**Supporting documents:** Project narrative document and Results Framework & Results Tracker, Annual Report 2017.

**Participants**

- Project participants and partners of the Breeding Better Bananas - "Improvement of banana for smallholder farmers in the Great Lakes Region of Africa" project
- Collaborators including project related MSc and PhD fellows
- Members of the Scientific Advisory Group (SAG)
- Members of CGIAR Root, Tuber and Banana Program (RTB)

**Objectives of the workshop**

- Assess whether we will achieve the 9 planned Primary Investment Outcomes (see Annex)
- Present, reflect and assess achievements to date
- Brief review on the BPAT
- Identify areas for strengthening within the project
- Strengthen team collaboration within and among the 5 work packages
- Determine clear projection for the coming 12 months
- Define communications objectives for coming year
- Provide information to the Steering Committee (SC) to assess progress and direction
- Facilitate interaction between SAG, SC and project staff

**Sunday 22nd April 2018**

| Time | Topic | Responsible |
|---|---|---|
| **All day** | Arrival of participants all day | Scola |
| **16.00** | Early Registration | Scola |
| **18.00** | Drinks reception for project arrivals at hotel | Scola |
| **19.30** | Dinner | Gold Crest Hotel |

**DAY 1: Monday 23rd April**

| Time | Topic | Chair or Facilitator |
|---|---|---|
| **8.00-8.45** | Transfer from hotel to NM-AIST Conference Room | Scola |
| **8.45-9.00** | Registration for local participants | Scola |
| **9.00-9.05** | Welcome from the Chair, Vice Chancellor NM-AIST | Prof K. Njau (Chair) |
| **9.05-9.15** | Remarks from IITA, the Lead center of the project, Danny Coyne & Rony Swennen | Prof K. Njau |
| **9.15-9.25** | BMGF Feedback - Jim Lorenzen | |
| **9.25-9.35** | Remarks Scientific Advisory Group, SAG Chair<br>Short introductions of SAG members | Prof K. Njau |
| **9.35-9.45** | Introducing the Banana Centre of Excellence at NM-AIST | Prof K. Njau |
| **9.45-9.55** | Opening Speech: Dr January Mafuru, Zonal Director, (Northern Zone) for Research and Development | Prof. K. Njau |
| **9.45-10.05** | Opening Speech: Dr Cyprian Ebong, Executive Secretary, ASARECA | Prof K. Njau |
| **10.05-10.15** | Group Photo | Scola Ponera |
| **10.15-10.45** | Coffee break<br>+<br>Press conference | Scola<br>Communication staff –Neema Muhando, Peres Muhagaze, NM-AIST; Catherine Njuguna, IITA |
| **10.45-13.00** | Work Package update reports ~20 mins per WP + questions:<br>WP1 – *Jerome*<br>WP2 – *Altus*<br>WP3 – *Brigitte*<br>WP4 – *Inge*<br>WP5 – *Lukas*<br>Discussion | Richard Sikora (Chair) |
| **13.00-14.00** | Lunch | NM-AIST |
| **14.00-17.00** | Transport to NM-AIST labs, banana fields then hotel | Scola |
| **18.30-20.00** | Welcome Cocktail evening | Blue Heron |

**Comments:** WP leaders – to use the annual report style/format for WP presentation
Partners to provide details of any additional outputs/students/connections/communication to be included and presented during WP feedback
Field trip to include demo of the data logging system by Trushar et al.
Posters to be hung in place at Conference Centre during registration/coffee/lunch

**DAY 2: Tuesday 24th April**

| Time | Topic | Chair or Facilitator |
|---|---|---|
| **8.00-8.40** | Practical arrangement/transport to NM-AIST | Scola |
| **8.40-8.45** | Day 2 details | Danny Coyne |
| **8.45-8.50** | Participant Introductions | Danny |
| **8.50-9.00** | Briefing: *Rony Swennen*<br>What should be discussed during WP meetings<br>Briefing on results tracker<br>Matters arising | Danny |
| **9.00-** | Individual Work Package meetings in parallel: discussion of progress and collaboration *WP leaders* | Danny<br>SAG members to sit in respective WP meeting<br>WP leaders to appoint rapporteur |
| **10.15-10.45** | Coffee break | |
| **- 12.30** | Work package meetings in parallel: continued…… | |
| **12.30-14.00** | Lunch | NM-AIST |
| **14.00-15.00** | Poster presentations – 1 min each presenter<br>Posters to be pinned up on day 1 at Conference Centre | Jim Lorenzen |
| **15.00-15.30** | Coffee break | |
| **15.30-17.30** | Report back and update on progress and forward planning for Work Packages:<br>- WP1 *Jerome*<br>- WP2 *Altus*<br>- WP3 *Brigitte*<br>- WP4 *Inge*<br>- WP5 *Lukas*<br>discussion | Steve Rounsley (Chair)<br>20 mins each |
| **17.30-19.30** | SAG and SC parallel meetings (room TBD) | |
| **20.00** | BBQ dinner at Life Fitness Center | Scola |

**Comments**

WP discussions/planning to consider:

Milestones of framework:

- Reporting
- Delays: how to handle
- Any change of the planned framework
- Communication within WPs
- Are WP supporting each other and interacting?
- How is a WP operating in between meetings?
- Interaction with SAG

**DAY 3: Wednesday 25th April**

| Time | Topic | Chair or Facilitator |
|---|---|---|
| **8.00-8.40** | Practical arrangement/transport to NM-AIST | Scola |
| **8.40-8.50** | Day 3 details | Danny Coyne |
| **8.50-10.15** | Inter-WP meetings<br>(WP1, WP3) + (WP2, WP4, WP5)<br>(WP2, WP3) + (WP1, WP4, WP5)<br>…… | Danny Coyne<br>60 mins each |
| **10.15-10.45** | Coffee break | |
| **10.45-12.45** | Inter-WP meetings<br>(WP4, WP3) + (WP2, WP1, WP5)<br>(WP2, WP1) + (WP3, WP4, WP5)<br>…… | Danny Coyne<br>60 mins each |
| **12.45-14.00** | Lunch | NM-AIST |
| **14.00-14.30** | Data platform update<br>*Trushar Shah, Lukas Mueller, Allan Brown* | Altus (Chair) |
| **14.30-14.50** | Communication – website, publications, data sharing, etc. *Danny Coyne/Laura Cortada*<br><br>Science Writing Course *Scriptoria* | Altus |
| **14.50-15.00** | Matters arising | Altus |
| **15.00-15.30** | coffee | |
| **15.30-16.30** | Poster competition results<br>- SAG /SC feedback – *Steve Rounsley*<br>- RTB Feedback – *Michael Friedmann*<br>- PL feedback – *Rony Swennen*<br>- BMGF feedback – *Jim Lorenzen*<br><br>+ other announcements?<br>Genomics Workshop – *Allan Brown*<br>Banana Agronomy meeting – *Jerome Kubiriba* | Inge (Chair) |
| **16.30-17.00** | Next meeting<br>Closing by *CornelMassawe* | Inge |
| **19.30** | Evening Dinner at ASILI | Scola |

**Comments**

- interactions between individuals and the SC and SAG members encouraged where and when suitable
- SAG and SC to discuss together over lunch. Steve to report back on behalf of both in absence of Victor Manyong (SC Chair)

**DAY 4: Thursday 26th April**

| Time | Topic | Responsible |
|---|---|---|
| **8.00-** | Science Writing Course<br>Gold Crest Hotel | Sciptoria |
| **8.00-** | Genomic Course<br>NM-AIST | Al Brown |
| **8.00-** | BMGF Banana Agronomy meeting<br>Gold Crest Hotel | Jerome |

**Comments**
All participants to ensure they are aware of their transfer times for the airport

# Practical information

## ARRIVAL AT THE AIRPORT

An airport assistant from IITA will be waiting for you outside carrying a sign with the inscription IITA. Please look for him. In the unlikely event that you don't see the assistant at the airport, please call directly to **Scola Ponera (+255682991550)** who is coordinating transportation service.

In most cases visas for Tanzania can be purchased on arrival in Tanzania. Each traveler needs to check and is responsible for their own situation however.
**Yellow fever vaccination is essential** for travelers to Tanzania; please ensure you travel with your vaccination card.

## ACCOMODATION

You will stay at Gold crest hotel (www.goldcresthotel.com) P.O Box 13285, Arusha, Tanzania, TEL.+255 27 2545302 MOBILE. +255 677 016774
This is a nice hotel which is located on Old Moshi Road, just 1 km from the city of Arusha. All the rooms have WIFI wireless Internet. A small swimming pool is available at the hotel and can be used at no charge.
Breakfast will be served from 06:30 hrs.

## CHARGES

Individuals and project partners with own contracts are responsible for their own costs.
SAG and SC: costs are covered
Accommodation B&B: $85 USD.
Airport transfer 50 USD per trip.
Late check-out: After 13.00 to 17.00 the charge is 50%. After 17.00 the charge is 100%.
(Late 'out-checkers' may consider to take the last shower in a room of a colleague).
All 'additional' (extra) costs at the hotel are the responsibility of the individual. Please ensure that all your extra bills are settled before you depart (i.e. laundry, phone calls, drinks etc.).

## VENUE

The meeting will be held at Nelson Mandela Institution of Science and Technology (www.nm-aist.ac.tz), which is a 20 minute drive from East African Hotel. There will be two coaster buses for transportation from the hotel to the venue and back. The bus will leave outside the hotel at 7:45am

## CURRENCY

The currency in Tanzania is Tanzania shillings (TZS)
1USD = 2200 TZS

USD bills as well as major credit cards are widely accepted in Tanzania.

## MEALS

Breakfast is included in hotel rate. Group lunch and two coffee breaks will be served during the meetings. Official dinner is organized for Tuesday and Wednesday evenings and cocktails on Monday. Participants are responsible for other evenings.

Please inform us if you have any special dietary requirements.

## EXPENSES

For SC and SAG members additional costs will be reimbursed against receipts. Please collect receipts for all taxis and out of pocket expenses to ease the process. Expenses and ticket refund can be collected on Monday 23rd during the coffee break.

**TIME ZONE**
Arusha is 3 hours ahead of Greenwich Mean Time (GMT).

**POWER SUPPLY**
Electric power in Arusha is 220 – 240 volts and the socket types are Type G & D:


Type G


Type D

**CLIMATE**
The average maximum daytime temperature in Arusha in April high –low is 25°C/ 18°C. It is also raining so please bring in rain season outfit.

**SOCIAL EVENTS**

- On Sunday 22nd April, there will be welcome drinks and delicious snacks available at the Gold crest hotel. There will be an open dinner.
- Monday 23rd April: Official Welcome cocktail is scheduled to take place at the Blue Heros at 19:00 hrs.
- Tuesday 24th April: BBQ dinner party is scheduled to take place at Life Fitness Center at 19:30 hrs. Transportation has been arranged leaving the hotel at 19:00hrs
- Wednesday 25th April: Closing dinner is scheduled at 19:30hrs at ASILI restaurant. Transportation has been arranged leaving the hotel at 19:00 hrs. Please be punctual.

**HEALTH NOTES**
We recommend that you drink bottled water while in Arusha.

If you are taking any medicine, do not forget to bring them with you.

**TRANSPORTATION TO THE AIRPORT**

We need your flight information in order to arrange transportation to the airport. Gold Crest Hotel is about 45 minutes to one hour from the airport and you are advised to be at the airport at least 3 hours before flight departure. Therefore, transportation will be arranged to depart from the hotel 4 hours before your flight time.

**Tourist information**

For tour safari please contact direct Ms. Scola Ponera.

**OTHER ASSISTANCE**

We will have a secretariat at the meeting venue and if you need any help with photocopying or printing, just let us know.

***Important telephone contacts for any emergency:***

Danny Coyne: + 254 714782436; D.Coyne@cgiar.org

Scola Ponera: + 255682991550; S.Ponera@cgiar.org

**We wish you a very pleasant stay!**

## ANNUAL MEETING ANNEX

### The nine planned Primary Investment Outcomes

1. Matooke and Mchare breeding pipeline performance increased by a 15-20% higher production of seeds facilitating larger progeny populations from which to select better performing and more pest and disease resistant hybrids (linked to WP1)
2. Accelerated Matooke and Mchare banana breeding through early identification of material resistant to Fusarium wilt, Sigatoka, nematodes and weevils (linked to WP2)
3. Genetics of resistance to *Fusarium oxysporum* f. sp. *cubensis* (Foc), burrowing nematode (*Radopholus similis*) and weevil determined in banana facilitating development of molecular markers for banana breeding (linked to WP3)
4. Breeders have better understanding of traits of importance to endusers and use this to orientate breeding strategies and early selection processes (linked to WP4)
5. Simplified, standardized protocol and tools for trial design and implementation, data collection and sharing implemented by all partners allowing meta-analyses across sites (linked to WP4)
6. Farmers participating in selection of new hybrids, with feedback driving changes to strategy and selection processes of breeding programs to improve tailoring of future improved hybrids (linked to WP4)
7. Farmers across Uganda and Tanzania and beyond growing their preferred NARITA cultivars, alongside local cultivars (linked to WP4)
8. Public access banana breeding database used on a daily basis by all project partners and regularly accessed by Musa researchers and breeders globally providing a virtual hub for information exchange, R&D collaboration and enhanced adoption of new hybrids (linked to WP5)
9. Project management

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

## Press Release and Project Overview

**Banana improvement project team meets to track progress**

**Arusha, Tanzania 23 April 2018.** A team of banana researchers will this week (23-27 April 2018) gather at Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha to review the progress made over the first three and a half years of the project and plan next years' activities. This international breeding platform coordinated by the International Institute of Tropical Agriculture and with its main basis in Uganda and Tanzania has links across 6 continents. Once a year they meet in the heartland of African banana to share progress, develop the next stage and observe their achievements in the field.

This international breeding platform is special for a number of reasons: banana is the most difficult crop to breed and so only a handful of banana breeding programs exist, which need to be united. These also tend to be national breeding programs, while 'Breeding Better Bananas' combines the expertise of scientists from across the world, including partners from national breeding programs. Researchers from Australia, Belgium, Brazil, Czech Republic, India, Kenya, Malaysia, South Africa, Sweden, Tanzania, Uganda and USA are involved in this unique project, and share their expertise, knowledge and plant material, towards improving this hugely important crop in the East Africa Region.

The platform is bolstering and strengthening the banana breeding programs in the two countries, with the aim of speeding up the development of new high-yielding and disease-resistant hybrid banana varieties. It especially focuses on the two most popular cooking bananas in the region - East Africa Highland banana (EAHB) also known as Matooke, and Mchare which is grown mostly in Tanzania. So far more than 250 Matooke hybrids have been selected for advanced yield and consumer trials and the first Tanzanian Mchare hybrids produced by hybridization with multiple disease resistant wild bananas have been planted in 2018.

Millions of smallholder banana farmers in Tanzania and Uganda rely on banana as a staple food and as a major source of income. The two countries produce over a half of all bananas grown in Africa, with the region's yearly banana crop valued at $4.3 billion. However, farmers are producing just a small proportion, about 9%, of what is possible, largely due to the devastating impact of pests and diseases. So, this project is focused on breeding varieties that farmers like, with resistance against the key problems. Bananas are difficult to breed though, because they are sterile and do not produce seeds. Breeders deal with this by using fertile parent varieties that do produce seed but this process takes a very long time. The researchers in this project are working together, using cutting edge techniques in state of the art laboratories across the world to overcome these issues, speed up the process and increase the generation of new varieties with good resistance to pests and diseases.
The major diseases that are being addressed by the project are Fusarium Wilt and Black Leaf Streak diseases (Sigatoka disease), while the major pests are the plant parasitic nematodes (microscopic worms) and banana weevils.

The project is also providing fundamental support to nurturing our next generation of banana researchers, through post-graduate student and technical staff training in advanced breeding techniques. The project is also facilitating the exchange of genetic plant material across countries and even continents in order to use the best material for developing improved hybrids, establishing the foundations of a globally connected banana breeding system.

“The team anticipates to develop hybrid banana varieties with a 30% higher yield and a 50% higher resistance to at least three of the major pests and diseases, compared to the current varieties grown by the farmers under the same on-farm conditions. The new varieties will also meet over 90% of the quality traits for consumers found in the current varieties”, said Prof Rony Swennen, Lead Banana Breeder at the International Institute of Tropical Agriculture (IITA) and the project’s team leader.

The project is led and coordinated by IITA but works hand-in-hand with the National partners in Tanzania and Uganda. The regional breeding activities are being conducted at the Nelson Mandela African Institution of Science and Technology (NM-AIST) in Arusha, Tanzania with close collaboration with regional Agriculture Research Institutes (ARI) in the banana growing areas and at the Uganda Banana Breeding Programme of the National Agricultural Research Organization (NARO), at Kawanda, and Sendusu, Kampala.

“Bananas are immensely important in Uganda and the region but are being heavily attacked by pests and diseases. This project is enabling us to link with other breeding programs across the world, to exchange banana varieties and use the best material in our breeding program. This is the first time this has happened on such a scale” says Jerome Kubiriba, Head of the Banana Program, NARO who heads up the breeding activities in Uganda for the project. He goes on to add that this project “will provide training and support to help create research teams in Uganda and Tanzania to develop improved high yielding hybrid varieties which our farmers are desperate for. Through this project, we will have a more efficient, faster and more vibrant banana breeding system across East Africa that can better respond to our immediate and to future challenges and especially now in the face of climate change.”

The project is also constantly attracting new partners who wish to join the team. This year has seen three new partner organizations joining in, continually expanding the team and contributing new skills. Meanwhile, the project is way ahead of schedule in a number of its activities. The first banana breeding program in Tanzania has been established through this project, paving the way for new Mchare varieties in the future. Matooke and Mchare seed production has already been increased 3-fold and in Uganda numerous Matooke hybrids resulting from crosses made in this project have been selected for yield assessment, way more than the year 3 target. Novel time-lapse movies, made over several days, are being explored and to aid in our understanding of the flowering process of Mchare and Matooke. For example the movies have shown that seed fertile banana varieties open their flowers earlier than seed sterile varieties and have helped in determining the most useful time to fertilize banana flowers. Meanwhile, the vast amount of data already collected and being currently collected in this project – is being amassed, organised and stored on a database, called Musabase, for everyone, everywhere, to access – preserving all this valuable information and saving time in the future.

**Building on past successes**

This is not the first time NARO, Uganda and IITA have teamed up. They successfully developed the first ever hybrids of the East African Highland Banana, named NARITA, 20 of which are being evaluated and promoted by the project in Uganda and Tanzania for local suitability and acceptance by farmers. There are 27 NARITA’s, two of which have already been released by NARO in 2010 in Uganda and which are now grown in over 15% of banana farms in Uganda.

“It took 18 years to generate these hybrids and represents a major milestone in breeding banana varieties in the region, but they can still be improved on, in particular in respect to pest and disease resistance. Our new project focuses on this while building on their success, taking banana breeding to the next level,” says Rony Swennen.

The project is unearthing the genetic foundation and diversity of existing banana varieties using the most modern cutting-edge science techniques to identify and better utilise sources of resistance to the major pests and diseases. This is being complemented by studies to understand the spread and damage caused by these pests and diseases, as well as to develop rapid diagnostic tools and faster screening mechanisms to quickly identify resistant varieties.

Through this project the exchange of banana genetic material from across the globe creates a rare and truly international network, with the most experienced banana researchers joining forces to enable the best efforts for improving Matooke and Mchare in East Africa.

The IITA Mchare breeding program is hosted at the Nelson Mandela African Institution of Science and Technology where other banana research is taken place. During this international gathering Prof Karoli Najau, Vice chancellor will explain his vision how his Institution is building a Banana Centre of Excellence at NM-AIST.

This project is being conducted within the framework of the CGIAR Research Program on Roots, Tubers and Bananas (RTB).

For more information see: http://breedingbetterbananas.org/

**Partners:**
Agricultural Research Institute, Department of Research and Development, Ministry of Agriculture, & Food, Tanzania

The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania

National Agricultural Research Organization, Uganda

International Institute of Tropical Agriculture-Uganda, -Tanzania, -Kenya

Bioversity International

University of Malaya, Malaysia

Swedish University of Agricultural Sciences, Sweden

Stellenbosch University, South Africa

Cornell University, USA

Katholieke Universiteit Leuven, Belgium,

University of Queensland, Australia

Empresa Brasileira de Pesquisa Agropecuária, Brazil

National Banana Breeding Program, India

Institute of Experimental Botany, Czech Republic

University of North Carolina at Charlotte, USA

David H. Murdoch Research Institute, USA

Weill Cornell Medical College, USA

ILLUMINA, USA

## POSTER PRESENTATIONS

### Abstracts Work Package 1

# POLLINATION AND SEED GERMINATION SUCCESS IN 'MATOOKE' BREEDING

**Batte, M.[1,2] *, Swennen, R. [3,4,5], Uwimana, B.[1], Akech, V.[1], Brown, A.[3], Lorenzen, J.[3Ω], Hovmalm, HP.[2], Geleta, M.[2], Ortiz, R.[2]**

[1] International Institute of Tropical Agriculture (IITA), P.O. Box 7878, Kampala, Uganda
[2] Swedish University of Agricultural Sciences (SLU), P.O. Box 101, SE 23053 Alnarp, Sweden
[3] International Institute of Tropical Agriculture (IITA), C/o The Nelson Mandela African Institution for Science and Technology (NM-AIST) P.O. Box 447, Arusha, Tanzania.
[4] Laboratory of Tropical Crop Improvement, Katholieke Universiteite Leuven (KUL), Willem De Croylaan 42, 3001 Leuven, Belgium.
[5] Bioversity International, Willem De Croylaan 42, 3001 Heverlee, Belgium.
[Ω] Currently with Bill & Melinda Gates Foundation, P.O. Box 23350, Seattle, WA 98102, USA
* Corresponding author: M.Batte@cgiar.org

East African highland bananas (EAHB) were originally regarded to be sterile. However, screening using 'Calcutta 4' revealed some fertile EAHB. This breakthrough led to EAHB crossbreeding at the international institute of Tropical Agriculture (IITA) and National Agricultural Research Organization (NARO) in Uganda. This study aimed at assessing the progress and efficiency of the EAHB breeding programme in the last 20 years, using the data collected at IITA-Uganda from 1995 to 2015. Month of the year had no significant effect on pollination success. Hence, banana pollination may be done throughout the year. Tetraploids were more female-fertile than triploids. Also, *Musa acuminata* subsp. *malaccensis* accession 250 had the highest pollination success when used as a male, followed by cultivar 'Rose'. These two accessions outperformed 'Calcutta 4', which was regarded as the best male-fertile parent. Thus, these two cultivars should be used in screening banana accessions for female fertility. Seed germination percentage was highest in $2x \times 4x$ (36%), followed by $2x \times 2x$ (22.8%), $3x \times 2x$ (11.1) and lastly $4x \times 2x$ (7.4%). Pollination should be optimized to boost seed set and embryo culture protocol should be improved to increase embryo germination rate.

# NUTRITIONAL AND SENSORY EVALUATION OF FIFTEEN LOCAL COOKING BANANA (MCHARE) CULTIVARS

**Dotto, J.[1], Matemu, A.[1],Ndakidemi, P[1] and Brown, A.[2]***

[1]The Nelson Mandela African Institution of Science and Technology, Old Moshi road, P.O. Box 447, Arusha, Tanzania, dottoj@nm-aist.ac.tz, athanasia.matemu@nm-aist.ac.tz, patric.ndakidemi@nm-aist.ac.tz;
[2]International Institute of Tropical Agriculture, c/o The Nelson Mandela African institution of Science and Technology, Old Moshi road, P. O. Box 447, Arusha, Tanzania,
* Corresponding author: A.brown@cgiar.org

Banana (Musa sp.) is an important fruit worldwide. In East Africa, cooking bananas are an important staple nutritional food and play a key role in addressing the issues of food security in the region. Considerable research is focused on the improvement of banana varieties with regards to good agronomic characteristics (e.g. disease resistance) but limited work has focused on sensory qualities. Nutritional value of the local bananas (particularly Mchare) have not been addressed. Additionally, consumer preferences differ greatly in many varieties of banana produced. Evidently, the need to evaluate the nutritional and sensory attributes of local banana varieties cannot be overemphasized. This study expects to identify the local banana cultivars with desirable characteristics with respect to nutritional value and sensory quality. This information is vital to banana breeders for developing improved banana cultivars that will be readily adopted by local farmers.

**Keywords**: Local banana, nutritional value, sensory attributes, consumer preferences

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

**Massawe,V.[1], Mduma, H. S[1], Swennen, R[1] and Brown, A[1*]**

[1]International Institute of Tropical Agriculture, c/o The Nelson Mandela African Institute of Science and Technology, Old Moshi Road, P.O. Box 447, Arusha, Tanzania

* Corresponding author: A.brown@cgiar.org

East African diploid cooking bananas include Mchare (syn. Mshare, Muraru or Mlali) is a staple crop for millions of subsistence farmers in Tanzania and other parts of East Africa. Several endemic pathogens are severe constraints to Mchare production. Sources of resistance to these pathogens have been identified but successful introgression of resistance is impeded by sterility issues that complicate breeding. The objective of this study was to assess quantity and viability of pollen among Mchare to identify the most fertile cultivars to be utilized in breeding schemes to provide farmers in East Africa with improved varieties. Pollen was collected once a month from fourteen genotypes (seven wild varieties and seven Mchare varieties) over a 12 month period. Quantification of pollen grains (3 replications per genotype) was accomplished by generating counts with image analysis software (ImageJ). Pollen viability was tested using TTC staining procedures. Wild (or unimproved) varieties such as 'Calcutta 4' and 'Borneo' produced the highest pollen counts and greatest percentage of viable pollen. Significant differences were observed among wild types, between wild and Mchare bananas, and among Mchare. Among Mchare varieties, 3 distinct groups could be observed with the most fertile cultivars (Huti White, Huti Green, and Mchare laini) performing significantly better than the other Mchare.

# MEASURING BANANA CANOPY COVER: TOWARDS MODELLING BANANA GROWTH WITH THE AQUACROP MODEL

**Stevens, B[a,b,c], Brown, A[b], Ndakidemi, P [c,], Diels, J[a], Vanuytrecht, E[a], R. Swennen, R[a,b]**

[a] KULeuven, Leuven, Belgium
[b] International Institute of Tropical Agriculture (IITA), Arusha, Tanzania
[c] Nelson Mandela African institution of Science and Technology (NM-AIST), Arusha, Tanzania.

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The biggest abiotic threat to banana production today is water stress. Banana plants 'mask' they are drought stressed, keep their leaves hydrated and show no easy indicators of water stress. Canopy cover and leaf growth are often considered the first physiological signs of drought, but farmers need an easy-to-use decision-support tool to better assess irrigation water use and its impact on yield.

In this research, the water-driven AquaCrop computer model, developed by the FAO, will be adapted for banana (Musa. spp.). To create a calibration dataset, 2 cultivars (Mchare-Huti Green and Cavendish-Grand Naine), are subjected to 2 moisture treatments: full irrigation (FI) and deficit irrigation (DI). Data collected contains climate, soil moisture and plant growth data as canopy cover (CC), biomass and yield. Imposing moisture treatments affected all growth parameters. CC started differing significantly and CCmax values of 75% (FI) and 65% (DI) were reached at flowering. Depending on incoming radiation, CC was found to change significantly diurnally and differences up to 17% have been found, hence pointing to the need of taking CC pictures in the morning. Plants are expected to be ready for harvest at June 2018, after which a first AquaCrop simulation run can be undertaken.

# IDENTIFICATION AND CHARACTERIZATION FOR MCHARE DIPLOIDS

**Jackline, U, Mduma, H. S and Brown, A.***

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*Corresponding author

Characterization of Mchare bananas (AA Mchare subgroup) was carried out following a descriptor protocol for bananas developed by Taxonomy Advisory Group. Eight cultivars were characterized using a set of 32 minimum descriptors with the aim of determining phenotypic variability and distinctiveness among Mchare cultivars. Results showed that that considerable phenotypic variation exists among Mchare in respect external colour of the pseudostem, colour of the male bud, number of hands per bunch, bract imbrication, and bract persistence. Preliminary data suggest that the phenotypic descriptors are adequate to distinguish among cultivars. Additional Mchare and Muraru cultivars will be described and a key developed for cultivar identification.

# SIMPLE SUGARS PERFORM BETTER THAN SUCROSE FOR IN VITRO AND *IN VIVO* GERMINATION OF BANANA POLLEN

**Waniale A.[1, 3, 4], Mukasa S.B. [1], Tugume A.K. [2], Tumuhimbise R.[3], Kubiriba J.[3] and Swennen R.[4]***

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Poor stigma receptivity limits seed set and consequently conventional improvement of East African Highland Bananas. This study sought to find pollen germination media (PGM) that can germinate banana pollen fast in vitro and *in vivo*. PGM was prepared by dissolving 0.01g $H_3BO_3$, 0.25g $MgSO_4.7H_2O$, 0.25g $KNO_3$, and 0.4g $Ca(NO_3)_2$ in a litre to along with a specific sugar type. Sucrose, glucose and fructose as well as glucose plus fructose and glucose plus fructose plus sucrose were varied at 1%, 3%, 5%, 10%, 15% and 20%. Banana pollen of Calcutta 4 and TMB2x 8075-7 was dusted on a cover slip and slowing lowered over four drops of PGM on a glass slide. The set up was incubated in a humid chamber for 3 hour and germinated pollen was counted using a light microscope at X40 magnification. Glucose (3%) had the highest germination of 48.9% whereas sucrose (20%) had the lowest of 1.3%. PGM with low sugar level generally performed better, sucrose had least means. Comparison of 3% glucose PGM with diluted banana nectar showed that 3% glucose had higher pollen germination means. Glucose is a more readily available energy source for pollen metabolism thus higher performance compared to sucrose and nectar.

## Abstracts Work Package 2

# OCCURRENCE AND DISTRIBUTION OF *PSEUDOCERCOSPORA FIJIENSIS* MATING TYPES IN UGANDA AND TANZANIA

**Kimunye, J.[1,2] George Mahuku, G.[1*] and Viljoen, A.[2]**

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*Pseudocercospora fijiensis* the causal organism of black sigatoka in banana is a heterothallic fungus that reproduces either sexually or asexually. Sexual reproduction occurs when the opposite mating type idiomorphs occur within the same geographical region at the same time. Occurrence and distribution of *P. fijiensis* mating types (MAT) in Uganda and Tanzania was investigated using 318 isolates collected from infected banana leaves. PCR analysis with MAT specific primers detected *P. fijiensis* MAT1-1 and MAT1-2 idiomorphs on the same plant, leaf and lesion. Of the 318 isolates, 59% were MAT1-1 and 41% were MAT1-2. Populations from Kawanda, Luwero, Mbarara and Bukoba conformed to the expected 1:1 (MAT1:MAT2) ratio revealing that *P. fijiensis* undergoes regular cycles of sexual reproduction which may play a major role in epidemiology and evolution of the pathogen. A slight departure from the expected ratio was observed in Arusha (P=0.02) while MAT1-2 was absent in Mbeya. An evolutionary process may have affected the mating type region in Mbeya and Bukoba as evidenced by the amplification profiles. Sequence analysis of these populations will provide further insights into significance of mating types in *P. fijiensis* evolution and epidemiology.

Key words: mating type, *P. fijiensis*, sexual reproduction, Sigatoka

# INFESTATION ASSESSMENT OF BANANA WEEVIL (*Cosmopolites sordidus* Germar) IN DIFFERENT BANANA-BASED FARMING SYSTEMS IN ARUSHA AND KILIMANJARO REGIONS, TANZANIA

**Mohamed, Y[1], Mbega, E. R.[1], Ndakidemi, P. A.[1] and Swennen, R.[2]**

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The present study was conducted to determine population size, infestation level and farmer's understanding of banana weevils in different banana-based farming systems (BFS) namely banana monoculture, banana-beans, banana-coffee and banana-maize. This was conducted by using banana pseudostem traps, coefficient of infestation method and standard interviewing. It was conducted from June to September 2017 in Nkoaranga, Mbuguni and Ngurdoto villages (Meru District) and Uduru, Uraa and Mbosho villages (Hai District) in Northern Tanzania. The data collected were analyzed by using GENSTAT 11th edition and SPSS Version 21.

There were significant differences ($P<0.05$) in the number of banana weevils in different BFS but not in coefficient of infestation. The highest weevil population per farm (29.2) was recorded in banana-maize followed by banana-beans (8.2); however, this reading was not significantly different from other BFS while highest damage level was recorded in banana-beans (31.25 %) followed by banana-coffee (24.5). The results also showed that banana weevil was ranked to be the first insect pest and a problem for about 68.8% of banana farmers. This study recommends more studies on factors responsible for the highest population in a banana-maize farming system unlike the rest and how banana weevils can be managed in Tanzania.

# ROLE OF PLANT PARASITIC NEMATODES (*Pratylenchus goodeyi* Sher and Allen) ON FUSARIUM WILT DISEASE INCIDENCE AND SEVERITY ON BANANA

**Mduma, H.S.[1], Brown, A.[1*], Ndakidemi, P.[2], and Mbega, E. R[2]**

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A pot culture experiment was conducted to study the role of plant parasitic nematodes (*Pratylenchus goodeyi*) on incidence and severity of Fusarium wilt disease (FWD) in banana caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) using selected susceptible and resistant cultivars. Results revealed that, treatments involved nematode inoculated 14 days prior to *Foc* and combined inoculation showed higher FWD incidence and severity on susceptible genotypes and hastened disease occurrence with a reduction in plant growth compared to untreated control. Such results suggest that nematodes play a detrimental role in the incidence and severity of FWD on *Foc* susceptible banana cultivars by acting as a predisposing factor for the fungal pathogen infestation. *Foc* resistant genotypes remain resistant regardless of presence of nematodes.

## Abstracts Work Package 3

# GENETIC ANALYSIS OF RESISTANCE AGAINST *Fusarium oxysporum* F. SP. *cubense* (FOC) IN SELECTED BANANA POPULATIONS

**Arinaitwe, I. K[1, 2, 4], Teo, H. C.[4], Ali Milton[2], Kayat, F[3], Uwimana, B[5], Tumuhimbise, R[2], Harikrishna, J.A.[1, 4] and Othman, R. Y[1, 4]**

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Fusarium wilt is one of the most disastrous diseases of banana, causing an estimated annual yield loss of 60 to 90%. Understanding and analysing Quantitative trait locus (QTL) in agricultural research is key in linking economically important traits to specific regions of a chromosome. Molecular markers aid breeders to identify early in the process of banana breeding the lines with high value QTL, thereby saving on resource costs and time. The aim of this study is therefore to identify the action, interaction, number and precise location of the fusarium wilt QTLs in diploid bananas. To achieve this, two unrelated diploid mapping populations were developed for fusarium wilt resistance screening. One population of Kokopo by Monyet was phenotypically screened for fusarium wilt resistance in a pot experiment and screened genotypically with molecular markers (IRAP, ISSR and SSR). Polymorphic and heritable markers were identified and used to analyse a preliminary QTL of 60/270 genotypes using GACD software (Zhang et al. 2015). A tentative QTL was found located between Markers AGMI 139-140_2 and AGMI 146-147_2. Screening and analysis of remaining genotypes is ongoing to achieve final QTL.

# HETEROBELTIOSIS IN BANANA BREEDING

**Batte, M.[1,2] *, Uwimana, B.[1], Swennen, R. [3,4,5], Akech, V.[1], Brown, A.[3], Hovmalm, HP.[2], Geleta, M.[2], Ortiz, R.[2]**

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Heterosis, or hybrid vigour, is the superiority of the hybrid for a certain trait over the mean of its two parents. Heterobeltiosis is a form of heterosis where the hybrid is superior to its best parent. Banana breeding is a tedious, time-consuming process, taking up to two decades to develop a hybrid. Exploiting heterosis in banana breeding will contribute to selecting breeding material with high compatibility, thus increasing banana breeding efficiency. Here we document heterobeltiosis by using the recently bred NARITA 'Matooke' hybrids and their ancestors. NARITA hybrids, their parents (4*x* and 2*x*), grandparents (3*x* and 2*x*), and local 3*x* 'Matooke' cultivar checks were planted in a rectangular lattice design with two replications. Yield and other agronomic data were collected at flowering and harvest. The NARITAs were compared with their 3*x* 'Matooke' grandmothers. Heterobeltiosis on bunch weight was calculated with the data of cycles 1 and 2. All the 23 NARITAs showed heterobeltiosis for bunch weight. NARITA 17 had the highest bunch weight (29.4 Kg) and the highest heterobeltiosis of 287%, followed by NARITA 23 (186%) and lastly NARITA 19 (7%). NARITA 7, the only released NARITA hybrid cultivar in Uganda so far, had a heterobeltiosis of 58%.

# UNDERSTANDING THE GENETICS OF RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* RACE 1 IN BANANA

**Mpina, M[1, 4], Viljoen, A[1], Mahuku, G[2], Uwimana, B[3] and Brown, A[4*]**
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Plant host resistance is a vital component in controlling Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Resistance to Foc race 1 exists in wild *Musa* accessions and is being utilized in multiple breeding programs. Effective breeding requires a thorough understanding of genetics of the trait. However, genetics of resistance to Foc race 1 in banana is still unclear. In this study, we used an F1 population comprised of 106 progenies derived from a 'Paliama X Borneo' cross, to assess the genetics of resistance to Foc race 1. Parents, $F_1$ progenies and control plants were inoculated with Foc race 1 using a millet seed technique, and symptoms were scored on the discoloration of leaves (1-5 scale) and inner corms (1-6 scale). Results revealed that Paliama and Borneo were statistically different in disease reaction ($P<0.05$) showing susceptibility and resistance respectively and their $F_1$ progenies segregated ($P<0.05$) for both leaf and corm symptoms. The continuous distribution observed suggests that a single-gene model previously described probably does not exist in this population. QTL analysis using saturated genetic markers (QTL analysis underway) is required to clearly understand the genetic nature of this resistance.

## Abstracts Work Package 4

# POST-HARVEST USE OF BANANA IN UGANDA AND TANZANIA: BANANA FOOD AND BEVERAGE PRODUCTS, PRODUCT CHARACTERISTICS AND CULTIVAR PREFERENCES BY FARMERS

**Marimo, P[1], Crichton, R[1], Van den Bergh, I[1], Caron, C[2], Karamura, D[1]**

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A preliminary overview of banana food and beverage product profiles, farmers' trait preferences for the products and cultivars used to make those products from six regions where the baseline data was collected in Uganda and Tanzania is presented. Understanding the characteristics of the various fresh foods or processed products, ingredients used, processing methods and end users' trait preferences of cultivars that are popularly used to produce the products can help breeding programs in priority setting and developing a selection strategy for example when prioritising which consumption traits to maintain or improve, and/or to provide context regarding why or why not certain cultivars are adopted or rejected. New cultivars must have traits that end users desire for fresh fruits or for producing their traditional/local products, and lack of these desired traits potentially affects adoption rates.

# PRELIMINARY INSIGHTS FROM SENSORY EVALUATIONS OF NARITAS AT THE KAWANDA FIELD SITE, UGANDA

**Marimo, P[2],, Nowakunda, K[2], Khakasa, E[2]**

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Sensory evaluations were conducted to assess consumer acceptability of NARITA hybrids at the Kawanda field site. The panelists who are staff at the institute, were provided with coded samples of four NARITAs plus one local check (Mbwazirume) and asked to rate each sample on a 5-point hedonic scale for the following attributes: color, texture, taste, aroma, flavour, and overall acceptability for a common local staple, *matooke*. Matooke is prepared by steaming peeled bananas in banana leaves and then mashing. The local check, Mbwazirume was highly preferred compared to all the NARITAs. The results indicate that NARITA 7, NARITA 18 and NARITA 24 are the most preferred among the NARITAs and have the potential to be taken for on farm trials.

## Abstracts Work Package 5

# BANANA BREEDING TRACKING TOOL: BTRACT

**Karanja, M.[1], Shah, T[1], Morales, N[2], Ouma, T[1], Bauchet, G[2], Ogbonna, A[2], Mduma, H. S[3], Massawe, V[3], Akech, V[4], Uwimana, B[4], Swennen, R[3,5], Mueller, L[2], Brown, A[3*]**

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The Banana Breeding Tracking Tool (BTracT) is a system that has been developed to enhance the data management, monitoring and reporting of activities within the Banana breeding programs. It utilizes technological frameworks which allow for data capture on handheld devices. The system also synchronizes data from various locations and allows for querying and analytics on a central dashboard.

The system was built on workflows that were mapped with extensive input from the breeding program on the critical steps and activities in both the field and the laboratory. BTracT is fully integrated to the global banana breeding database (Musabase) and the data flow is seamless.

BTracT has been implemented in Arusha and will be rolled out in Sendusu and Ibadan.

| Summary of Poster tittle | Authors | Presenter | Work Package |
|---|---|---|---|
| **POLLINATION AND SEED GERMINATION SUCCESS IN 'MATOOKE' BREEDING** | **Batte**, M., Swennen, R. Uwimana, B., Akech, V. Brown, A., Lorenzen, J., Hovmalm, HP., Geleta, M., Ortiz, R. | **Michael Batte** | WP1 |
| **NUTRITIONAL AND SENSORY EVALUATION OF FIFTEEN LOCAL COOKING BANANA (MCHARE) CULTIVARS** | Joachim **Dotto**, Athanasia Matem, Patrick Ndakidemi, and Allan Brown | **Joachim Dotto** | WP1 |
| **POLLEN VIABILITY AND SEASONAL VARIATION IN SELECTED WILD MUSA (AA) DIPLOIDS AND MCHARE CULTIVARS** | Veronica **Massawe**, Hassan Mduma,Rony Swennen and Allan Brown1 | **Veronica Massawe** | WP1 |
| **MEASURING BANANA CANOPY COVER: TOWARDS MODELLING BANANA GROWTH WITH THE AQUACROP MODEL** | B. **Stevens**, A. Brown, P. Ndakidemi, J. Diels, E. Vanuytrecht, R. Swennen | **Bert Stevens** | WP1 |
| **IDENTIFICATION AND CHARACTERIZATION OF MCHARE DIPLOIDS** | Jackline **Ulotu**, Hassan S. Mduma, Allan Brown | **Hassan S. Mduma** | WP1 |
| **SIMPLE SUGARS PERFORM BETTER THAN SUCROSE FOR IN VITRO AND *IN VIVO* GERMINATION OF BANANA POLLEN** | **Waniale** A., Mukasa S.B., Tugume A.K., Tumuhimbise R., Kubiriba J., and Swennen R. | **Allan Waniale** | WP1 |
| **OCCURRENCE AND DISTRIBUTION OF *PSEUDOCERCOSPORA FIJIENSIS* MATING TYPES IN UGANDA AND TANZANIA** | Janet **Kimunye**, George Mahuku, and Altus Viljoen | **Janet Kimunye** | WP2 |
| **INFESTATION ASSESSMENT OF BANANA WEEVIL (Cosmopolites sordidus Germar) IN DIFFERENT BANANA-BASED FARMING SYSTEMS IN ARUSHA AND KILIMANJARO REGIONS, TANZANIA** | **Mohamed**, Y, Mbega, E. R., Ndakidemi, P. A. and Swennen, R. | **Yussuf Mohamed** | WP2 |
| **ROLE OF PLANT PARASITIC NEMATODES (*Pratylenchus goodeyi* Sher and Allen) ON FUSARIUM WILT DISEASE INCIDENCE AND SEVERITY ON BANANA** | Hassan S. **Mduma**, Allan Brown, Patrick Ndakidemi, and Ernest R.Mbega | **Hassan S. Mduma** | WP2 |

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|---|---|---|---|
| **GENETIC ANALYSIS OF RESISTANCE AGAINST *Fusarium oxysporum* F. SP. *cubense* (FOC) IN SELECTED BANANA POPULATIONS** | Ivan Kabiita **Arinaitwe**, Chee How Teo, Ali Milton, Fatimah Kayat, Brigitte Uwimana, Robooni Tumuhimbise, Jennifer Ann Harikrishna, and Rofina Yasmin Othman | **Ivan K. Arinaitwe** | WP3 |
| **HETEROBELTIOSIS IN BANANA BREEDING** | **Batte**, M., Uwimana, B., Swennen, R., Akech, V., Brown, A., Hovmalm, HP., Geleta, M., Ortiz, R. | **Michael Batte** | WP3 |
| **UNDERSTANDING THE GENETICS OF RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* RACE 1 IN BANANA** | Mohamed **Mpina**, Altus Viljoen, George Mahuku, Brigitte Uwimanan, and Allan Brown | **Mohamed Mpina** | WP3 |
| **POST-HARVEST USE OF BANANA IN UGANDA AND TANZANIA: BANANA FOOD AND BEVERAGE PRODUCTS, PRODUCT CHARACTERISTICS AND CULTIVAR PREFERENCES BY FARMERS** | Pricilla **Marimo**, Rhiannon Crichton, Inge van den Bergh, Deborah Karamura | **Priscilla Marimo** | WP4 |
| **PRELIMINARY INSIGHTS FROM SENSORY EVALUATIONS OF NARITAS AT THE KAWANDA FIELD SITE, UGANDA** | Pricilla **Marimo**, Kephas Nowakunda, Elizabeth Khakasa | **Priscilla Marimo** | WP4 |
| **BANANA BREEDING TRACKING TOOL (BTracT)** | Margaret **Karanja**, Trushar Shah, Nicolas Morales, Turry Ouma, Guillaume Bauchet, Alex Ogbonna, Hassan Mduma, Veronica Massawe, Violet Akech, Brigitte Uwimana, Rony Swennen, Lukas Mueller, Allan Brown | **Margaret Karanja** | WP1, 5 |

## 1.3 Opening Remarks by Guest of Honour, Dr J. Mafuru

**OPENING REMARKS BY GUEST OF HONOUR- TANZANIA**
**ANNUAL REVIEW AND PLANNING MEETING**
**THE BMGF EAST AFRICA BANANA BREEDING PROJECT**
**'Breeding Better Bananas'**
**AT NELSON MANDELA-AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY**

**23 – 25 APRIL, 2018- ARUSHA**

Vice Chancellor of NMAIST, **Prof. Karoli Njau**
Deputy Vice Chancellor of NMAIST, **Prof. Joram Buza**
Executive Secretary ASARECA, **Dr Cyprian Ebong**
Project Management team,
Members of the Project Steering Committee
Members of the Science Advisory Group;
Distinguished Invited Guests
**Ladies and Gentlemen Good morning na Karibuni sana**

On behalf of the Directorate of Research and Development of the Minister of Agriculture of Tanzania, it gives me immense pleasure to address you and welcome the participants to Arusha and to this **Opening Ceremony** of the three-day Annual Review and Planning meeting of the East Africa Banana Breeding Project entitled **"Improvement of banana for smallholder farmers in the great Lakes Region of Africa".**

The Organizing committees have requested me to express, on their behalf, their thanks and gratitude to the management of the Nelson Mandela-African Institution of Science and Technology, for hosting this gathering and IITA for supporting this workshop.

**Ladies and Gentlemen**
The Department of Research and Development is aware of the tremendous effort made, and the large investment in funds and scientific effort by the project partners in enhancing banana productivity, particularly in Tanzania in order to keep pace with increasing human population and the many problems banana growers face in regards to diseases and other serious constraints of production.

**Ladies and Gentlemen**
It has become commonplace to say, and repeat saying, that banana productivity has failed to keep pace with increasing food demand despite the steady increase in banana acreage over the years in East and Central African (ECA) region. Banana is a primary food staple as well as an essential cash crop for the region's smallholder farmers. Over 50 million people in the East African region depend on highland bananas for their food and/or income. However, average smallholder banana

productivity has remained low to less than 30% of attainable. This declining yield has compromised food and income security of our communities. In selected banana-growing areas, farmers have begun to adopt improved varieties that have only relatively recently become available from a very limited number of active banana breeding programs in the world.

**Ladies and Gentlemen**
Several factors contribute to reduced banana production in Tanzania. I am optimistic that findings from this project will certainly assist to bridge this productivity gap.

It is reassuring to learn that the goal of this project is aligned with the Second Five Year Development Plan (2010-2015) in Tanzania, particularly in regard to increasing farm productivity through providing smallholder farmers' greater access to superior seeds/planting materials, more effective tools and farm management practices, locally relevant knowledge and reliable markets through value chain analysis for livelihood of the communities.

**Ladies and Gentlemen**
The organizing committee of this meeting aims to bring the best of the science in banana crop together in an environment where everyone in the banana fraternity can benefit. Embedded within the context of this meeting, feedback from project activities is essential to be channeled directly into the scientific pool of knowledge, assist in breeding programs and networkingthe partners involved to learn and develop more in their carriers.

I am excited to hear that the first Mchare breeding program in the world has been developed by IITA here in Arusha, particularly at this campus. This effort has created the first 200 Mchare hybrids existing today. The materials have just been transplanted to field for adaptation testing. While Matooke, desert banana and plantain are important in other parts of Tanzania, in this part of the country (Arusha and Kilimanjaro), the bananas the farmers want are Mchare. Mchare is a uniquely Tanzanian banana and there is no other place in the world where this banana is receiving the attention that it gets here.

It is very pleasing to learn that NM-AIST is becoming an active partner in this work, helping to conduct consumer preference and quality testing.

**Ladies and Gentlemen**
It is encouraging to know that IITA is playing such a vital leadership role in agricultural development in Tanzania. IITA and the National Agricultural Research Systems (NARS) in Tanzania have had a long-term collaboration in several research disciplines from the time of their establishment. Successful and significant research cannot be conducted in isolation, but demands the integration of many partners with many skills. We in Tanzania, look forward to the strengthening of these collaborations with IITA and with all the partners present here today. We are always stronger together.

**Ladies and Gentlemen**

As the Ministry of Agriculture, we are responsible for the formulation of policy and regulations and creating an enabling environment for the agricultural sector. As a result, we have developed the Agricultural Policy Action Plan (APAP) which seeks to provide both a long-term vision, and focused interventions in a five-year rolling schedule. Furthermore, it presents institutional arrangements of collaborating with development partners for achieving our targets and objectives of lifting our communities out of food insecurity.

The Department of Research and Development will continue working with all regional and International organizations to enhance banana productivity and to prioritize food security and agrarian transformation in the economic development agenda of the country

**Ladies and Gentlemen**
I would like to express my gratitude to all who have so generously worked beyond normal expectations to make this meeting a success. There are far too many to thank all individually, but in particular I wish to thank the Organizing committee and our hosts from Nelson Mandela, without whom this event could not have taken place.

**Ladies and Gentlemen**
I would like also to express my appreciation to the Funding body of this project which aims at benefiting the smallholder farmers and public welfare.

Finally, I am delighted to formally declare the official opening of this Annual Banana Breeding Project meeting.

I sincerely hope you will enjoy today and the next two days of feedback and networking. Wish you well in your deliberations.

**Thank you, Karibuni Arusha**

## 1.4 SAG Feedback to SC Meeting Minutes

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

**Annual Project Planning Meeting**

**23-26th April 2018**

**SAG Feedback Meeting, 25th April 2018**
**NM-AIST, Arusha**

**Present**
SC: Jerome, Altus, Inge, Brigitte, Lukas, Rony, Danny
SAG: Klaus, Richard, Eva, Steve

**Duration**
Started: 12.50
Finished: 13.29

**Feedback**
In essence, the project is in a much better state and situation than this time last year – with credit due to Rony, who enables and provides direction to the team. There are of course issues, but the project is definitely moving in the right direction and all appears to be in good state.

Few items for cause for concern to keep eye on.

- Trying to do too much. Too many opportunities will spread the team too thin. Need to restrict the focus to prevent being spread too broad.
- WP structure. Possibly getting in the way and in its current form, is a concern to the SAG, even if a little too late to change at this point. But in any follow-on phase, need to consider changing this structure.

**Eva**
Enjoyed the pre-workshop meeting for WP4 with the breeders. Good to see the breeders being engaged, and towards an end-user perspective. Important therefore over next 18 months to consider the end user much more.

Important to be clear with the perspective of people who wish to use the data being generated in the farm trials. There is a need for considerable capacity to analyze the data that is currently being collected. Need to consider this and ensure sufficient effort is apportioned to analyze the data properly.

Maintain the goals for the individual analysis.

**Richard**
There are two overarching goals to the project: 1) breed new bananas of the types people want to eat; 2) and breed banana that can grow in the face of pest and disease

challenge. We need the team to reach these goals by working together and streamlining the process (pipeline) towards ensuring this.
Speeding up screening protocols sometimes, just cannot be done. However, there are two aspects to increasing the efficiency of screening protocols: reducing the overall length of time of the screen and increasing the throughput. If the timeframe cannot be reduced but the ability to increase the throughput can be achieved, then we have progress.

**Klaus**
Comments mainly on the organization of the team and project.

A highly dedicated and motivated group of people, which is very good.

Given the geographic and disciplinary spread of the project, it is unique that cohesion is maintained and communication kept to an apparent optimum.

Would be good to have a flowchart developed of when and how many lines / hybrids are expected to be screened by the various sections / disciplines.

Question raised as to how we can get a good dialogue between breeders and those conducting the screening.

Need to remember that not everything can be screened, and we cannot do everything, and so therefore the priorities need to be established.

**Steve**
Appears that genomic selection has gravitated towards WP3, but can the tool be applied and how and where in the breeding scheme would it fit. If this project is to become a molecular breeding program, then a molecular breeding lab should be considered – especially in any follow-on phase.

In general, good progress is apparent but there is need to be vigilant of a couple of issues. Communication will always be a recurring problem and is an issue to watch, including from WP leaders to their respective team members. And this is one area where there should be continued investment.

A flowchart of numbers for screening would be helpful to plan and accommodate the volume of plants and lines required for screening, to ensure it is possible – or so this can be planned for. And this in itself is a communication device. So mapping out the various schemes would help plan and determine project ability to cope.

Are we building the structure to enable the system to breed better bananas, or actually breeding the better bananas???

Comment – we are not just breeding for the best banana, but to enable a greater selection of bananas with broader scope of improved properties.

For the objectives of this project to have long term sustainability there is need to think about the next WP leaders and the upcoming generation of banana breeders.

There should be an understanding built into the pipeline as to how better bananas are disseminated and distributed.

**Jim**
Reiterated how there needs to be consideration for the development of a dissemination system for new bananas.

Regarding the project lifetime, and the idea is to build and put in place a system which is durable and effective in the long term. Consequently, we need to consider developing a pipeline that will work in the long term, and not restrict ourselves to defining the process by the timeframe of the project.

While there are current promises or guarantees for a second phase of the project there is optimism for a follow-on phase.

## 1.5 World Banana Forum conference statement

A record 300 delegates from over 40 countries across all continents registered in the Third Global Conference of the World Banana Forum (WBF) on November 8 and 9, 2017 at the International Conference Centre, Geneva, Switzerland. The meeting was preceded by a well-attended meeting on "Gender Equity in the Banana Industry" on November 7 and followed on November 10 by a workshop on "Engaging stakeholders in combating the Fusarium wilt disease (TR4)", which poses a major threat to global production.

The Conference had balanced representation of all players involved in banana production and trade – producers, workers' unions, retailers, importers, exporters, civil society and consumer organisations, research institutions as well as governments and inter-governmental organisations. The meeting was hosted by the Swiss Confederation and facilitated by the Food and Agriculture Organization (FAO) of the United Nations in which the Secretariat of the WBF is based. The International Labour Organization (ILO) provided key technical support to the Conference.

**Attendance at the Conference demonstrated the growing reach and influence of the WBF. The Conference celebrated the main achievements of the Forum including:**

- A Manual on Occupational Health and Safety, launched at the Conference by the Ecuadorean Minister of Labour, to train workers, company staff and growers.
- A set of best practices for sustainable production.
- A web portal from which these practices and other useful materials are easily accessible to all stakeholders.
- A practical guide for measuring and reducing the carbon and water footprints of growers
- A global multi-stakeholder Task Force to combat Fusarium wilt Tropical Race 4, influence key industry players and collaborate with global programmes against banana diseases.
- A search for a commonly agreed method for calculating decent standards of living for workers at exporting-country level.
- The facilitation of successful labour relations dialogue in Peru and West Africa

These achievements resulted from collaboration between all sectors of the industry. The Conference endorsed this approach and committed itself to seek even wider representation in the Forum, including from Africa and Asia, as well as more retailers.

The major areas of discussion focused on gender issues; labour rights; health and safety; sustainability, the environment and the impact of climate change; fair distribution of value; and combating the TR4 disease. A number of wide-ranging recommendations were made by the Conference for implementation by the Steering Committee of the Forum. The conference emphasized the need for integrated approaches for prevention and management of Fusarium wilt TR4. It supported the launch of the global programme led by FAO in partnership with the WBF, Bioversity International and the International Institute for Tropical Agriculture (IITA).

**The World Banana Forum wishes to express its gratitude and appreciation to the Swiss Confederation, FAO and ILO, the many sponsors, and the participants who all contributed to the success of the meeting.**

## 1.6 Global program on banana Fusarium wilt disease

Food and Agriculture Organization of the United Nations

# Global Programme on Banana Fusarium Wilt Disease

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

Bioversity International

IITA Research to Nourish Africa

World Banana Forum WBF

Fazil Dusunceli, Inge Van den Bergh,
Rony Swennen, Pascal Liu
*Fazil.Dusunceli@fao.org*

3rd Conference of the World Banana Forum
7-10 November 2017, Geneva

Food and Agriculture Organization of the United Nations

# Prioritised actions

## based on risk levels and production systems

(32 Work packages and 128 specific activities)


**Prevention**
- Awareness raising
- Regulations
- Preparedness
- Risk assessments
- Contingency planning
- Seed system support

**Preparedness and response**
- Risk assessments
- Regulations
- Contingency planning
- Seed system support
- Resistant / tolerant varieties
- Research and surveys

**Management / Rehabilitation**
- Resistant / tolerant varieties
- Seed systems support
- Research and dev.
- Agronomic practices
- Cropping system
- Regulations

Food and Agriculture Organization of the United Nations

# Coordination / Collaborations

## – Provisional, non exclusive

- **FAO** including its decentralized offices & IPPC
- **Bioversity** International
- **IITA** -International Institute of Tropical Agriculture
- **World Banana Forum**

- **International** organizations & institutions
- Advanced universities and agricultural **research** centres
- Regional Plant Protection Organisations (**RPPO**'s)
- International and regional banana **networks**

- **National** institutions, phytosanitary authorities, NPPOs
- **Producer and organizations**, industry & NGOs

# The way forward

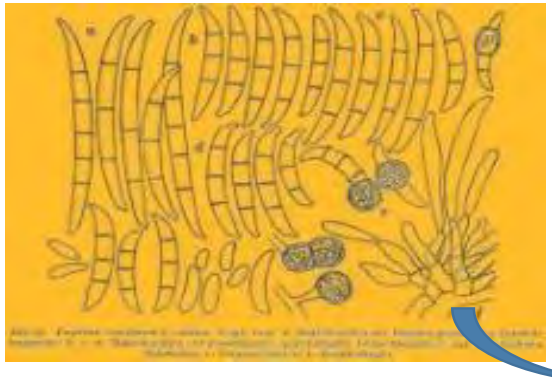
- **Principles:**
  - Complement existing efforts, promote synergies
  - Prioritized activities based on production systems and risk levels.
- **Coordination**
  - Based at FAO, run in collaboration with partners
  - Steering committee (FAO, Bioversity Int., IITA, Regional representatives: Asia, Africa, Near East, Latin America and Caribbean)
  - Programme implementation team and specialists
- **Follow up**
  - Presentation and launch at the CFS meeting on 13th November
  - Efforts to promote partnerships and mobilise the estimated requirement of USD 98 million
  - Further advocacy and publicity
  - Implementation planning
  - http://www.fao.org/food-chain-crisis/how-we-work/plant-protection/banana-fusarium-wilt/en/


Not panic but vigilance
Prevention best
Threat is real – Act !!
Thank you !
Integrated management & diversity needed
Collaboration is key
Food and Agriculture Organization of the United Nations

# The disease: Fusarium wilt

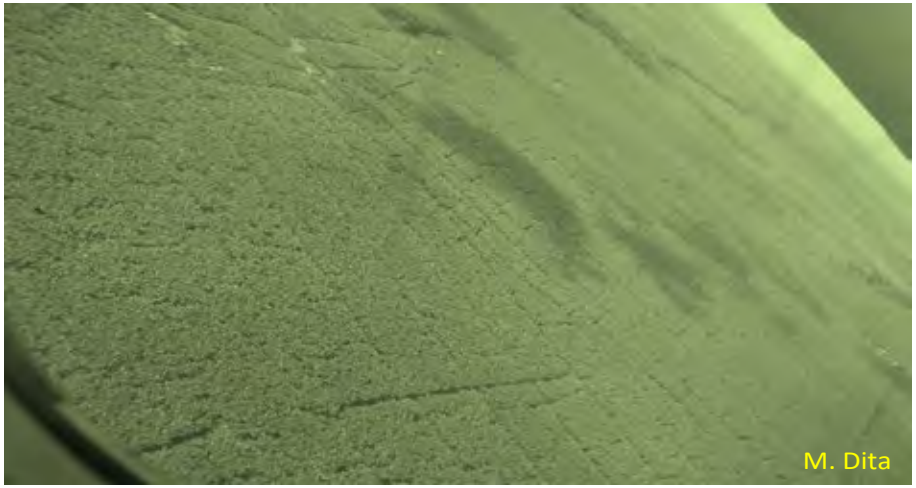
*Fusarium oxysporum* f.sp. *cubense* – *Tropical race 4 (TR4)*

- **Soil borne fungus**
- **Spread**:
  - Planting materials
  - Soil particles: Vehicles, shoes, tools….
  - Water: Irrigation, drainage, floods



# Major challenges in controlling TR4

- Extremely **aggressive**
- **No chemical** control option
- **Containment** is difficult
- Cropping systems **vulnerable**
- Lack of resistant **varieties**



M. Dita

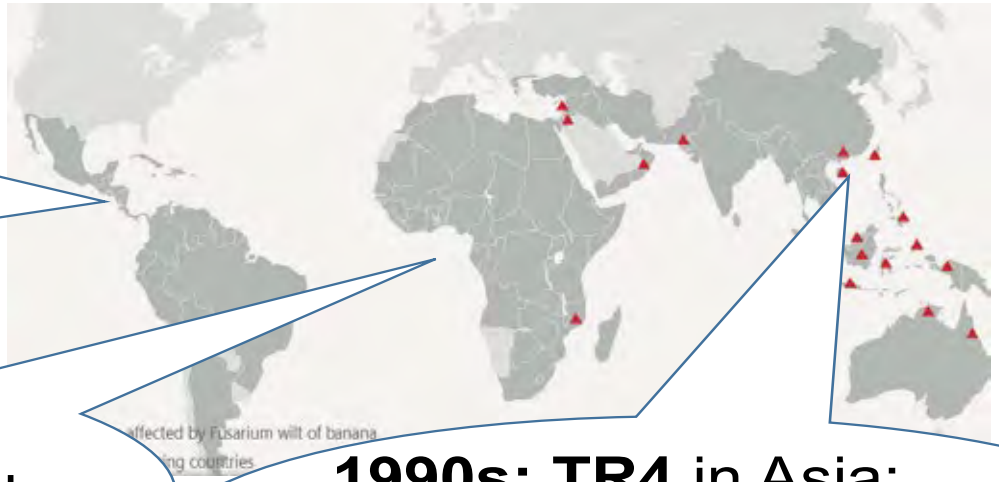
# Major challenges in controlling TR4

- Cavendish most susceptible
- Supplying around 50 % of world`s bananas

M. Dita

# Fusarium Wilt Disease – What can it do ?

**Past lessons** with Race 1: USD 2,3 billion

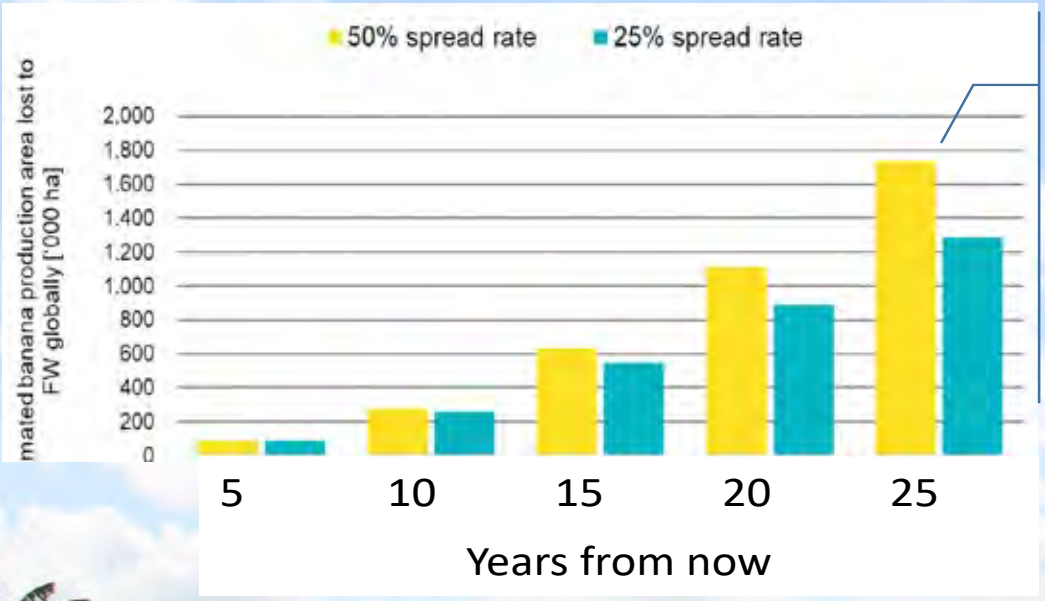



**2010s: TR4** in Near East, South Asia and Mozambique

**1990s: TR4** in Asia: Reported losses sum up USD 390 million

Food and Agriculture Organization of the United Nations

# TR4 –Assessments for future spread * ?




**By 2040**

- 1,6 million ha
- 17 % of current area
- 36 million tonnes valued USD 10 billion

* Assessments by Bioversity International scientists (Sheerer *et al*, 2016)

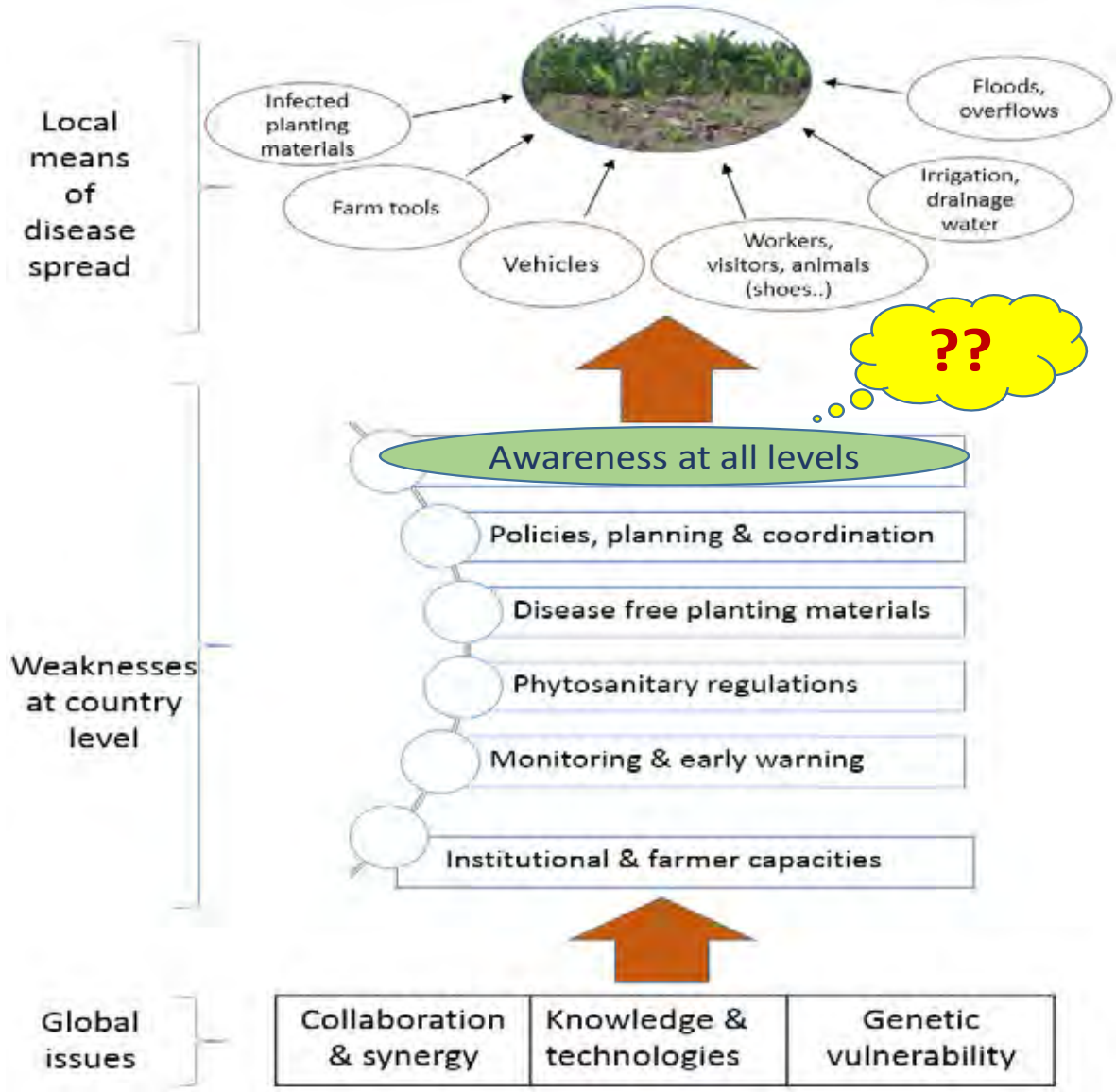
Food and Agriculture Organization of the United Nations

# Factors contributing to spread

Local

Country level

Global



# Global Programme on Banana Fusarium Wilt

## A multi disciplinary partnership

Governance, policies, national and farmer linkages

Research, Knowledge, IPM and biodiversity

Linkages with producers, NGOs and industry

Research in Africa and breeding resistant varieties

+ Other international and national institutions, Universities

# Global Programme on Banana Fusarium Wilt

**More resilient banana systems with reduced disease risks and impact**

**Improved prevention**

- **Surveillance**, monitoring and early warning conducted
- Risk **analysis** and phytosanitary **regulations** improved
- **Containment** improved

**Improved integrated management**

- Germplasm and v**arieties with resistance** developed
- **Integrated** disease management improved

**Enhanced synergies, capacities and policy environment for improved prevention and management of Fusarium wilt disease**

- International **collaboration** strengthened
- Policies, **strategies**, awareness enhanced
- National **capacities** improved

## 1.7 ISHS ProMusa workshop on TR4

*Symposium workshops*

*Growing and Marketing Banana under Subtropical Conditions*

http://www.ihc2018.org/en/S04.html

# Developing commonly agreed guidance for the prevention and containment of banana Fusarium wilt TR4

### Workshop date, time and venue

To take place 15 August from 08:30- 10:30; the room will be posted at the symposium

### Organizers

Food and Agriculture Organization (FAO), Bioversity International, International Institute of Tropical Agriculture (IITA) and World Banana Forum (WBF)

### Abstract

Fusarium wilt is among the major diseases of banana. The Tropical race 4 (TR4) of the soil borne fungus (*Fusarium oxysporum* f.*sp. cubense*) is currently of a major concern as it continues to spread to new geographies. Since eradication and management is challenging, the most effective means to protect bananas is prevention of spread and prompt containment. In this respect, countries, producers and other stakeholders require sound strategies and guidance on the policies, measures and actions needed for prevention and containment. The workshop will be conducted to review and finalize the prevention and containment elements of the two guidance documents i) Policy and technical guide for prevention and management of Fusarium wilt disease; ii) Recommendations for travellers to prevent the spread of Fusarium wilt disease TR4.

The workshop is organized in context of the ``Global Programme on Prevention and Management of Banana Fusarium Wilt Disease`` jointly by Food and Agriculture Organization (FAO), Bioversity International, International Institute of Tropical Agriculture (IITA) and World banana Forum (WBF).

### Purpose

- To review the available knowledge and gaps in prevention and containment of TR4, and develop practical recommendations for policy makers and other actors

*Symposium workshops*

*Growing and Marketing Banana under Subtropical Conditions*

http://www.ihc2018.org/en/S04.html

## Specific objectives:

- To review update the guidance on `prevention and containment` aspects of the "Policy and technical guide for prevention and management of Fusarium wilt disease"
- To review and establish consensus on the document "Recommendations for travellers to prevent the spread of Fusarium wilt disease TR4"

## Programme:

- Review of the available knowledge and gaps in prevention and containment of TR4 (Miguel Dita, Inge Van den Bergh, Bioversity International) - 15 min
- Presentation of the `Global Programme on Prevention and Management of Fusarium wilt Disease` (Fazil Dusunceli, FAO) – 10 min
- Presentation of the two guidance documents i) Policy and technical guide and ii) Recommendations for travellers, developed by FAO, Bioversity International, IITA and WBF, presented by Inge Van den Bergh, Bioversity International – 10 min
- Working group discussions: (60 min)
  - Group A: Policy and technical guide for prevention and containment of spread
  - Group B: Recommendations for travellers
- Presentations back to plenary (20 min)
- Summaries and conclusions (10 min)

## Working documents:

- Policy and technical guide for prevention and containment of spread: (PDF 512 kB)
- Recommendations for travellers: (PDF 384 kB)
- Comments form: (PDF 186 kB)

## 1.8 Final BPAT Report: Banana and Plantain

# Breeding Program Assessment of Banana Breeding Programs at IITA (Arusha, Tanzania, and Sendusu, Uganda) and NARO (Kawanda, Uganda) and Plantain Breeding at IITA Ibadan/Onne

## May 25th – April 1st 2017 and Nov 27-28th 2017

## Final report to the Bill and Melinda Gates Foundation
## Written by the BPAT team
## (Yilma Kebede, Andre Drenth, Errol Corsan, Mark Cooper and Chris Lambrides)

### Introduction

In May/June of 2017, the BPAT team (André Drenth, Yilma Kebede and Chris Lambrides) assessed the IITA/NARO banana breeding programs operating in East Africa. The assessment included site visits to the IITA Mchare program in Arusha, Tanzania, the IITA Matoke program in Sendusu, Uganda and the NARO Matoke program in Kawanda, Uganda. An expanded BPAT team (André Drenth, Yilma Kebede, Chris Lambrides, Mark Cooper and Errol Corsan) assessed the IITA plantain breeding program at Ibadan, Nigeria.

East Africans get about 30% of their calories from banana. The East African Highland banana also known as 'Matoke' are sterile triploids (AAA) used primarily in Uganda for cooking, roasting, beer production and desserts. In Tanzania, the 'Mchare' are sterile diploids (AA) used primarily as a cooking banana in the Arusha-Kilimanjaro region. The yield gap between actual and potential production in farmer fields is large for both Mchare and Matoke bananas. For example, in Uganda production is about 10t/ha/year on average while the yield potential is 60 t/ha/year. There are multiple reasons for low yield, including susceptibility to pests and diseases such as nematodes, weevils, Black Sigatoka, Fusarium wilt (Race 1 for Mchare), Xanthomonas wilt. Production is affected by combinations of abiotic factors like declining soil fertility, low nutrient inputs and drought.

Fifty percent of global plantain production is in Africa where 70 million people use it as an important food crop. Plantain is not grown in plantations but predominantly in smallholder garden-type plots. Typically, yields are very low and performance of newly planted plantains don't persist and reduce after the second cycle. The yield decline leads to more frequent replanting of plantain compared to Matoke in East Africa. Poor ratooning of plantain is to some degree due to the mat growing out of the ground which makes it highly susceptible to

nematode damage. The yield gap between actual and potential production in farmer fields is large in West Africa; 8 t/ha/year has been reported compared to the potential of 35 t/ha/year. Plantain originated in Asia but has been cultivated in central Africa for hundreds or may be thousands of years producing diversity within the introduced clonal set. At present approximately 120 varieties of plantain are recognised. There are three major types of plantain, French, false horn, and horn. Although the genomic make up of plantain is reported as AAB it has recently been found that the B genome contains a large amount of A, most likely due to past recombination events.

The BPAT team recognised that there are a number of major challenges to banana and plantain breeding in Africa. These relate to low fertility and seed set of breeding materials, poor germination of seed and the need to select for a complex trait like parthenocarpy (fruit without seed) and most importantly a consumer quality profile that at this point is yet to be properly defined.

Collectively the IITA/NARO programs demonstrated that there was excellent capacity to carry out banana breeding in East Africa and plantain breeding in West Africa, particularly with regard to the mechanistic aspects of the crossing and testing activities in the field and clonal propagation via embryo rescue and tissue culture in the laboratory. Without exception, the research staff were early to mid-career, enthusiastic and clearly dedicated to their designated roles. The research stations were, by enlarge, well equipped or had the potential to be so, in the near future. The Mchare program in Arusha was established recently and only just begun to produce materials for testing. The Matoke programs in Uganda were more advanced with the NARO program being the most mature having operated for more than 25 years. A plantain breeding program was run by IITA from 1987 to 2005 at Onne in Nigeria after which it received little to no funding and all activities stopped until it was re-established through funding from USAID in 2013 and is now based primarily at IITA Ibadan. The Onne research station is also being brought back into operation.

In terms of achieving genetic gain, the BPAT team felt the programs could be improved in many areas, and currently they are not producing elite materials in sufficient quantity to make significant genetic gain. Areas for improvement are detailed in the following sections of this report. The report will start by introducing ‘Product concepts’ which will aid in framing the design and focus of the breeding programs. This will be followed by more specific detail about the Breeding and Testing programs, Variety release, Benchmarking genetic gain, and other issues around Communication, Alignment of expectations, Collaborations and Access to literature.

**Product Concepts**

Product concepts form the framework through which all components of the breeding program would be aligned. While the breeders are clear about their breeding objectives there was no formalised documentation setting out the type of products to be developed. Current focus of programs (IITA and NARO) is on production zones with political boundaries delineated by west, central and east zones for EAHB (East African Highland Banana). The stated breeding objectives are mainly determined by constraints such as pest and disease screening. There is a need to focus on product specification for the main production areas and consumers. Production areas in east Africa with regards to EAHB and W Africa with regards to plantain need to be clearly defined along agro-ecological zones and suitable sites identified to determine the productivity of current varieties EAHB and Narita 7 as standards for EAHB and suitable plantain standards to have a benchmark for new varieties.

Delineation of different Matoke bananas by zones is not well documented. Western Uganda is the main production area but Black Sigatoka, weevil and nematodes are not key production constraints rather compact bunches are required for transportability. Such kind of product concept information in one form or another does exist with the breeders in IITA and NARO but

it should be formalized, through proper documentation and sharing across disciplinary teams. Quality descriptions are also needed for plantain. Product concepts are based on unique product specifications taking into account area of adaptation, maturity, traits, farmer preference and consumer attributes. It provides a way to determine whether the program is lined up with the important production requirements, smallholder and consumer needs. That is, for a program responsible for various production areas and consumer needs the proportional efforts in the breeding program should be reflected in the size of nursery, yield tests, and screening for defensive traits addressing each of the product concepts. Product profile/concept is not only about documenting various attributes but is essential as an accountability framework, and a look back tool to assess progress and bring focus to a product development program. It provides clarity regarding priority of breeding targets and managing the product lifecycle (introduction/adoption & retirement of products). It will help align the breeding program with all other disciplines so everyone is aware of the program's direction including leadership of the organization. Meaningful ownership of the product concepts by all staff involved in the program, interaction across discovery, development, validation and deployment is essential to realize intended outcomes.

Product concepts help decide:

- What types of product(s) to emphasize and potentially what to stop/drop.
- Trait prioritization based on market size as well as nursery and yield test entry lists to reflect breeding targets
- The combination of products required by stakeholders (growers, processors, marketers, consumers) to ensure the program is addressing stakeholder needs.
- The future new products that the breeding program can address
- The extent to which product development interventions are consistent with beneficiary requirements, country priorities and policies.

**Recommendation**: Key product concepts need to be formally documented and used to drive decision making for all banana and plantain improvement efforts. (Example, see Table 1). These should be discussed and agreed to by the crop team and confirmed by survey data. It may take some back and forth with other disciplines where crop teams need to document status, discuss priorities and requirements as well as costs to implement the required activities. It is important to jointly plan and establish expectations and time frames for achieving them. The scientists ought to be organized as a multi-disciplinary team to deliver on the product concept.

Establish a specification with regards to relative size, maturity range, defensive traits and current products under cultivation. Effort in a breeding program addressing various product concepts and user needs should be proportional to the size of each area of adaptation and product concept.

**Breeding and Testing Program**

***Breeding conundrum*** All programs are severely constrained by having to breed new banana and plantain cultivars with a complex quality profile equivalent to that of either Mchare or Matoke landraces adopted by East Africans and plantain types adopted by West Africans for hundreds and maybe thousands of years. Fruit quality ideotypes for Mchare, Matoke and the different plantains should be established, formulated and used to guide the breeding programs. Existing landraces are infertile and have been difficult to cross and efforts need to be made to reset the genetic clock in terms of fecundity (Jim Lorenzen pers. comm.). The

breeding programs are also complicated by the need to select for parthenocarpy (fruit without seeds) the inheritance of which is known to be oligogenic and controlled by 3 or more loci. Breeders should consider breeding commercial cultivars with ploidy types other than those traditional for the Mchare, Matoke and plantain landraces.

***Genetic base*** The germplasm base for Mchare, Matoke and plantain breeding is narrow and strategies for increasing the genetic variation in the breeding program should be considered a high priority. These may include, a wider search for clonal variants of Mchare, Matoke and plantain landraces, more extensive germplasm collections of progenitor lines that led to the creation of the landraces and/or collection of wild diploids with appropriate quality attributes, even if they are prolific seeders. For plantain, false-horn types are now preferred but the breeding program does not have a large number of these to breed from because of their fertility issues.

Several disease resistance traits such as Black Sigatoka, Fusarium and nematode resistance are available from wild Musa germplasm but low male fertility often stands in the way of making successful crosses. Common use is made of diploids such as Calcutta 4, Borneo, cultivar "Rose" and improved diploids from FHIA and EMBRAPA. Calcutta 4 is a wild diploid with very small seeded fruit, good male fertility, excellent Black Sigatoka resistance, good drought tolerance and an important source of Fusarium resistance in several breeding programs around the world including IITA and NARO. A pedigree analysis of breeding materials and the improved diploids may reveal a common genetic background and the continuous use of this material may constrain future genetic gain.

Often seeded diploids have higher levels of fertility and may have potential to be used as male parents in the breeding program. However, their use is based more on performance as pollen donors and less on genetic value as parents in the breeding program. Broadening the genetic base of materials used for Fusarium resistance has commenced with the use of diploid seeded bananas from Prof Aitken at the University of Queensland. This material should also be screened for potential resistance/tolerance to other pests and diseases such as Black Sigatoka, nematodes and weevil borers in an effort to expand the current Mchare material for use as female parents in crosses. Other East African diploids including Muraru (Kenya), Mlali (Madagascar Is) need to be considered. As a long-term activity, the programs should obtain material from other parts of East Africa as well as Indonesia, Philippines and Malaysia and determine their breeding value as female parents.

**Recommendation**: A thorough review should be made of the germplasm being used in the banana/plantain breeding programs of IITA/NARO. The over reliance on parents such as Calcutta 4 should be addressed. Diploids that produce both reduced and unreduced gametes should be exploited further which may help increase the number of new triploids available for breeding. In the case of plantain a more extensive list of false-horn types should be incorporated into the program. The plantain breeders are also contemplating doubling existing diploids to produce new 4n parents to be used in the secondary cross. This strategy should be fully embraced and used across all banana/plantain programs. The active introduction of new diploid germplasm for banana/plantain programs should continue and be expanded. In particular diploids from Malaysia may provide a good source of nematode resistance.

***Population size*** Small population sizes constrain the effectiveness of the breeding program despite the high number of crosses completed. In the case of Mchares a total of 22 parents, 11 male and 11 female, are used in the breeding program. Of the 11 closely related Mchare varieties only 6 are female fertile and reliance on fewer parents will no doubt constrain genetic gain. For Matoke the IITA program relies on 11 closely related triploid Matoke landraces, 10 diploids (FHIA, Sendusu, Ibadan), 13 tetraploids (8 from NARO, 5 from Sendusu). The Matoke program at NARO uses 10 tetraploids and 30 diploids all of which are used to make secondary triploids. For both Mchare and Matoke breeding programs the relationship among the clones may be higher than anticipated because of the extensive use of Calcutta 4. This clone has

high male fertility and multiple resistance to pests and diseases including resistance to Tropical race 4 of Fusarium wilt.

***Breeding for quality*** Selection intensity is high primarily because many clones fail to reach quality standards equivalent to those of Mchare, Matoke and plantain landraces. In addition, many clones have unfavourable bunch characteristics, are not parthenocarpic and have inappropriate maturity. As an example of high selection intensity, in the 2017 NARO Matoke testing program, 2000 clones were tested in the EET and only 28 in the next level of testing. This high attrition rate occurs primarily because new clones fail to meet the production and quality standards.

In the case of the Matoke programs the failure to reach quality benchmarks was not surprising given that non-Matoke diploid parents are used twice in the crossing program including; a non-Matoke diploid (frequently Calcutta 4) to introgress disease resistance to make the primary cross and an improved non-Matoke diploid to make the secondary triploid. Consequently, strategies to accumulate more alleles for quality should be considered in the breeding programs and this can be best achieved by developing Matoke quality diploids that can be used to cross to Matoke triploids in the primary cross or to Matoke 'type' tetraploids in the secondary cross. To this aim, the IITA/NARO breeders may want to consider some complementary breeding strategies that are presented in Figure 1. These strategies make use of the occurrence of 'diploid segregates' that result from 3n x 2n primary crosses. These segregates have potential to be used further in the breeding program particularly if they have Matoke quality attributes and disease resistance. For example, they could be used by; (i) crossing them to a tetraploid segregate from the primary cross and selecting out a sterile triploid progeny with appropriate characteristics (Figure 1) or (ii) crossing them to a source of resistance e.g. Calcutta 4, selecting out a Matoke quality disease resistant segregate and backcrossing to the original diploid segregate. This BC1F1 Matoke quality, disease resistant, diploid material can then be crossed to a Matoke triploid landrace that may result in tetraploids with a combination of desired characteristics. These tetraploids could then be crossed to the BC1F1's described above to give triploid progeny with Matokeness and disease resistance. To facilitate this process all lines of the breeding program should be extensively genotyped to reveal those clones with a high proportion of Matoke genome content. A consequence of using this strategy might be the occurrence of inbreeding depression due to the lack of genetic diversity among the Mchare and Matoke clones sets, however, the benefits of attaining quality benchmarks sooner would hopefully outweigh any reductions in bunch yield.

The extensive use of diploids in the breeding program lends itself to the possibility of breeding commercial diploid Matoke cultivars as is the case with plantain. This would require developing diploid populations of Matoke types segregating for parthenocarpy. Other ploidy types for banana/plantain should be considered.

Varietal acceptability in the market place is almost exclusively dependent on the attainment of appropriate quality i.e. Mchareness or Matokeness for EAHB and market acceptance for plantain. To date, the physico-chemical characters of the banana fruit that underlie Mchare, Matoke and plantain quality are unknown let alone the understanding of their inheritance. Yield *per se*, may not be as important as quality and disease resistance in the breeding program and therefore resources should be allocated accordingly. Because quality benchmarks are so stringent all existing quality data should be analysed retrospectively and interrogated thoroughly to determine germplasm lines that may have Mchare/Matoke/plantain qualities.

**Recommendation**: Breeding values (in a quantitative genetics sense) for quality should be determined for all parents used in the breeding program. Note that seeded diploids may still contribute genes for improved quality but this needs to be checked by crossing them to appropriate parthenocarpic testers. This strategy is analogous in dairy cattle improvement where the breeding value of bulls is determined by measuring the milk yield of the cows they

bare. There is likely to be sufficient data in existing breeding programs to genetically map quality attributes in both banana and plantain.

In the breeding programs of NARO Uganda a score is given out of 100 for Matokeness. It is unclear how this score is achieved and where the threshold of acceptability lies. These quality characteristics seem to consist of colour, texture, firmness, and taste and are determined through sensory evaluation by testing panels consisting of 40-60 people. This testing involves a lot of time, effort and material and is not suitable for high throughput. Since the different components of these quality characteristics are not clearly defined or quantified, fruit quality cannot be accurately evaluated preventing the accurate prediction of breeding values and genetic gain in general. We are aware that a new project is planned which seeks to determine the physico-chemical bases of essential consumer traits which would aid in accurate phenotyping of bananas for quality although the funding may not be adequate.

**Recommendation:** Since the quality characteristics of Mchare, Matoke and plantain are poorly defined some research is needed to better define and quantify these characteristics which will make it easier to evaluate clones and assign reliable scores. It may be that multiple scores are needed for the different quality characteristics, as it is highly likely that many genes are responsible for the overall profile. It is important to be involved in new projects in this area to improve the accuracy of phenotyping as it appears RTB will not provide substantial funding for this purpose. The plantain breeders should investigate further their new strategy of focusing on quality in the primary cross and disease resistance in the secondary cross. Their approach to inter-cross a set of diploid lines (Mchare, Pisang Lilin, Banksii) using a factorial design to develop new segregating material for quality should be prioritised.

***Crossing nurseries: quality not quantity*** The programs have set some ambitiously high targets with regards to numbers of breeding crosses considering the low fertility/seed set/germination of breeding parents. There appeared to be a heavy focus on the mechanical aspects and process of breeding with the aim to produce a large number of total seeds rather than a set number of seeds/cross. Breeding crosses appear to be made at random and depend on when parental genotypes 'nick' (i.e. have coincidental flowering), consequently a level of redundancy was observed in all programs. The focus should shift from quantity of seed to the genetic value of the seed produced. All crossing programs should be studied retrospectively to identify duplicated and redundant crosses. Importantly, this analysis should align with the outputs of the testing program so that pedigrees that produced lots of seeds but inferior clones are not continually resampled for further testing.

A crossing schedule is needed where it is clear which traits are to come from which parents. Some resistances like Fusarium and Black Sigatoka are present in more than one diploid parent and some are already in Matoke germplasm such as resistance to Fusarium wilt race 1. At this stage it is not known if these resistances share the same genetic background.

The programs are in urgent need of more input from floral biologists to understand basic physiological processes that underpin the difference in flowering habit, pollen quantity/quality and style receptiveness among clones to make them more valuable in the crossing program. To date, a restricted number of parents are used because they are relatively easy to cross. There is a need to develop methods to extend the period that clones can be crossed to enable a greater suite of crosses to be made. This period could be extended by long term storage of pollen and manipulations of irrigation/plant density of males to prolong pollination. In the past, Calcutta 4 has been used extensively as a source of disease resistance primarily because of its excellent pollination characteristics.

All effective plant breeding programs have a common feature in that they are cyclical. This means that breeding materials are intermated, selection pressure is applied, new lines are fixed genetically, superior genotypes are selected over multiple generations and then at some point the best lines are intermated to start the process again. The last step effectively closes

the breeding cycle. There was no evidence of a closed cycle in any of the breeding programs nor did any of the breeders provide a future plan for doing so. There appeared to be a prevailing sentiment that all future clones could be generated from the existing breeding strategy without recycling newly developed material.

**Recommendation:** The focus on number of crosses should shift towards a more targeted approach based on combining the best genetic attributes. The parents to be used and the design of the pollen blocks need to be prioritised according to what the traits of highest priority. Include only parents of high breeding value for traits of interest. A long term breeding plan needs to be developed that demonstrates a closed breeding cycle.

***Pollination biology*** There are a number of challenges in banana and plantain breeding that relate to low fertility, parthenocarpy, low seed set and poor germination of the obtained seed. For example, on average about 6 seeds are produced per pollination in Mchare highlighting the fact that a significant effort is required to produce seed of breeding crosses. Pollination characteristics of different clones need to be better understood including on a diurnal basis, at different times of the year and under different growing conditions. One cannot assume that the best place to grow bananas is also the best place for breeding activities and pollen production and fertilisation is likely to be different between regions and seasons. A retrospective analysis of all past pollinations in the breeding programs may be helpful in improving seed production. For plantain, pollination is more successful at Onne although it's unclear why this is.

**Recommendation:** Investigate pollen production in different male germplasm in different areas at different times of the day with the aim to increase pollen production. Likewise, investigate stigma receptivity of different female parents. Utilise the linkages with other breeding programs, for example EMBRAPA (Dr Edson Amorin) to acquire further knowledge on the mechanics of banana pollination. For plantain, the reasons for greater pollination success at Onne should be explored, including the possible effects of local weather conditions and the micronutrient (e.g. Boron) status in the soil due to low pH. The breeders are retrieving records from past breeding efforts (1987-2005) and these should be thoroughly investigated to provide insights into pollination characteristics of existing clones.

***Testing program*** There are considerable opportunities to reduce the breeding program cycle time, currently about 10-17 years, by significantly reducing the length of the field testing program. As an example, in the mature Matoke program conducted by NARO, a three stage testing program is in place; clones are tested 3 years EET (Early evaluation trial), 3 years PYT (Preliminary yield trial) and 3 years AYT (Advanced yield trial). As of 2017 the number of clones at each stage of testing was, 2000 in EET, 28 in PYT and 10 in AYT. Reducing the EET by one year and eliminating the PYT altogether would reduce the testing program by 4 years.

The field testing program has peculiarities that need to be addressed. Because the EET is made up of progeny from 4n x 2n crosses not all entries in the field are 3n and often 2n types are recovered. It is recommended that the ploidy of each genotype be checked prior to entering the field program. Ratooning is another practise that complicates the yield assessment as variation for suckering can indirectly affect bunch yield and confound the interpretation of yield data.

Greater value could by extracted from yield test data by using a quantitative genetics approach to understand the underlying genetic variance that is observed across years and locations. The data could be analysed using classical designs, e.g. North Carolina Design 1 and 2, to provide values of GCA (general combining ability) and SCA (specific combining ability) for yield and quality attributes. These data would need special consideration given that (i) each F1 plant made by crossing two parents is a unique genotype and (ii) most experiments will

have unbalanced data sets due to clone death and/or poor recovery from embryo rescue and tissue culture.

**Recommendation:** Breeders should consult with a quantitative geneticist/biometrician to assist with data analysis and subsequent selection of clones to advance in the breeding program.

***Molecular breeding*** Considerable resources have been directed to marker assisted breeding. However, these efforts in some cases have been slowed by the difficulties in obtaining high quality phenotypic data particularly for Fusarium, bacterial wilt, weevil borers and nematode resistance. Black Sigatoka resistance is easily scored in the field. GWAS (Genome wide association studies) experiments targeting drought resistance are being established although the methodology for drought screening has not been developed. GWAS has been initiated and the success of these will largely depend on the quality of the supportive phenotypic data.

There would be great value in finding QTL's linked to parthenocarpy that can be used to develop markers for routine selection in the breeding program. Tracking parthenocarpy/seededness is particularly important given some past commercial releases have had issues with seed production in farmer fields.

**Recommendation:** GWAS should only be attempted after breeding program fundamentals are in place and assays to screen for the various resistances have been optimised. Understanding the inheritance of parthenocarpy is very important and finding linked markers are a high priority.

***Trait identification/prioritisation*** The current low yields of East African banana are probably a combination of pest (nematodes, banana weevil) and disease pressure (Black Sigatoka, Fusarium wilt, and bacterial wilt), erratic drought stress, lack of clean planting material, reduced soil fertility combined with poor agronomic practices and low levels of fertiliser use. Low yields of plantain are largely due to Black Sigatoka, nematode damage in combination with the above mentioned abiotic factors.

Black Sigatoka is a leaf disease where disease pressure is higher in higher rainfall areas and at lower altitude as has been observed in Latin America. The severity of Black Sigatoka was lower in the Arusha area. There could be several reasons for this such as altitude, climatic conditions and the upright stature of Mchare varieties, which greatly reduce leaf wetness. In Mchare, resistance to Fusarium wilt R1 and potentially TR4 is required combined with nematode and weevil resistance/tolerance. Although other diseases such as the Banana Bunchy Top Virus (BBTV) and Xanthomonas wilt can have major limitations to production of EAHB there are no effective resistances known for these diseases although recent findings with regards to BBTV are of interest. However, clean planting schemes and good agronomic practices go a long way to controlling these diseases as has been convincingly shown elsewhere. In plantains, tolerance to nematodes combined with better mat structure and better suckering helps to reduce yield decline in ratoon crops compared to the plant crop.

**Recommendation:** It is important that traits are prioritised. An analysis of the economic and social impact of pest, diseases and drought would provide insight into these issues. Although Matoke is resistant to race 1 of Fusarium wilt, Mchare is susceptible and both may be considered susceptible to Fusarium wilt Tropical race 4 as limited, if any, rigorous data exists to the contrary. It is also important to ensure that no susceptibility to these Fusarium strains is introduced through the breeding program.

***Disease Screening and capacity*** A very important component of the program is the ability to screen materials for resistance/tolerance to a range of pests and diseases. Since the overall aim of the breeding program is to improve yield due to reduced impact of pests and diseases in EAHB and plantain it is paramount that resistant phenotypes can be accurately identified.

To do this effectively, screen houses are needed to evaluate young plants under artificial conditions in addition to field sites where the disease or pest is present at sufficiently high levels and environmental conditions are conducive to disease development. With regard to soil borne diseases such as Fusarium wilt an additional complication is the distribution of inoculum in the soil. Field sites for disease screening remote from research stations need to be chosen for their suitability as screening sites in combination with ease of access, security etc.

For Mchare breeding the weakness is the lack of rapid and effective screening for diseases and pests. Since this is a relatively new program the capability for disease screening needs to be built at Arusha, with protocols documented and adhered to. Although pathologists are part of the program they are not actively involved in the day to day operations or the development of the disease screening. Useful material has been provided in the form of workshops and training manuals, however, these should be viewed as first steps in general training and capacity building. Especially at Arusha hands-on help and guidance from an experienced pathologist involved in day to day operations followed up with regular visits to the station would help to improve the actual screening methods and procedures and at the same time build highly relevant pathology skills.

Nematode resistance screening is conducted by IITA in Uganda. The screening assay is complex and very time consuming and the variation between experiments is high. There clearly is a need for a less labour intensive and a more streamlined approach to screening for nematode resistance.

In Tanzania and Uganda only Fusarium wilt race 1 is present so screening for this can be conducted locally. Screening in Tanzania is currently done in collaboration with the Tanzanian Agricultural Program although this institution was not evaluated for its ability to conduct this part of the research. A dual system of screening in the glasshouse linked with a field screening site away from the research station, to prevent infection of the site with Fusarium, is needed to enable effective screening of Mchare types for resistance to Fusarium wilt race 1. To ensure that data collection is done according to IMTP (International Musa Testing Program) guidelines, specific pathology input to develop and implement disease screening procedures are recommended.

Since TR4 is now present in Mozambique and screening could be potentially be done there to safeguard the EAHB from this major problem. However, due to a lack of expertise, security, infrastructure and controls in Mozambique this does not seem to be a viable option. Other options involving screening in Asia for TR4 resistance should be considered.

Plantains are susceptible to Black Sigatoka which is a major problem along with banana streak virus for which the impact is largely unknown. Plantains suffer from damage by nematodes due to their susceptibility and growing of the mats out of the ground makes them more vulnerable. Although problems with different species of nematodes such as *Radopholus similis*, *Pratylenchus spp.* and *Meloidogyne* have been well established there is currently no effective screening for nematode resistance in the plantain breeding program. It is known that nematode resistance is present in some of the FHIA hybrids and diploids originating from Malaysia and it is understood that different sources of resistance are required for each nematode species. Robust assays are needed for assessment of parental and breeding material.
Plantains are resistant to currently known races of Fusarium wilt. Their resistance to Xanthomonas wilt is unknown as that pathogen is confined to East Africa. The plantain breeders should ensure that susceptibility is not introduced to, for example, Fusarium wilt when focussing on other disease resistances.

Screening for disease resistance of advanced material should be done in different agro-ecological zones (not regions). For example, the disease pressure for Black Sigatoka in

general declines with increasing altitude such that screening for disease resistance is best conducted at lower altitude due to higher disease pressure.

With regard to plantain, screening for Black Sigatoka can either be done using detached leaves in containers or in the field, or ideally both. Field conditions in Ibadan are not ideal for Black Sigatoka development compared to Onne where the higher level of rainfall and humidity provides a much more conducive climate for development of disease and thus screening.

**Recommendation:** The capability in pathology needs to be strengthened particularly on screening for disease resistance which is one of the main objectives of the breeding programs. Hands-on input is needed from pathologists to rapidly improve disease-screening capability in Arusha and Ibadan with special attention to screening for Black Sigatoka, Fusarium wilt, weevil borer, nematodes and Xanthomas wilt.

***Agronomic traits*** Agronomy is closely linked to breeding and a good understanding of the banana and plantain grower and what they require in a new banana variety is important to be successful. Due to the difference in plant stature of the diploid Mchare varieties which have a far more upright leaf orientation compared to triploid Matoke types and different ways of preparation and consumption of the fruit means that agronomic practices between these banana varieties are different. In addition to these differences, the breeders should bear in mind that farmers only apply small amounts of nutrients to their fields and primarily in the form of organic matter.

For some agronomic characters such as suckering ability of clones, decisions need to be made based on what is acceptable to growers. Suckering is needed to produce the next pseudostem but growers may not favour varieties with very high levels of suckering. It would be worthwhile considering developing an index of selection and prioritise the agronomic traits. Local growers need to get involved in parts of the selection process to fine tune the desirability of characters.

**Recommendation:** In addition to disease resistance, a selection index that contains attributes of yield and agronomic traits acceptable to growers needs to be developed**.**

***Mechanisation*** The programs lack good systems of mechanisation particularly in terms of electronic data capture. Data is mostly captured onto excel spreadsheets and there appears to be limited data analysis and sharing. Much time and effort is spent on collecting passport type data that have limited value to the breeding program. The breeders spoke of labelling issues of clones either in the field or in tissue culture emphasising the clear need for barcoding and electronic cataloguing of materials through the breeding program to maintain clone integrity.

**Recommendation:** Electronic data capture should be implemented by the breeding programs as a matter of priority.

***Increase understanding of floral biology*** Since fertility is so low in bananas any improvement in fertilisation, seed set, seed viability and germination would make a significant improvement to banana breeding. Some studies are underway in Arusha to look at pollen viability and to determine which germplasm makes good male parents. From past work, the diploids, Calcutta 4, Borneo, Pahang, and cultivar “Rose” are good pollen donors while among the Mchare varieties Huti White is the best pollen producer at Arusha. Due to low seed germination and survival, embryo rescue is used to obtain the hybrid plants. It has been noted that especially the Mchare varieties show some differences for their ability to grow in tissue culture which may affect recovery after embryo rescue. In Matoke the stigma is more receptive before the flowers open and that seed set mainly occurs in the lower hands. If this is consistently the case one needs to consider only pollinating the lower hands that would give a higher return for time and effort invested in obtaining viable seed.

A study could also be conducted to determine where seed-set preferentially occurs in the fruit. Because some evidence exists in other species that fertilisation and seed set is a function of style length because it represents the distance needed for the pollen tube to travel before reaching the ovule. One could hypothesise that most seed would be found in the part of the fruit closest to the end that the perianth and stigma/style were attached as this represents a shorter distance for pollen tubes to grow. With regards to floral biology the existing strong international links with Dr David Turner and Dr Jane Gibbs is strongly encouraged and may need to involve some exchange of staff.

Some student projects include the study of fertility and this work has found that high fertility genotypes tend to have receptive stigmas prior to the bud opening and seed set occurs mostly in lower hands. Applications of a Sucrose solution has been shown to improve seed set. The wetness and stickiness of pollen needs consideration when extracted from closed flowers as this is difficult to apply to the female flowers.

**Recommendation:** To gain further insight into poor female fertility, intensify and resource the collaboration and exchange of staff with Dr David Turner and Dr Jane Gibbs. An improved understanding of the genetics underlying sterility would be a very useful long term goal as this is one of the key factors that limits genetic gain in banana breeding programs.

**Variety release and dissemination**

Development of improved varieties will not contribute to improved on-farm productivity without a reliable and continuous supply of planting material to growers. An adequate delivery system appears lacking and there are no good links between public and private sectors for delivering banana cultivars to farmers. Planting materials are not pure because private tissue culture laboratories do not use elite and pathogen tested material from NARO or other parties for multiplication. Currently, there is one or probably two recognized commercial producers of planting material in Uganda that operate quite independently from NARO. Tissue Culture (TC) companies go to farmers' fields instead of NARO to get their source material. It was clear from discussions that the limited supply of true to type disease-free quality planting is a large bottleneck. Recycling using suckers from previous plantings, leads to build up of pests and diseases.

Adoption of tissue culture (TC) technology remains relatively low in East Africa. Reports indicate that in East Africa bananas are traditionally propagated through suckers. In Kenya, only 7% of the banana production area has been established using TC plantlets. In Uganda, Tanzania and neighbouring countries, the area cultivated with TC plantlets is even lower.

For plantain some FHIA lines as well as lines from the breeding program have been released from the previous plantain breeding program years ago but no information with respect to their adoption is available. The target for plantain is resistance to Black Sigatoka, good bunch, parthenocarpy and marketability of the fruit.

Investing in improved varieties is a critical step towards increasing yield and improving livelihoods. Planting material of improved varieties should be available to farmers on a continual basis, at the right quantity, quality, time, and price to realize the increased performance. Most banana farmers have very limited access to high quality, improved released varieties with desirable traits. Some of the specific challenges include the limited capacity and lack of role clarity among the different participants.

**Recommendation:** Banana in smallholder farmer systems is traditionally propagated by using suckers, which could transmit pests and diseases. Farmer access to clean planting material from tissue culture plantlets or tissue culture plantlets multiplied in clean fields is a key bottleneck. Tissue culture technology requires a different skill-set and knowledge and training in agronomic and technical expertise. Developing small business enterprises similar to what

AGRA has done for cereal/legume seed through financial, advisory, and training support to producers is worth exploring for multiplication of quality planting material.

Collaboration between NARO and IITA has generated 27 Matoke hybrids designated as NARITA (NARO-IITA). Two of these were released by NARO in 2010 in Uganda and are now being grown reportedly in at least 15% of the banana farms in Uganda. Varieties have been released but adoption is low. Why the reluctance to change and what hurdles are limiting widespread uptake of new varieties among smallholder farmers in Uganda? This could be a result of any number of things; varieties selected on research stations may not have the performance advantage under farmer management, varieties may not have the combination of traits that are needed or preferred by farmers, farmers may not have access to information about planting material of new varieties, or it could be unavailability of affordable and clean planting material.

Planting material provided through a network of public and private entities covers a small percentage of the total land area. Farmer to farmer or other less formal commercial entities producing and exchanging their own material dominate the source of supply. Thus in the short term the majority of smallholder farmers will only be reached through the informal sector. To be effective, formal interventions need to be coordinated among governmental, private sector, and non-governmental implementation partners. Each intervention requires activities to be owned by different stakeholders in the planting material production chain. These stakeholders include NARO, extension service, public/private seed enterprises, farmers, and regulatory bodies.

Involvement of the public extension system in popularizing new varieties appears low. A stronger interaction between NARO, extension, and planting material producers will be essential for popularization of new varieties and ensure that farmers are aware and educated about improved varieties. This includes effectively identifying the target farmers and agro-ecologies that will benefit from the new variety. This will require sustained engagement with stakeholders to develop strategies for varietal dissemination.

Variety release and registration needs to be organised in an effective manner. It is essential to develop variety release and registration guidelines detailing steps and processes of varietal evaluation, release and registration. Procedures associated with such process need to ensure that varieties released meet farmers' specific needs. Best practice requires that older varieties should be retired from the system as newer and higher performing varieties are released.

A big hurdle for commercial tissue culture plant production is the limited use of certification for quality and health, which is important to avoid the spread of pathogen and pests. In the absence of a well-developed regulatory system, the current system faces various challenges thus it is essential to establish standards to enforce quality control for planting material producers. Establishment of regulatory and enforcement guidelines for quality control is critical. This will require updating the existing relationships, facilities and personnel.

**Benchmarking genetic gain**

In the banana breeding programs of both IITA and NARO there has been little or no effort to measure genetic gain within the programs. Neither organisation has actively sought information about the performance of lines after release. There is a need to develop a system of obtaining this information involving other stakeholders in this sphere.

Genetic gain is the measurable increase in performance of varieties achieved by a crop improvement program. Investments in breeding can only be justified if there is genetic gain over time, thus the need to embark on system changes that improve the ability of a research system to generate and deliver products efficiently and timely. Crop improvement is a cyclical process where the new generation is built on the previous elite material. As much as genetic

gains could be tracked over long periods, it is what is accomplished every year that adds up. Thus, it is essential that the breeding program assess its progress every year/season by examining what new germplasm is being added, quality and quantity of data that goes into selecting parent lines, precision of data collection, size of breeding generations and test entries, applying the right selection pressure and using appropriate benchmarks when making advancement decisions.

Product and trait pipelines have to be planned well for a successful program. The product pipeline would be optimal when a product (variety, hybrid) is released its potential replacement is at the observation or PYT stage, whereas a trait pipeline relies on the alignment of trait teams (breeders, physiologists, pathologists, etc.) Integrating these complementary activities towards a common goal of achieving genetic gain efficiently in the shortest time possible will contribute to advancing IITA and NARO's vision of increased food production, better nutrition and climate resiliency.

Achieving genetic gain is not necessarily about more investment but more often, it is a redirection. Some changes do require resource allocation/reallocation e.g. use of molecular markers, multi-location yield tests, mechanization etc. Some require a mindset change (efficiency in breeding, data management,) others require leadership in aligning efforts.

**Recommendation:** Feedback on the performance of new varieties ought to be monitored and followed on a regular basis. Specific data on amount of planting material produced, yield and area under production of released varieties are required. A data driven approach to monitor the performance of new varieties to gain confidence on the contribution of the breeding program as well as to better align with what is required by growers and consumers is needed.

It is especially important that the bananas meet consumer preferred attributes (taste, aroma, color, texture) found in traditional bananas. "Matokeness" needs to be better understood and clearly defined so it can be selected for to improve marketability. Preference traits are hard to quantify though NARO has made every effort to use taste panels to get to this issue. Taste panels may not be practical for a large number of entries.

***Genetic Gain Drivers*** To maximise the benefits of a breeding program it is important to consider the drivers of genetic gain.

- Develop product concepts, based on unique product specification adaptation, trait, and customer preference to drive breeding objectives.
- Increase Program Size (improving odds)
    - Determine size of nursery that will result in appropriate lines for testing: number of entries, populations, plots, etc.
- Improve the quality of breeding populations
    - Better parent selection and strategic crossing focused on defensive and consumer desired traits
- More accurate selection (higher heritability)
    - Identify representative test locations, better experimental designs and analysis,
- Mechanization, automation, digitization (improve accuracy)
    - Electronic data capture, field books and bar coding, etc.

- Reduce breeding cycles (rapid variety turnover)
    - Yield testing at earlier generations
- Measure periodic product performance gains rather than number of releases
    - Raising the bar for releases
- Build adequate screening capability for major defensive traits.
    - Potential use of marker assisted selection

Against a backdrop of the complexity of banana and plantain breeding, it is worth addressing serious bottlenecks that prevent the breeding program from achieving genetic gain:

- Lack of diverse germplasm: Explore potential for sourcing germplasm from the center of origin especially of Matoke and plantain types to obtain new germplasm. It is unclear what the genetic diversity is among the NARITA varieties and among the pipeline hybrids as they seem to trace back to a narrow germplasm base.
- Crossing requires lots of labor with few or no seed from most crosses. Concentrate on making crosses that result in diverse and desirable combinations rather than duplicating easy to make crosses.
- Extended generation time delaying variety identification. Once the pipeline is full this may not be an issue. However, cost and efficiency require exploring early generation testing and determining ploidy level before planting in field.
- Need for large plots to assess performance of clones. Determine optimum number of plants that would be ideal to determine performance.

***Measuring genetic gain*** There are indirect and direct measures of genetic gain. The indirect measures of genetic gain would be how and to what extent the breeding program is adopting/implementing changes in internal efficiencies (detailed under genetic gain drivers above) that will set the breeding program on a path of genetic gain. In terms of direct evidence it is important that this be actively monitored and could constitute any of the following activities, which constitute internal and external measures.

Internal measures would include assessing the performance of newer products by conducting performance tests on previously released, existing and new varieties. Establishing performance benchmarks and formal criteria for advancing or releasing varieties to move material through the system.

External measures would include yield trend analysis over many years especially if released varieties have been adopted. Performance of released varieties including proportion of cropped area under improved varieties should be tracked to determine impact of new varieties.

**Other Issues**

***Communication*** Given the geographical separation of the breeding teams that operate at IITA/NARO across three countries the leadership team may consider having a website or a regular e-mail update to alert staff of activities and events and news worthy items. It is important that all staff in the teams take ownership of the program and that they feel part of a highly successful team.

The mentoring of especially young staff provides development opportunities especially for those based at remote stations. At some locations bi-weekly laboratory meetings are held while on alternate weeks topical scientific publications are discussed. These weekly team meetings are strongly encouraged.

Another part of communication concerns, the growers of Mchare, Matoke and plantain. There appears very little interaction between the breeding program and the growers. Having growers involved in the program to get a better understanding of what they are looking for and get better adoption of new varieties is recommended.

Communication with international breeding programs varies. Excellent collaboration exists with EMBRAPA in Brazil. Limited collaboration and communication exist with FHIA and CIRAD although some of the past improved clones of FHIA and material of CIRAD are being used and collaboration with India is under development.

***Alignment of Expectations*** In addition to the details provided in various funding applications it is important that everyone is clear on what is expected of them. A common problem encountered with project funded activities is that the expectations of the funding body and the staff at the research provider are not fully aligned. In order to achieve outcomes of the project in harmony with the organisation entrusted with performing the activities one solution is to develop individual work plans for each major subprogram and for each individual researcher working in the program. These then need to be written in such a way that each researcher can see how his or her activities contribute to the overall product concept. Individual contributions need to be acknowledged but it is equally important that all understand that the breeding of improved banana varieties is a team effort. In this case it is also important that the activities conducted by the different national and international organisations are planned such that complementarity is maximised and duplication is minimised. The point of where the project should be at the end needs to be better described along the product concept guidelines. For example, yield is mentioned as a phenotype but there is no benchmark set for what the yield target should be, making it difficult to measure success. It is also important that the incentives for individuals and teams are well devised and that the institute and project incentives are aligned. It could be argued that more time should be spent by breeding leads on interrogation and retrospective analysis. This may be achieved by delegating some tasks currently held by breeding leads to junior staff. As a specific example a manager could be appointed to oversee the new lab facility being built at IITA Sendusu.

The plantain breeding strategy has not significantly changed from the original breeding activities at IITA. The strategy may not be the problem but its execution needs to be questioned by the breeders with regards to the diversity being utilised. It may be necessary to broaden the genetic base of the program and consideration given to diploid breeding and pre-breeding. Medium to long term it is also unclear what the future of the plantain breeding program is with regard to program funding. Not having a shared vision and long term strategy is of concern to many of the staff members involved in the program.

***Collaboration/duplication Leadership*** Overall excellent teams have been built across IITA/NARO that work well with a very high level of commitment to banana and plantain breeding. The capacity to do banana breeding is in place and new field, greenhouse and laboratory facilities have been, or are in the process of being, established.

To optimize outcomes for farmers there needs to be complementarity between national and international program activities to avoid duplication. While IITA and NARO appear to work closely together a level of redundancy in the breeding programs was observed, with the same parents and crosses being made at both organisations. It is clear that NARO has a strength and capacity in banana breeding as their program was established decades ago. IITA should be more actively involved in capacity building, importing diploids from various parts of the world, producing improved diploids suitable for local conditions. IITA activities, especially in

Uganda, should focus on improving the enabling environment and give the NARO program a boost through access to novel germplasm, producing improved diploids, provision of scientific (e.g. floral biology) and technical assistance and support that is often more difficult to obtain by national programs. However, some level of overlap is required to be able to future proof the programs and underpin the collaboration and create a shared understanding of the challenges in banana breeding.

***Students*** A number of staff involved in the banana programs are also enrolled as MSc or PhD students. Although this is a good sign and important for capacity building in Africa the downside of this is a more narrow approach to breeding. Some higher degree students focus on only a very narrow aspect of the program. Especially molecular oriented programs have a very narrow focus and may not produce the problem solvers Africa needs in the future. An important question to ask is if these staff members get evaluated and rewarded for scientific papers instead for breeding outcomes. It is important that the leadership team aligns the incentives for students, but also the staff with the outcomes of the project.

***Access to Literature*** The access to literature by research staff is limited at Arusha and Sendusu. In the current era of electronic access to global literature through Universities or research organisations the project leadership team needs to make it a priority to provide access to literature for all key scientists based in Africa. This can either be done through the essential electronic agricultural library (TEEAL) run out of Cornell, or via portals of the IITA or the University of Leuven.

Table 1 The product concept will be unique considering the following attributes: (an example)

| Product concept (adaptation, end use resistances etc.) (an example) | Estimated area (ha) | Area/ effort (%) | Target environment and spillover agro-ecologies | Agronomic Traits (height, maturity etc.) | Resistance/ tolerance required | Other criteria, incl. consumer preferences | Product development goals | Benchmark Products |
|---|---|---|---|---|---|---|---|---|
| **(1) Short duration "Matoke" with resistance to foliar fungal disease resistance** | | | **Target geographies:**<br><br>**Potential Spillover:** | | **Biotic stress:** Resistance to foliar fungal and soil borne diseases<br><br>**Abiotic stress:** Tolerance to water deficit stress. | **Must have traits: "matokiness"**<br><br>**Nice to have traits:** | **Yield:** At least 10% higher than the standard check).<br><br>**Resistance level:** BS score: 2-3; FUS score: 2-3; Nematode | |
| **(2) Medium duration varieties with resistance to foliar fungal diseases and soil borne, and dual purposes** | | | | | | | | |
| **(3) Long duration varieties resistance to foliar fungal and soil borne diseases for food and other purposes.** | | | | | | | | |

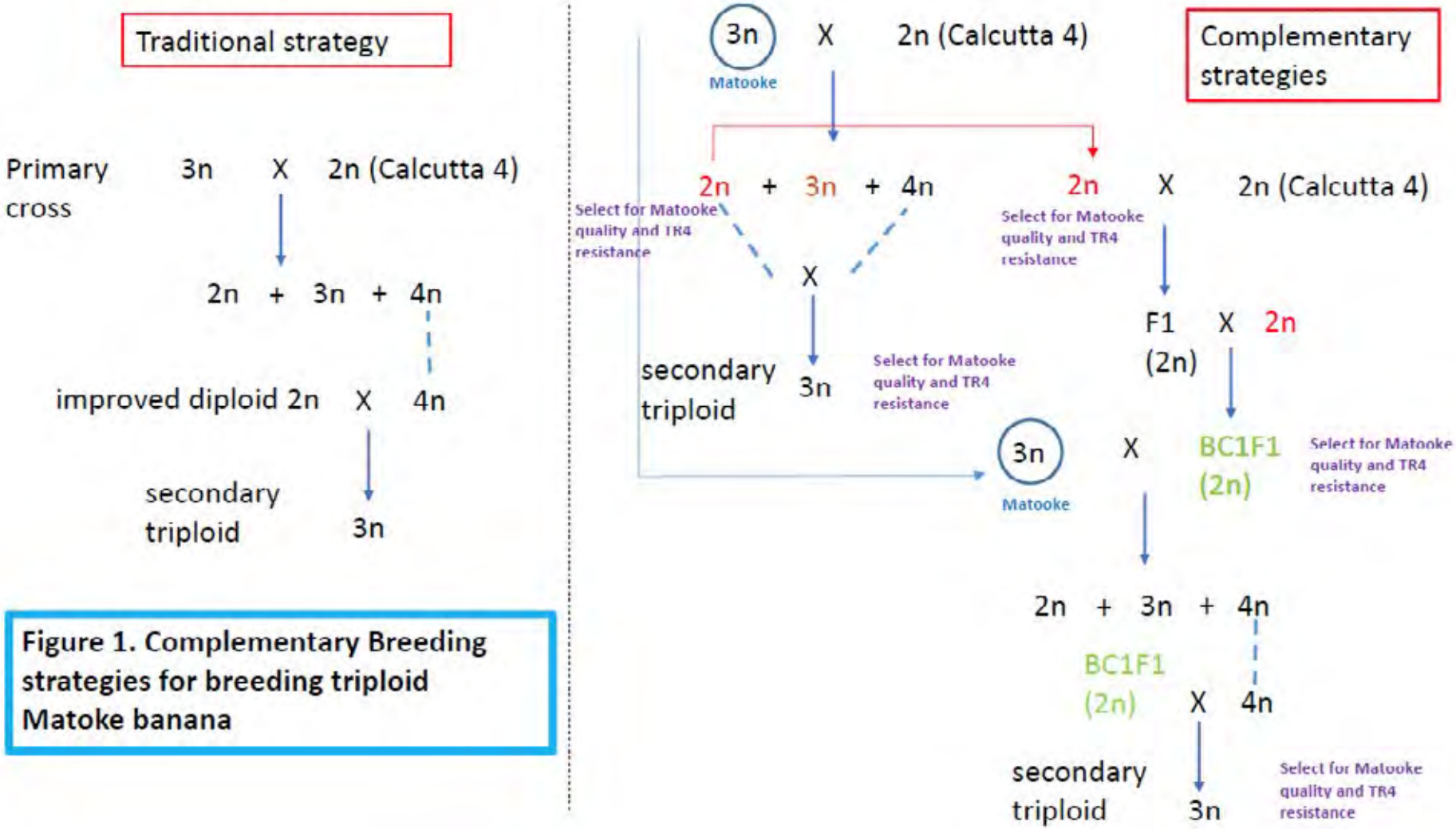



**Figure 1. Complementary Breeding strategies for breeding triploid Matoke banana**

## 1.9 Final internal IITA audit report of the Breeding Better Bananas Project



**PJ-002013: Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa – Breeding Better Banana Project**

**DATE:** **10 NOVEMBER, 2017**

**REPORT STATUS:** **FINAL**

**REPORT NO.:** **R7-17/PJ**

| **Distribution List:** | |
|---|---|
| Chairman, Audit and Finance Committee | IITA |
| Director General | IITA |
| DDG, R4D | IITA |
| Director of Finance | IITA |
| Director, R4D - EAH | IITA |
| Project Coordinator, Banana Breeding | IITA |
| Project Manager, Banana Breeding | IITA |
| Head, Finance and Administration, EAH | IITA |
| Head, Project Development and Administration unit (PDAU) | IITA |
| Financial Controller | IITA |
| Station Administrator - Uganda | IITA |

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| Audit Team | Patrick Kamau<br>Zainab Alatishe |

**Transmittal Memo**

10th November, 2017

Dr. N. Sanginga, Director General

**Report on an Audit of IITA's "Breeding Better Bananas Project"**

Attached is a report on an internal audit of "PJ-002013: Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa – Breeding Better Bananas" covering the period, 1st January 2016 to 30th July 2017. The audit is part of the approved Annual Internal Audit Plan for the Financial Year 2017.

PJ-2013 now commonly known as Breeding Better Bananas is a 5 year project that commenced in October 2014 and is funded by the Bill and Melinda Gates Foundation under the W3 funding category of the CGIAR. This project focuses on breeding superior Matooke and Mchare bananas. It is supported by the Breeding Program Assessment Tool through collaborative research between two CGIAR centers, the national programs of Uganda and Tanzania, six universities and national breeding programs in Brazil and India. Its activities are carried out in three countries namely Tanzania, Uganda and Kenya with the Project Management based in Arusha Tanzania, the Uganda activities are based at the Sendusu Research Station and banana database work being carried out in Kenya. The project life budget is US $13,873,600 and it has received US $ 8,596,914 less 2% cost sharing percentage (CSP) of US $175,447 as at end of June 2017.

**Audit Conclusion and Overall Ratings**
The overall rating from our review of the internal control system within the project is **Some Improvements Needed** (the rating scale used is defined in the report rating on *Annexure 3*).

The attached report details the results of our findings on the audit work undertaken. We have also recommended ways to address the identified shortcomings and improve controls and operations.

We would like to thank the Project Management Team and staff at Arusha, Dar es Salaam, Sendusu and Kampala Stations for the co-operation given to the Internal Audit Team during the course of this assignment. Should you have any queries, please contact the undersigned.

Sincerely,

Takawira Fumhe
**Head, Internal Audit**

## Executive Summary

### A. Objectives and Scope

The overall objective of the audit was to evaluate the adequacy, effectiveness and efficiency of the governance, risk management and internal control systems over the project. The audit entailed reviewing the project from a financial, operational and strategic perspective for the period January 2016 to June 2017. The audit incorporated visits to the project national partners in Arusha and Kampala for a health check on project and financial management. The project had also undergone an extensive external technical review funded by the donor in the month of May 2017.

In performing our work, we tested on a sample basis transactions and information made available to us by the project team.

### B. Positive Practices and Initiatives

As part of the audit process, we noted the following internal controls/and good practices instituted by the Project Management Team:

| Best Practices |
|---|
| Project implementation level for period 2014-2017 as at end of March 2017 (mid of the project timeline) was 66% with year 3 period of October 2016 to March 2017 having a high execution rate of 72%. This is above the mid-term target and shows that the project may close Year 3 with an even higher implementation level than the last two previous years. |
| Periodic sharing of the OPD transactions by the Project Account Officer at HQ to CC budget holders enables the Project Management Team and budget holders to monitor transactions being posted to their accounts and correct any miss-postings as well as monitor the burn rate of the overall project. There is need to upscale this initiative to all projects especially for those with wide reporting period e.g. Semi-annually and annually and make the frequency of sharing monthly from Finance. |
| Good interaction between the Science Advisory Group (SAG), Project Steering Committee (SC) and work package (WP) leaders and team during annual meeting. A project management meeting has been introduced that combines the work package leaders meeting and Project Steering Committee members to discuss cross-cutting issues and work packages. This has created good interactions and common understanding of the mission of the project among all stakeholders including partners. |
| Investment by the project approximated at **US$ 115k** in the refurbishment of the facilities at Sendusu Station and the Nelson Mandela African Institution of Science and Technology, with new labs for tissue culture, nematology and molecular work, screen-houses and irrigation systems. This will go a long way in supporting the research work for the project as well as the Institute in its mission of Transforming African Agriculture. |
| There is great synergy, team work and coordination among the project staff and the implementing partners in providing required support to the project activities being conducted in various countries from which the project stakeholders are operating from. |
| Development of the banana breeding website (http://breedingbetterbananas.org/) to capture the activities of the project, such as reports, discussions accessible by the project stakeholders. The website also serves as a marketing tool of the work done by the project to banana breeding programmes across the world. In addition, the project is also developing a Musabase (https://musabase.org/about/index.pl), a breeding database designed for advanced breeding methods in banana breeding and which is directly accessible through the project website. |

## C. Key Risk Issues

We have included recommendations on shortcomings identified on some operations of the project as well as the station administration support provided to the project. We believe that implementing the recommendations would improve the overall internal control environment and mitigate the identified risks from materializing. The key issues identified during the audit are summarized below:

| Key Issue | References | Management Action |
|---|---|---|
| National partners have challenges with regard to submission of the financial reports on time due to capacity issues. Majority of the financial reports are submitted after the deadline required by the institute, thus delaying dispatch of funds to partners. Delays in remitting funds negatively effects the delivery of project milestones and the institutes burning rate. | 1.1 | Project management will request for financial reports to be simplified in terms of need for copying with all costings, especially where the partner is independently audited. This particularly refers to NARO, who complain that they do not request the same of IITA. Further we shall liaise Finance Directorate and PDAU for advice on most useful form of training to be provided to partners that are failing to submit reports on time due to capacity issues. |
| Funds requested and issued as advance in Uganda were posted directly as expenses and not as advances as required by the finance policy. The expenses posted to OPD did not have supporting justification. This creates room for misappropriation of the funds issued as no follow up is done to confirm if the funds were used for the intended purpose once the funds have been expensed. | 3.2 | The Uganda station notes the gaps identified in the posting of advances. There will be keenness in the posting of advances given and the wrongly posted transaction mentioned will be reversed to the correct Account. |

Furthermore, some low-risk issues were identified with potential to become significant issues if they are not properly attended to. These issues were discussed with the Project and Station Management Teams and are listed in ***Annexure 1*** as low risk which do not feature in the main body of our report. We will monitor developments on the issues to ensure their risk levels do not change adversely.

## DETAILED FINDINGS:

### 1. PARTNERS

#### 1.1. Financial Reporting

Internal audit carried out visits to two national partners namely HORTI Tengeru in Arusha and NARO Kawanda in Kampala as part of the audit scope.

The Financial reports submitted by the partners for the period October 2016 to March 2017 had the following gaps;

**a) HORTI TENGERU**

- Non-adherence to the provided reporting format.
- No budget line per expenditure to monitor over and under-expenditure within the cost categories.

**b) NARO**

- The report only captured the accumulated expenditures since the project started and not the expenditure for the period being reported.
- The report captured expenditure for the period November 2016 to April 2017 and not October 2016 to March 2017 as stipulated under sub agreement Article 10. This is attributed to the movement by one month of the reporting period during reporting of October 2015 to March 2016 period which included expenditures for the period October 2015 to April 2016.
- The travel budget as per the life budget has already been surpassed by 43% while the project still has 30 months to go. This is contrary to Article 10 of the sub agreement that prohibits budget cost category change of more than 10%.
- The purchase of the vehicle being used by NARO by IITA reduced the Year 3 contribution to NARO but this has not been adjusted in the life budget by NARO.

A review of submission of reports (Financial and technical) by NARO and HORTI Tengeru for the period October 2014 to March 2017 as at 30th August 2017 showed that 45% of them were received later than one week after the submission deadline as summarized in the table below;

| Particulars of Submission | Technical Report | Financial Report | Total |
|---|---|---|---|
| Before deadline | 3 | 4 | 7 |
| Within one week | 3 | 1 | 4 |
| 1-2 Weeks | 1 | 0 | 1 |
| 3 weeks – 1 Month | 1 | 1 | 2 |
| 1 – 2 Months | 0 | 3 | 3 |
| 3 Months | 1 | 0 | 1 |
| Above 3 Months | 0 | 1 | 1 |
| Cancelled | 1 | 0 | 1 |
| **Total** | **10** | **10** | **20** |

NARO reports accounts for 78% of the reports submitted later than one week after the submission deadline.

The Project Management Team noted that late submission of the reports has been a major challenge in the past affecting the project deliverables. The challenge in financial reports is attributed to inadequate skills and financial system at the partner's organization; this in turn leads to submission of bulky reports which are time consuming during preparation as well as during review of justification of expenditure by PDAU and Finance.

***Cause:*** *Systems and capacity of partner organizations.*

| **Risk** | **Recommendation** | **Management Comments & Action** | |
|---|---|---|---|
| • Non-adherence to reporting timelines.<br><br>• Delay in delivery of project activities and outcome. | • Project Management in liaison with PDAU and Finance should consider:<br>a) Training partner's staff on financial reporting requirements.<br>b) Finding an amicable solution with the partners on the reporting requirements to ensure the requirements do not infringe on the delivery of the project outputs.<br>• Continued engagement of the partners with regards to early submission of reports should be sustained.<br>• Project Management should discuss with NARO regarding the over-expenditure of the travel budget and reallocate funds to the cost category. | There has always been consistent engagement of partners and reminders towards timely submission of reports. In latest sub-contract agreements, the submission dates for technical reports was brought forward by 2 weeks to enable a greater time buffer to account for late delivery and better facilitate compilation of Annual Technical Report to BMGF.<br>• Project management agrees and will request advice from Finance Directorate and PDAU on: a) which training and how, for partners to improve delivery and standard of reports; b) request that reports from partners (e.g. NARO) do not require bulky reproduction of all costings to avoid delays associated with this at both the partner and IITA.<br>• Project management will assess the over expenditure of NARO travel budget and take this into account in the budget forecast for next contract period (year 4). | |
| **Risk Rating** | **Priority Ranking** | **Responsible Official** | Project Management, Finance Directorate and Head, PDAU |
| **Significant** | 1 | **Due Date** | 30th November 2017 |

## 2. PROJECT MANAGEMENT

### 2.1. Risk Management

Risk assessment and profiling is yet to be incorporated into the project management activities. A couple of risk assumptions were included in the project proposal categorized as per the work packages and there are discussions by the various work packages on the challenges being faced in the achievement of the project deliverables, but this in itself cannot replace a formal risk management which will include: risks that could impact achievement of project objectives, probability of occurrence, impact if they materialize, mitigation plans, and responsible parties for the action.

A number of risks that were included in the project proposal have since occurred include:

- Loss of key staff by partner,
- Effect of changes in climatic conditions,
- Limitation of importation of germplasm from the partner breeding programmes,
- Destroyed field experiments due to construction of power lines.

Risks not yet formally documented that could impact the delivery of the project milestone would include but not limited to the following:

- Delay in dispatch of funds from the donor and subsequently to the partners;
- Discontinuation/Delay of activities by strategic partner; and
- Data loss.

**Cause**: *Risk management not well embedded in the project.*

| **Risk** | **Recommendation** | **Management Comments & Action** | |
|---|---|---|---|
| Inadequate risk management processes. | • Project Management should develop a risk register and share it with all key project stakeholders and the Institute's Risk Management Committee.<br>• Discussions on the risks can be included in the annual review and planning meeting of the Project Steering Committee team (SC) and Science Advisory Group (SAG). | Project Management takes note and will take into consideration for development of a risk register, including through the annual review and planning meeting in June 2018 when the SC and SAG can contribute. | |
| **Risk Rating** | **Priority Ranking** | **Responsible Official** | Project Manager |
| **Medium** | 2 | **Due Date** | June 2018 |

## 3. FINANCE REVIEW

### 3.1. Accumulation of Overdue Advances

As at 31st of August 2017, the OPD showed outstanding advances totaling $27,693. The advances balance is made up of Cash Advance Account 1526 ($9,164) and Travel Advance Account 1527 ($18,529). Refer to the summary of the project CC below:

| CC | Cash Advance $ | Travel Advance $ | Total $ |
|---|---|---|---|
| **5733** | 5,563.05 | 592.09 | **6,155.14** |
| **5734** | 768.93 | 12,164.61 | **12,933.54** |
| **5735** | 1,500.54 | 1,520.04 | **3,020.58** |
| **5736** | 1,757.50 | 3,853.58 | **5,611.08** |
| **5737** | (426.39) | 0.00 | **(426.39)** |
| **5738** | 0.00 | 399.03 | **399.03** |
| **T O T A L** | **9,163.83** | **18,529.35** | **27,693.18** |

More information on the individual CC balances is given in ***Attachment 1***.

The above August 2017 balance has increased by 14% from the July 2017 balance of **US$ 24,271**. The increase is largely attributed to erroneous double posting of corrections to the staff advance accounts by the Kampala station and Expense Claims Unit in the Finance Directorate on the 29th August 2017 and the 30th August 2017 respectively.

Some of the reasons for these outstanding advances include;

- Advances are given to staff with earlier advances not yet retired.
- Un-cleared advance accounts for ex-staff.
- Piecemeal retirement of advances.
- Advances taken by students posted to general staff accounts without capturing the names of the students. This makes it difficult to follow up on the outstanding amount.
- Wrongly posted justifications for advances taken to different staff accounts, justifications posted to the project CC while the staff do not relate to the project and interchanging of the advance accounts i.e. cash advance retired as travel advance.
- The balances comprised of balance rolled over from 2015 to 2017 for which justifications are yet to be submitted.
- Submitted justifications posted without the corresponding advance issued posted to the advance account resulting in negative balance in the staff accounts.

The Institute's advance policy requires that advances be justified/retired not more than two weeks after the completion of the activities for which the advance was obtained.

***Cause:***

- *Non-adherence to advances standard operating procedures.*
- *Lack of clarity on the process for handling students advances.*
- *Unresolved exchange rate differentials.*
- *Erroneous posting of justification.*

| **Risk** | **Recommendation** | **Management Comments & Action** | |
|---|---|---|---|
| • Under-reporting of period. expenditures.<br>• Cash rolling.<br>• Financial loss. | • Investigate the double postings and correct the errors once the reasons of the initial errors are understood by the stations or expense claim units.<br>• Careful analysis of the outstanding amounts should be conducted and necessary actions taken to get the account cleared.<br>• Consider including the advance accounts report in the periodic expenditure report shared to the project CC's budget holders to ensure that outstanding issues are promptly resolved. | Project Management appreciates the risk of these overdue advances, but cannot take responsibility of station accountants and FD staff. This is an institute issue with relevance to institute policy as opposed to being a project specific issue. Project Management therefore request that FD deals with this within the FD under institute policy to ensure that this specific project does not suffer further and that current overdue advances are resolved.<br><br>The station accountants will follow up advances with staff and Accounts Unit to ensure that advances are accounted for/retired correctly | |
| **Risk Rating** | **Priority Ranking** | **Responsible Official** | Project Manager and Dar and Kampala Station Accountants |
| **Significant** | 1 | **Due Date** | 31st December 2017 |

### 3.2. Advances

Funds requested and issued as advance in Uganda were posted directly as expenses and not as advances as required by the finance policy. At the time of posting the transactions, the expenses did not have supporting justification as shown in the samples below:

| Voucher/ Cheque # | Description | Amount (UGX) | Observation |
|---|---|---|---|
| 2549 | WAGES FOR CASUALS AND LUWERO FIELD ADVANCE | 2,573,000 | The cash issued to a student was directly posted as an expense to a wrong Account 7016 for student support. The student later submitted expense claim showing there was a balance that remained. The expense claimed were neither checked nor approved. |
| 2452 | FUEL SUPPLIES AND PERDIEM-MBARARA | 550,000 | Travel advance to Mbarara was directly expensed to fuel and travel expense accounts without supporting receipts. |
| 2591 | BBN SUPPLIES | 2,985,000 | Cash paid to the procurement officer for purchase of supplies as requested by the project staff was directly expensed without confirmation that the said supplies were bought and received by the project. |

The above practice leaves room for misappropriation of the funds issued as no follow up is done to confirm if the funds were used for the intended purpose once the funds have been expensed.

It was also noted that there is no segregation of duties in the cash advance process relating to the payment of casuals and procurement of certain lab supplies by the project staff in Arusha as noted below:

- The project staff who oversees the hiring, assignment of jobs and monitoring of the casual workers, is also responsible for the payment of the casuals e.g. banana field watchman.
- The purchase of the lab supplies by the lab technician who is also the requester as well as the end user of the item being purchased.

Segregation of duties reduces fraud risks and is important in ensuring value for money is achieved.

***Cause:*** *-Gaps in the advance treatment.*
*-Lack of segregation of duties in the cash payment and procurement processes.*

| Risk | Recommendation | Management Comments & Action | |
|---|---|---|---|
| • Misappropriation of advanced amount as no justification is required.<br>• High advance balances<br>• Cash rolling | • All funds issued to staff or students as advance should be treated as such and posted to the relevant advance account. Expensing should only be done after submission and approval of the expense claims.<br>• Payment of casuals working at the project fields in Arusha should be done by the Project Administrator who also acts as the cashier.<br>• Purchases by cash should be minimized and carried out by the Project Administrator. | • Project Management agrees with the issues on payment of casuals in Arusha by the Project Administrator and will direct Project Administrator accordingly, although it may not be physically ideal for the Project administrator to directly pay casuals up country.<br>• Project Management agrees that cash purchases should be minimized and carried out by Project Administrator but as for above point, may be unavoidable in some circumstances and may not be physically ideal for the Project Administrator or Country Administrator to directly purchase.<br>• There will be keenness in the posting of advances issued to staff and the wrongly posted transaction mentioned will be reversed to the correct Account by the Uganda Station. | |
| **Risk Rating** | **Priority Ranking** | **Responsible Official** | Kampala Station Accountant and Project Administrator |
| **Significance** | 1 | **Due Date** | 30th November 2017 |

### 3.3. Project Assets

Due to the location and activities of the project, the project staff utilizes and manages assets bought by the project funds as well those bought by other projects. A review of the assets records provided by the project, Dar es Salaam and Kampala administration for the assets located at the project offices at Nelson Mandela Africa Institute of Science and Technology (NM-AIST) in Arusha and Sendusu Station in Kampala with the actual assets *(see Attachment 2)* revealed the following:

a) There were assets in the project list which were physically verified during the audit but were not included in the Dar es Salaam and Kampala administration shared assets list.
b) Assets captured on the administration asset list and were verified during the audit but were not included in the project assets list.
c) Some assets have been captured more than once on the project and the administration lists.
d) Some assets purchased by the project but could not be traced to either the project or the administration asset lists.

Some of the gaps noted in the assets management process include:

- No evidence of periodic assets verification by both the project staff and station administration;
- Non-updating and reconciliation of registers by the project and teams at stations; and
- Inconsistent processes followed in the updating process when new project assets are bought.

The asset registers maintained by HQ, station administration and the Project team should mirror one another to ensure all assets have been properly captured and safeguarded.

***Cause:*** *Inconsistencies in the management of assets.*

| **Risk** | **Recommendation** | **Management Comments & Action** | |
|---|---|---|---|
| • Loss and misuse of assets<br>• Incomplete assets record. | • The Project team in liaison with the station administration in Tanzania and Uganda should update the asset lists and communicate the same list to Fixed Assets Unit of FD to upload into the Oracle System.<br>• Periodic assets verification exercises should be enforced and reports filed by the Station Administrator and Project Manager.<br>• Tagging and updating of the new assets should be done consistently and in a timely manner. | • All new assets procured are normally tagged after purchase, but for this time the purchase was done during the construction of the Mandela New office that could not be tagged before completions of the office and locate assets on specific locations e.g. Labs.<br>• This has been noted. We will work together with Assets Management Team to ensure that Assets register reconciles with the record at HQ. | |
| **Risk Rating** | **Priority Ranking** | **Responsible Official** | Project Manager and Station Administrators |
| **Medium** | 1 | **Due Date** | 31/12/2017 |

### 3.4. Posting of Project Expenses

From a sample of 2017 project expenditures in OPD posted from Dar es Salaam Station, expenditures made through cash advance amounting to TZS 3M (US $ 1,343) were consolidated as one amount and posted to a wrong Account 6651 (allocated administrative costs) as summarized in the table below:

| Reference | Description | Amount (TZS) |
|---|---|---|
| EC/282 | Purchase of office groceries, stationaries and internet bundle | 882,000 |
| EC/294 | Purchase of internet bundle, office groceries, repair of water dispenser and water tank | 858,100 |
| EC/297 | Purchase of internet bundle, stationaries, office groceries, cartridge and send doc by DHL | 578,100 |
| EC/327 | Purchase of internet bundle, stationaries, office groceries, cartridge and send doc by DHL | 685,300 |

The consolidation of the expenditure limits monitoring of the individual costs such as internet as per the budget lines.

It was also noted in the 2017 OPD posting from Kampala station that expenditures with independent account line amounting to UGX 16.8M (US $ 4.6k) were posted to Accounts 6645 (Other Admin Costs) and 6784 (Other Supplies) as summarized in the table below:

| Reference | Description | Amount (UGX) | Account Posted |
|---|---|---|---|
| 2545 | BMGF Meeting- Transport Services | 240,000 | 6645 |
| 2667 | Airport Transfers for May.17 | 80,000 | 6645 |
| 2474 | BBN Lab Supplies | 1,470,000 | 6784 |
| 2572 | Lab Electrical Works | 10,009,940 | 6784 |
| 2591 | BBN Supplies | 5,022,475 | 6784 |

We further observed that the descriptions captured in Oracle for the transactions are inadequate as they do not indicate, the payee, invoice number and specifics of supplies. This may make it difficult for Project Management to monitor the cost lines or identify any erroneous posting.

***Cause:*** *Oversight in the posting of transactions.*

| Risk | Recommendation | Management Comments & Action | |
|---|---|---|---|
| • Overrun on the budget lines.<br>• Inability to correctly analyze transactions. | All project expenditures should be posted to the appropriate account line. This will enable detailed analyses and monitoring of costs charged to the project. | Project management agrees with recommendations in relation to postings and labelling of postings. This aspect has been raised by project staff previously, and more detailed or appropriate labeling of postings will help project budget officers keep track of costings and budgets. In addition, the appropriate labelling should be in line with the posting requirements of the donor (to facilitate reporting to the donor) but which deviates from the IITA postings.<br>This will be revered to their independent Account code lines. | |
| **Risk Rating** | **Priority Ranking** | **Responsible Official** | Dar and Kampala Station Administrators and Accountants |
| **Medium** | 1 | **Due Date** | 30th November 2017 |

**Annexure 1: Audit Findings with 'Low' Risk Ratings**

| S/N | Issue | Risk | Proposed Action to Monitor risk |
|---|---|---|---|
| 1 | **Occupational Health and Safety**<br>With the refurbishment of the facilities at Sendusu station, there were concerns with regards to the health and safety of the staff and facilities as noted below:<br>a) There were only 3 fire extinguisher which were yet to be placed at properly identified and labelled areas of the facilities.<br>b) The fire extinguisher had not been inspected since January 2017.<br>c) The presence of dispensable drugs in the first aid kitty may be subject to abuse by personnel as the kitty is not locked or monitored by a trained first aider.<br>d) There is no trained first aider or fire marshal at the station.<br>e) The power inverter system has been placed on the entrance of the tissue culture lab which has no alternative exit.<br>f) The designated assembly point is yet to be marked and shared to all stations users.<br><br>The Project/Station Management must ensure, as far as is reasonably practicable, that everyone is aware of the health and safety arrangements.<br><br>***Cause:*** *The refurbishment of the building is yet to be fully completed.* | • Damage to property.<br>• Injuries to staff.<br>• Loss of lives. | The project/station in liaison with the Regional Facilities Manager may consider;<br>• Carrying out a safety audit of the new facilities to gauge the level of compliance to health and safety standards.<br><br>Training of first aiders and fire marshals. |
| 2 | **Accumulation of Staff Leave Days**<br>As at end July 2017, some project staff in Uganda had accumulated high leave balances as shown below;<br>**Staff No.** / **Leave balance**<br>50343 / 42<br>50211 / 39<br>50342 / 33<br>50339 / 31<br><br>The accumulation of leave by staff may lead to the staff forfeiting their leave days as well as disruption of the project activities if they decide to take all the leave days.<br><br>The project team has noted the accumulated leave days by staff and have devised a plan for staff not to take all accumulated leave at once but spread it over the year.<br><br>***Cause:*** *Staff not conscious of the need to take leave.* | • Low staff productivity rate.<br><br>• Forfeiture of accrued leave above the terms of employment. | Project Management in liaison with the Station Administrator should prepare annual leave schedule showing when various staff will take leave. This will encourage staff with high accumulated leave days to go on leave and will also facilitate better planning of project activities. |

## Annexure 2: Definition of Risk Ratings

**Risk Ratings**

Risks are a composite of the impact of the risk which the recommendation is addressing and the likelihood of that risk occurring if the recommendation is not implemented.

For analytical purposes, composite risk will be classified as follows:

| | |
|---|---|
| High | Failure has the potential to significantly damage or destroy the effective functioning of the process and/or Center/Office or its future viability. The risk would require attention and action by senior management and a clear action plan developed. |
| Significant | Failure has the potential to damage important aspects of the Center's/Office's functions or future viability, which would require significant management effort and time to recover. Management responsibility would need to be identified to manage the risk. |
| Medium | Failure has the potential to damage particular aspects of the Center's/Office's functions, diverting management effort if an adverse event occurred, but not expected to damage the overall medium-long term operations of the Center/Office. Management by specific monitoring or response procedures would be required. |
| Low | Failure would have limited impact on the process or Centre's/Office's functioning. It would be unlikely to require specific application of resources and may be dealt with by routine procedures. |

**Priority Ratings**

The priority ratings influence the setting of target dates for implementing the recommendations.

| | |
|---|---|
| **1** | ***Immediate*: Recommendations should be actioned immediately. These recommendations meet one of the following criteria:**<br>▪ **Pertain to high-risk, serious or materially significant audit findings;**<br>▪ **Control weakness; due to the seriousness or significance of the matter, immediate attention and appropriate corrective action is warranted; or**<br>▪ **Can be implemented easily.** |
| **2.** | *Within 3 months*: The recommendation should be implemented within 3 months of the release of the audit report. The recommendations relate to high or moderate risks that could evolve to higher risk If not addressed promptly. |
| **3.** | *Within 6 months*: Recommendations should (a) be completed, (b) have achieved significant progress, or (c) have a schedule for completion within six months of the release of the audit report. Although implementation of these recommendations has already begun or should begin upon the release of the audit report, significant implementation or measurement of the results may take up to six months. This rating is applicable to recommendations relating to low risk findings that could evolve to higher risk if not addressed over a reasonable period. |
| **4.** | *Over 6 months*: The recommendation requires long-term process changes. While these recommendations address serious issues concerning the Office/Centre's operations as identified in this report, they are either long-term goals or are dependent upon the implementation of other recommendations. The Centre/Office should be prepared to report the initial steps taken to implement these recommendations during a one-year management audit update. |

**Annexure 3: Report Ratings**

| | |
|---|---|
| **Effective Controls** | Internal controls evaluated are adequate, appropriate, and effective to provide reasonable assurance that risks are being managed and objectives should be met. |
| **Some Improvement Needed** | A few specific control weaknesses were noted; generally however, controls evaluated are adequate, appropriate, and effective to provide reasonable assurance that risks are being managed and objectives should be met; |
| **Major Improvement Needed** | Numerous specific control weaknesses were noted. Controls evaluated are unlikely to provide reasonable assurance that risks are being managed and objectives should be met; |
| **Unsatisfactory Controls** | Internal controls evaluated are not adequate, appropriate, or effective to provide reasonable assurance that risks are being managed and objectives should be met. |

## 1.10 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. Brown, A., Tumuhimbise, R., Amah, D., Uwimana, B., Nyine, M., Mduma, H., Talengera, D., Karamura, D., Kuriba, J., and Swennen, R. 2017. The genetic improvement of bananas and plantains (*Musa* spp.) In Genetic Improvement of Tropical Crops (H. Campos, and P.D.S. Caligari, eds): Springer, Cham, pp 219-240.
2. Coyne, D.L., Dubois, T. and Daneel, M. 2017. Integrated Pest Management in Banana and Plantain. In: *Integrated Pest Management in Tropical Regions*. CAB International, UK. Pp. 229-245. https://www.cabi.org/bookshop/book/9781780648002.
3. Coyne, D. and Kidane, S. 2018. Nematode pathogens. In: Jones, D. (ed) *Diseases of Banana, Abacá and Enset*. 2nd Edition. CAB International, Wallingford, UK, pp. 429-461.
4. Coyne, D.L., Nicol, J.M. and Claudius-Cole, B. 2018. *Practical plant nematology: a field and laboratory guide*. 3rd edition. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Pp 82. ISBN: 978-978-8444-91-6
5. Sikora, R.A., Coyne, D.L., Quénéhervé, P. 2018. Nematode parasites of bananas and plantains. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. 3rd Edition. CAB International, Wallingford, UK. pp. 617-657.
6. Viljoen, A., Ma, L.-J. and Molina, A.B. Fusarium wilt (Panama disease) and monoculture banana production: Resurgence of a century-old disease. Emerging Plant Diseases and Global Food Security. APS Press, St Paul, USA.

#### Peer Reviewed Journal Articles

1. Adheka, J.G., Dhed'a Djailo, B., Karamura, D., Blomme, G., Swennen, R., and De Langhe, E. 2018. The morphological diversity of plantain in the Democratic Republic of Congo. Scientia Horticulturae, 234, 126-133. https://doi.org/10.1016/j.scienta.2018.02.034.
2. Adheka, J.G., Komoy Losimba, J., Tamaru, C., Sivirihauma, C., Dhed'a Djailo, B., Karamura, D., De Langhe, E., Swennen, R., and Blomme, G. 2018. Banana and plantain diversity in Oriental provinces, north-eastern Democratic Republic of Congo. Acta Horticulturae, 1196, 255-264. https://doi.org/10.17660/ActaHortic.2018.1196.31.
3. Aguayo, J., Mostert, D., Fourrier-Jeandel, C., Cerf-Wendling, I., Hostachy, B., Viljoen, A. & Ioos, R. 2017. Development of a hydrolysis probe-based real-time assay for the detection of tropical strains of *Fusarium oxysporum* f.sp. *cubense* race 4. PLoS One 12(2): e0171767. Doi 10.1371/journal.pone.0171767: 1-20.
4. Alakonya, A.E., Kimunye, J., Mahuku, G., Amah, D., Uwimana, B., Brown, A., and Swennen, R. 2018. Progress in understanding *Pseudocercospora* banana pathogens and the development of resistant *Musa* germplasm. Plant Pathology 67:759-770. https://doi.org/10.1111/ppa.12824.
5. Amah, D., van Biljon, A., Brown, A., Perkins-Veazie, P., Swennen, R., and Labuschagne, M. 2018. Recent advances in banana (Musa spp.) biofortification to alleviate vitamin A deficiency. Critical Reviews in Food Science and Nutrition, 1-39. https://doi.org/10.1080/10408398.2018.1495175.

6. Batte, M., Mukiibi, A., Swennen, R., Uwimana, B., Pocasangre, L., Hovmalm, H.P., Geleta, M., and Ortiz, R. 2018. Suitability of existing Musa morphological descriptors to characterize East African highland 'matooke' bananas. Genetic Resources and Crop Evolution, 65, 645-657. https://doi.org/10.1007/s1072.
7. Baiyeri, P.K., Aba, S.C. and Ortiz, R. 2018. Germplasm evaluation and cropping systems enhanced sustainable plantain and banana production in Nigeria. *Nigerian Journal of Crop Science* 5, 62–68
8. Campos, N.A., Swennen, R., and Carpentier, S. 2018. The plantain proteome, a focus on allele specific proteins obtained from plantain fruits. Proteomics, 18(3-4). https://doi.org/10.1002/pmic.201700227.
9. Chittarath, K., Mostert, D., Crew, K.S., Viljoen, A., Kong, G., Molina, A.B. & Thomas, J.E. 2018. First report of *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 (VCG 01213/16) associated with Cavendish bananas in Laos. Plant Disease 102 (2): 449-450.
10. Christelová, P., De Langhe, E., Hřibová, E., Čížková, J., Sardos, J., Hušáková, M., Van den houwe, I., Sutanto, A., Kepler, A.K., Swennen, R., Roux, N., Doležel, J.: Molecular and cytological characterization of the global *Musa* germplasm collection provides insights into the treasure of banana diversity. – Biodivers. Conserv. 26: 801-824, 2017.
11. Coyne, D.L., Cortada, L., Dalzell, J.J., Claudius-Cole, A.O., Haukeland, S., Luambano, N., Talwana, H. 2018. Plant Parasitic Nematodes and Food Security in Sub-Saharan Africa. *Annual Review of Phytopathology, 56*, 381-403 https://doi.org/10.1146/annurev-phyto-080417-045833
12. Friedmann, M., Asfaw, A., Anglin, N.L., Becerra, L.A., Bhattacharjee, M., Brown, A., Carey, E., Ferguson, M.E., Gemenet, D., Lindqvist-Kreuze, H., Rabbi, I., Rouard, M., Swennen, R., and Thiele, G. 2018. Genomics-Assisted Breeding in the CGIAR Research Program on Roots, Tubers and Bananas (RTB). Agriculture, 8, 89. https://doi.org/10.3390/agriculture8070089.
13. Hung, T.N., Hung, N.Q., Mostert, D., Viljoen, A., Chao, C.P. & Molina, A.B. 2018. First report of Fusarium wilt on Cavendish bananas, caused by *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 (VCG 01213/16), in Vietnam. Plant Disease 102 (2): 448.
14. Karangwa, P., Mostert, D., Ndayihanzamaso, P., Dubois, T., Niere, B., Felde, A., Schouten, A., Blomme, G., Beed, F. & Viljoen, A. 2018. Genetic diversity of *Fusarium oxysporum* f.sp. *cubense* in East and Central Africa. Plant Disease 102 (3): 552-560.
15. Komoy Losimba, J., Adheka, J.G., Dhed'a Djailo, B., Karamura, D., Blomme, G., Swennen, R., and De Langhe, E. 2018. The complex distribution of plantain cultivars (Musa sp., AAB subgroup) in the Bas-Uele province of the Democratic Republic of Congo. African Journal of Agricultural Research, 13(26), 1358-1373. https://doi.org/10.5897/AJAR2018.13202.
16. 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., Mostert, L. & Viljoen, A. 2017. The distribution and host range of the banana Fusarium wilt fungus, *Fusarium oxysporum* f.sp. *cubense*, in Asia. PLoS One 12 (7): e0181630. https://doi.org/10.1371/journal.pone.0181630.
17. Nakato, G.V., Christelová, P., Were, E., Nyine, M., Coutinho, T.A., Dolezel, J., Uwimana, B., Swennen, R., and Mahuku, G. (in press). Sources of resistance to Xanthomonas campestris pv. musacearum , the causal agent of banana Xanthomonas wilt. Plant Pathology. https://doi.org/10.1111/ppa.12945.
18. Němečková, A., Christelová, P., Cizkova, J., Nyine, M., Van den houwe, I., Svačina, R., Uwimana, B., Swennen, R., Dolezel, J., and Hribova, E. 2018. Molecular and cytogenetic study of East African Highland Banana. Frontiers in Plant Science 9: 1371. http://journal.frontiersin.org/article/10.3389/fpls.2018.01371/full?&utm_source=Email_to_authors_&utm_medium=Email&utm_content=T1_11.5e1_author&utm_campaign=Email_publication&field=&journalName=Frontiers_in_Plant_Science&id=339101

19. Nyine, M., Uwimana, B., Blavet, N., Hribova, E., Vanrespaille, H., Batte, M., Akech, V., Brown, A., Lorenzen, J., Swennen, R., and Dolezel, J. 2018. Genomic prediction in a multiploid crop: genotype by environment interaction and allele dosage effects on predictive ability in banana. The Plant Genome, 11(2). http://dx.doi.org/10.3835/plantgenome2017.10.0090.
20. 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
21. van Wesemael, J., Hueber, Y., Kissel, E., Campos, N., Swennen, R., and Carpentier, S. 2018. Homeolog expression analysis in an allotriploid non-model crop via integration of transcriptomics and proteomics. Scientific Reports, 8(1), 1353. https://doi.org/10.1038/s41598-018-19684-5.

**Peer Reviewed Articles in process**

1. Marimo, P., Caron, C., Van den Bergh, I., Crichton, R., Weltzien, E., Ortiz, R. and Tumuhimbise, R. Trait preferences for banana cultivation and use in Sub-Saharan Africa, with specific focus on gender: Implications for banana breeding. Submitted to the journal Agriculture and Food Security (*under review*).
2. Tumuhimbise, R. et al. (2018). Early variety selection in cooking banana breeding in Uganda (*under development*).
3. Waniale, A., Mukasa, S.B., Tugume, A.K., Tumuhimbise, R., Kubiriba, J. and Swennen, R. Simple sugars perform better than sucrose for *in vitro* and *in vivo* germination of banana pollen. Acta Horticulturae (*under review*)

**Thesis**

1. Arinaga, E. 2018. Characterization of Target Population of Environments for East African Highland Banana Using a Multi-criteria Decision Methods. MSc Thesis, KU Leuven, Belgium, pp. 83.
   Thesis: http://breedingbetterbananas.org/wp-content/uploads/2018/10/BMGF-BBB-2018.-MSc-thesis-Emile-Arinaga.pdf
   Appendix to the thesis: http://breedingbetterbananas.org/wp-content/uploads/2018/10/BMGF-BBB-2018.-MSc-thesis-Appendix-Emile-Arinaga_Appendix.pdf
2. Mohamed, Y. 2017. Infestation assessment of banana weevil (*Cosmopolites sordidus* Germar) in different banana-based farming systems in Arusha and Kilimanjaro Regions, Tanzania. MSc Thesis, Nelson Mandela African Institution of Science and Technology, Tanzania, pp. 57. http://breedingbetterbananas.org/wp-content/uploads/2018/10/BMGF-BBB-2018.-MSc-thesis-Yusuph-Mohamed.pdf
3. Nyine, M. 2018. Genomic Selection to Accelerate Banana Breeding: Genotyping by Sequencing of Banana Hybrids. PhD thesis, Institute of Experimental Botany. Palacký University, Olomouc, Czech Republic, pp. 163. http://breedingbetterbananas.org/wp-content/uploads/2018/10/BMGF-BBB-2018.-PhD-thesis-Moses-Nyine.pdf
4. Shabani, H. 2017. Role of plant parasitic nematodes (*Pratylenchus goodeyi* Sher and Allen) on fusarium wilt disease incidence and severity on banana. MSc Thesis, Nelson Mandela African Institution of Science and Technology, Tanzania, pp. 58. http://breedingbetterbananas.org/wp-content/uploads/2018/10/BMGF-BBB-2018.-MSc-thesis-Hassan-Meduma-1.pdf

**Technical Briefs/ Protocols/**

1. Crichton, R., Ainembabazi, J.H., Caron, C. and Van den Bergh, I. 2017. Baseline intra-household survey: Tools for understanding the agricultural production systems and their socio-economic context in target regions for the introduction of new banana cultivars: baseline intra-household survey. Montpellier (France): Bioversity International, 27pp.
2. Crichton, R., Fliedel, G. and Van den Bergh, I. 2018. Trait preferences for banana products and varieties focus group discussion: Tools for understanding the agricultural production systems and their socio-economic context in target regions for the introduction of new banana cultivars: Trait preferences for banana products & varieties focus group discussion. Montpellier (France): Bioversity International, 11pp.
3. Crichton, R., Albertson, E., Caron, C. and Van den Bergh, I. 2018. Seasonal calendar focus group discussion: Tools for understanding the agricultural production systems and their socio-economic context in target regions for the introduction of new banana cultivars: Seasonal calendar focus group discussion. Montpellier (France): Bioversity International, 14pp.
4. Crichton, R., Albertson, E., Caron, C. and Van den Bergh, I. 2018. Weekly and daily calendar focus group discussion: Tools for understanding the agricultural production systems and their socio-economic context in target regions for the introduction of new banana cultivars: Weekly & daily calendar focus group discussion. Montpellier (France): Bioversity International, 12pp.
5. Crichton, R., Ajambo, S. and Van den Bergh, I. 2018. Community wealth ranking focus group discussion: Tools for understanding the agricultural production systems and their socio-economic context in target regions for the introduction of new banana cultivars: Community wealth ranking focus group discussion. Montpellier (France): Bioversity International, 12pp.
6. Viljoen, V., Ndayihanzamaso, P. and Mostert, D. Greenhouse inoculation of banana plantlets for Fusarium wilt resistance. 2018. Dept. Pathology, Stellenbosch University.

**Extension Materials**

1. Aitken, Chen, Czislowski, Warman and Anderson. 2018. Fusarium wilt Tropical Race 4 Research Program. A poster detailing ongoing work in the Plant Pathology lab at UQ was presented at the 2018 Australian Banana Roadshows held in North Qld, NSW and WA. The poster included aspects of the molecular marker work funded by IITA as well as some co-funding from Horticulture Innovation Australia (HAIA) and HIA funded work on SIX genes.

## CONFERENCE OUTPUTS

**Invited Presentation**

1. Chen, Morgan, Fraser-Smith, Zander, Ruperao, Batley, Edwards, Hamill and Aitken. 2018. Genetic dissection of resistance to Fusarium wilt disease in *Musa accuminata* ssp. *Malaccensis,* Fusarium wilt of Banana Side Meeting, International Congress of Plant Pathology, Boston, USA, July.
2. Coyne, D. The War on Worms in Africa: Plant Parasitic Nematode Threats to Food Security. The Inaugural J.N. Sasser Lecture: *Honoring a Lifetime of Science with Global Impact,* North Carolina State University, USA, 13 March 2018.
3. Marimo, P. and Tumuhimbise, R. Integrating gendered knowledge into banana breeding. Invited presentation at the Webinar: Ensuring gender-responsive plant and animal breeding: A practical decision checklist
4. Swennen, R. 2018. Banana-based systems in eastern Africa. MUSA2020 Project Workshop, Kampala, Uganda, 27-29 March.

5. Tumuhimbise, R., Kuriba, J. Musa breeding in Uganda. MUSA2020 Project Workshop, Kampala, Uganda, 27-29 March.
6. Viljoen, A. 2017. Future of bananas: Managing the risks of Fusarium wilt TR4 in Africa. 3rd Conference of the World Banana Forum, Geneva, Switzerland. 8-9 November.
7. Viljoen, A. 2017. A global perspective on the introduction and spread of Fusarium wilt TR4 in Mozambique. 1st National Conference on Recent Biological Invasions in Agriculture in Mozambique: Impact, Prevention, Detection, Eradication and Mitigation". Maputo, Mozambique. 19 and 20 October.
8. Viljoen, A., Mostert, D and Rose, L.J. 2018. Conventional and unconventional breeding of banana for Fusarium wilt resistance. South African Plant Breeders meeting, Umhlanga Rocks, South Africa. 12-14 March.
9. Viljoen, A. and Molina, A.B. 2018. Current efforts to mitigate the impact of Fusarium wilt (Foc TR4). ACORBAT meeting, Miami FL, USA. 2-4 May.
10. Viljoen, A., Mostert, D. and Ma, L.-J. 2018. The evolution of and diversity in the banana Fusarium wilt fungus *Fusarium oxysporum* f. sp. *cubense*. International Mycological Congress, San Juan, Puerto Rico. 16-20 July.
11. Viljoen, A. 2018. Global status of Foc TR4 epidemics, research and development. International banana conference, Davao, Philippines. 10-12 October.

**Oral Presentation**

1. Aitken, E. Fusarium wilt of Banana: a 100 Year War. James Cook University, Cairns, March 2018. Guest presentation.
2. Aitken, E. Fusarium wilt of Banana. CIRAD, Montpellier, France, April 2018. Guest presentation.
3. Aitken, E. Fusarium wilt of Banana: a 100 Year War. EARTH University, Costa Rica, July 2018. Guest presentation.
4. Bauchet, G. et al., Musabase: A Phenotyping and Breeding Database for Banana. Plant and Animal Genome conference, San Diego, USA, 13-16 January, 2018. https://pag.confex.com/pag/xxvi/meetingapp.cgi/Paper/31633
5. Coyne, D. The War on Worms in Africa: Plant Parasitic Nematode Threats to Food Security. Iowa State University, USA, 19 March 2018.
6. Coyne, D. Current and future status of biocontrol of pests and diseases of banana in Africa. Annual Project Planning meeting for MUSA2020, EARTH University, Costa Rica, 17-19 September, 2018.
7. Datta, S., Jankowicz-Cieslak, J., Davson, A., Chao, C.-P., Huang, S.-H., Viljoen, A., Nielen, S., Ingelbrech, I. and Till, B.J. 2017. A low coverage NGS based pipeline for rapid screening of gamma irradiated mutant bananas for resistance to Fusarium Wilt TR4. 4th International Symposium on Genomics of Plant Genetic Resources, Giessen, Germany.
8. Dita, M., Teixeira, L., O'Neill, W.T., Pattison, A.B., Li, C., Zheng, S., Staver, C. Viljoen, A. 2018. Current state of Fusarium wilt of banana in the subtropics. ISHS meeting on banana in the subtropics, Turkey. 13-15 August.
9. Mostert, D., O'Neill, W.S., Perry, Mostert, L. and Viljoen, A. 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.
10. Ndayihanzamaso, P., Karangwa, P., Mostert, D., Mahuku, G. and Viljoen A. 2017. Molecular markers for the detection of *Fusarium oxysporum* f.sp. *cubense* in East and Central Africa. Congress of the Southern African Society for Plant Pathology, Champagne Sports resort, Drakensberg, South Africa. 16-18 January.
11. Nyine, M., Uwimana, B., Blavet, N., Hribova, E., Vanrespaille, H., Batte, A., Akech, V., Brown, A., Lorenzen, J., Swennen, R., and Dolezel, J. 2018. The Benefits, Challenges

and Prospects of Genomic Prediction in Polyploid Banana. Plant & Animal Genome Conference XXVI (PAG). San Diego, USA. 13-17 January 2018. Abstract. https://pag.confex.com/pag/xxvi/meetingapp.cgi/Paper/30796

12. Tenkouano, A., Lamien, N., Agogbua, J., Amah, D., Swennen, R., Traoré, S., Thiemele, D., Aby, N., Kobenan, K., Gnonhouri, G., Yao, N., Astin, G., Sawadogo-Kabore, S., Tarpaga, V., Issa, W., Lokossou, B., Adjanohoun, A., Amadji, G.L., Adangnitode, S., Igue, K.A.D. and Ortiz, R. 2018. Disseminating promising high-yielding tetraploid plantain bred-hybrids in West Africa. In *FOOD 2030 International Congress: Towards Sustainable Agri-Food Systems*. Abstracts of Free Presenters. Univ. Hohenheim, Germany, 5-6 September 2018. University of Hohenheim, Germany. p. 20
13. Tumuhimbise, R., Kubiriba, J., Tushemereirwe, W.K., Barekye, A., Nowankunda, K., Akankwasa, K., Swennen, R., Karamura, D., Karamura, E. and Lorenzen, J. 2018. Breeding of East African Highland Bananas (*Musa* spp.) in Uganda: From Nearly Impossible to Routine Release of Varieties. Food and Agriculture Conference, Stockholm, Sweden, June 25-27, 2018. https://foodandagriculture.conferencesnest.com/scientific-speakers
14. Uwimana, B., Batte, M., Arinaitwe, I.K., Vuylsteke, M., Swennen, R. 2018 Molecular tools for banana breeding: QTL mapping for weevil resistance in banana. Symposium: Advances in Plant Genomics for Crop Improvement, Institute of Experimental Botany, Olomouc, July 13, 2018
15. van Wesemael, J., Hueber, Y., Campos, N., Kissel, E., Swennen, R., Roux, N., and Carpentier, S. 2018. Detection of variety specific alleles and correlation to their phenotype: a proof of principle in the Bioversity International collection. Plant & Animal Genome Conference XXVI (PAG). San Diego, USA. 13-17 January 2018. Abstract. https://pag.confex.com/pag/xxvi/meetingapp.cgi/Paper/30794.
16. Viljoen, A. 2017. Effective management of banana wilts has remained elusive but a global priority. Banana Wilts Cluster workshop, Kampala, Uganda. August 30-September 1.
17. Viljoen, A. 2017. Generation and evaluation of mutant Cavendish bananas against *Fusarium oxysporum* f.sp. *cubense* race 4. 2nd FAO/IAEA Research Coordination Meeting (RCM) on CRP D22005: Efficient Screening Techniques to Identify Mutants with Disease Resistance for Coffee and Banana. Lisbon – Portugal. 28 May-02 June.
18. Zorrilla, J., van Wesemael, J., Do, H., Uzilday, B., Ozgur, R., Turkan, I., Swennen, R., and Carpentier, S. 2018. Insights into the root fermentative metabolism during growth in banana. COSTFA1306 Final Meeting 'Plant phenotyping for future climate challenges'. Leuven, Belgium. 20-21 March 2018. Poster abstract. https://www.plant-phenotyping.org/cost-meeting_leuven18.

**Poster Presentation**

1. Arinaitwe, IK, CH Teo, A Milton, ANA Mustafa, F Kayat, B Uwimana, R Tumuhimbise, JA Harikrishna, RY Othman. Genetic Analysis of Resistance Against *Fusarium oxysporum* f.sp. *cubense* (FOC) In Banana Populations using Molecular Markers, 7th International Molecular Biology and Biotechnology Congress, Necmettin Erbakan University, Faculty of Science, Turkey, 25 - 27, April 2018
2. Arinaitwe, IK, CH Teo, A Milton, F Kayat, B Uwimana, R Tumuhimbise, JA Harikrishna, RY Othman. Preliminary QTL mapping for *Foc* race1 Resistance in Banana, BBB 4th Annual Meeting, Arusha, Tanzania, 23 – 25 April 2018
3. Batte, M., Uwimana, B., Swennen, R., Akech, V., Brown, A., Hovmalm, H.P., Geleta, M., Ortiz, R. Heterobeltiosis in banana breeding. IITA R4D Week, Ibadan, Nigeria, 20 – 25 November 2017
4. Batte, M., Uwimana, B., Swennen, R., Akech, V., Brown, A., Hovmalm, H.P., Geleta, M., Ortiz, R. Pollination and seed germination success in “Matooke” breeding. BBB 4th Annual Meeting, Arusha, Tanzania, 23 – 25 April 2018

5. 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.
6. Chen, Morgan, Fraser-Smith, Zander, Ruperao, Batley, Hamill, Edwards and Aitken. 2018. Genetic dissection of resistance to Fusarium wilt disease in *Musa accuminata* ssp. *Malaccensis,* International Congress of Plant Pathology, Boston, USA, July 2018. Poster abstract.
7. 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.
8. Waniale, A, Mukasa, S.B., Tugume, A.K., Tumuhimbise, R., Kubiriba, J. and Swennen, R. 2018. Simple sugars perform better than sucrose for *in vitro* and *in vivo* germination of banana pollen. Presented at the 30th International Horticultural Congress (IHC 2018) in Istanbul, Turkey between 12th and 16th August 2018, full text article was submitted to Acta Horticulturae for review.
9. Van Wesemael, J., Kissel, E., Swennen, R., and Carpentier, S. 2017. Using lab models and on-line transpiration monitoring to select water efficient cultivars: Proof of principle in the banana (*Musa* spp.) bio(di)versity. Plant Phenotyping Forum: integrating European plant phenotyping community. Tartu, Estonia. 22-24 November 2017. Poster abstract. https://eppn2020.plant-phenotyping.eu/index.php?index=49.

**Film/Video**

1. September 2018: Movie on banana breeding

   for youtube:
   https://drive.google.com/open?id=1MG8CiMgo_s0Uw5XlNdxX6olRztjtra8s
   https://www.youtube.com/watch?v=BzhNKTLN73c

   for social media version:
   https://drive.google.com/open?id=1B-e1VttNxNNyrRZ8_1_d7ZlxbhLu3dMt
   http://bulletin.iita.org/index.php/2018/10/06/journey-better-mchare/
   http://breedingbetterbananas.org/

2. September 2018: BBB website

   Product profiles of Mchare and Matooke:
   http://breedingbetterbananas.org/index.php/2017/01/24/work-package-1-banana-breeding-pipeline/

   Distribution maps of Sigatoka, Fusarium and nematodes in Uganda and Tanzania, displayed:
   http://breedingbetterbananas.org/index.php/2017/01/24/work-package-2-pest-and-disease-control/

**MEDIA**

**News Reports, Newspaper articles, Television Reports**

1. 2 October 2017. IITA News. Rony Swennen leading guests on an inspection tour of the renovated banana laboratory. http://www.iita.org/wp-content/uploads/2017/10/Bulletin_2402.pdf
2. 18 October 2017. Global programme seeks to contain serious threat to the world's bananas, FAO, Rome. http://www.fao.org/news/story/en/item/1044761/icode/
3. 18 October 2017. FAO and partners join forces to fight serious threat to the world's bananas. http://www.onuitalia.com/2017/10/18/fao-partners-join-forces-fight-serious-threat-worlds-bananas/
4. 20 October 2017. The Leuven Hundreds. http://thewordmagazine.com/thehundreds/?c=leuven&cat=scienceinnovation
5. 27 October 2017. IITA leads breeding efforts in a global programme that seeks to contain serious threat to the world's bananas. http://www.iita.org/news-item/iita-leads-breeding-efforts-global-programme-seeks-contain-serious-threat-worldsbananas/
6. 28-29 October 2017. Het-Belang-van-Limburg: 12-13
7. 28 October 2017. Twitter. "FAO and partners join forces to fight serious threat to the world's bananas – OnuItalia
8. 30 October 2017. Global program to fight fungus that threatens bananas. http://www.freshplaza.com/article/184069/Globalprogram-to-fight-fungus-that-threatens-bananas
9. 31 October 2017 (Fresh Plaza) - Fears around containment of Fusarium TR4 in Africa and Asia http://www.freshplaza.com/article/184078/Fears-around-containment-of-Fusarium-TR4-in-Africa-and-Asia
10. 8 November 2017. Radio Télévision Suisse reportage Banane. Broadcasted on 5 December 2017.
11. 9 November 2017. Campaign to tackle new fungus strain threatening bananas launched http://www.jamaicaobserver.com/news/campaign-to-tackle-new-fungus-strain-threatening-bananaslaunched_114487?profile=1373
12. 2017. J. Doležel: Talk show in "Hyde Park Civilizace", Czech TV,
13. 2017. Television Report: "Banány odolné vůči škůdcům a nemocem", Czech TV.
14. 2017. Television Report: "Češi šlechtí odolnější odrůdy banánů" in "Věda 24", Czech TV
15. 8-12 January 2018: Press: IITA News no 2417. IITA success story: Beneficiary sends 4 sons to university; is now a consultant for plantain seedlings in South-South Nigeria. http://bulletin.iita.org/index.php/2018/01/21/beneficiary-sends-sons-university/
16. 9 January 2018: Der schleichende Tod der Banane. Swiss Radio. https://www.srf.ch/news/wirtschaft/aggressiver-pilz-der-schleichende-tod-der-banane
17. 9 January 2018 (Business Daily) - The Banana Apocalypse? http://www.bbc.co.uk/programmes/w3csw8d4
18. 1 February 2018 (BBC News) - Battling to save the world's bananas http://www.bbc.com/news/business-42777803
19. 18 February 2018: Genomic prediction: http://bulletin.iita.org/index.php/2018/02/18/researchers-dna-prediction-banana-breeding/
20. 20 February 2018: Researchers use DNA prediction models to speed up banana breeding. IITA news: http://www.iita.org/news-item/researchers-use-dna-prediction-models-speed-banana-breeding/
21. 16 March 18: IITA and partners conduct first proteomic investigation in plantain and banana: http://blogs.iita.org/index.php/iita-and-partners-conduct-first-proteomic-investigation-in-plantain-and-banana/

22. 17 March 2018 (Queensland Government) - Expert praises TR4 biosecurity program efforts Queensland Government 17 March 2018
23. 9 April 2018: Taking stock of efforts to address Pseudocercospora banana pathogens of three deadly banana diseases. http://bulletin.iita.org/index.php/2018/04/09/taking-stock-of-efforts-to-address-pseudocercospora-banana-pathogens-of-three-deadly-banana-diseases/
24. 16 April 2018 Taking stock of efforts to address Pseudocercospora banana pathogens of three deadly banana diseases
25. http://bulletin.iita.org/index.php/2018/04/09/taking-stock-of-efforts-to-address-pseudocercospora-banana-pathogens-of-three-deadly-banana-diseases/@IITA_CGIAR @RTB_CGIAR @kslopez77 #BBetterBanana
26. 22 April 2018, Msumba news link: Watafiti mbalimbali wakutana Katika Tasisi ya Sayansi ya Nelson Mandela Jijini Arusha http://www.msumbanews.co.tz/2018/04/watafiti-mbalimbali-wakutana-katika.html
27. 22 April 2018, The Citizen Link : www.thecitizen.co.tz/zephania ubwani or www.thecitizen.co.tz/banana
28. 22 April 2018; hosting workshop on seed set in banana, Arusha, funded by BMGF
29. 23 April 2018: Banana experts meet to discuss hybrid varieties. Daily News. Tanzania.
30. 23 April 2018: Banana experts converge in Tz for meeting. The Citizen, Tanzania.
31. 23 April 2018: Banana improvement experts track progress at Arusha meet. The Guardian, Tanzania.
32. 23-25 April 2018; organising and hosting, Project Planning and Annual meeting of Breeding Better Bananas, Arusha, funded by BMGF
33. 26 April 2018: Experts say Tanzania can do better in banana production. http://www.xinhuanet.com/english/2018-04/26/c_137136925.htm
34. 2-4 May 2018: Invited and funded key speaker at ACORBAT, Miami, USA: Breeding new banana varieties for Africa with strategies of global relevance.
35. Received the only award given: http://bulletin.iita.org/index.php/2018/05/23/iita-banana-breeder-honored-musa-meeting/
36. 18 May 2018: Musa project develops hybrids of local Tanzanian cooking banana “Mchare http://bulletin.iita.org/index.php/2018/05/18/project-develops-hybrids-local-tanzanian-cooking-banana-mchare/
37. 23 May 2018: IITA banana breeder honored at international Musa meeting http://bulletin.iita.org/index.php/2018/05/23/iita-banana-breeder-honored-musa-meeting/
38. 24 May 2018: Musa project develops hybrids of local Tanzanian cooking banana “Mchare” http://bulletin.iita.org/index.php/2018/05/18/project-develops-hybrids-local-tanzanian-cooking-banana-mchare/ … @IITA_CGIAR @RTB_CGIAR @kslopez77 #BBetterBanana
39. 13 July 13, 2018: Breeding new bananas varieties for Africa with strategies of global relevance. Symposium: Advances in plant genomics for crop improvement, Institute of Experimental Botany, Olomouc, Czech Republic.
40. 2018. Bulletin of University Palacký (UP Žurnál) and CR Hana bulletin: “Doktorand z Ugandy získal během studia v Olomouci důležité poznatky pro šlechtění banánovníku”, http://www.cr-hana.eu/3579/doktorand-z-ugandy-ziskal-behem-studia-v-olomouci-dulezite-poznatky-pro-slechteni-bananovniku/
41. 2018. Bulletin of University Palacký (UP Žurnál) and CR Hanan bulletin: “Vědci se podělí o novinky ze studia dědičné informace pšenice a banánovníků” https://www.zurnal.upol.cz/nc/zprava/clanek/vedci-se-podeli-o-novinky-ze-studia-dedicne-informace-psenice-a-bananovniku/

42. 2018. J. Doležel: News Report in czech radio “Banány a pšenice mohou nakrmit lidstvo. Pokroky ve výzkumu představují vědci z celého světa v Olomouci”, Czech radio. https://olomouc.rozhlas.cz/banany-a-psenice-mohou-nakrmit-lidstvo-pokroky-ve-vyzkumu-predstavuji-vedci-z-7565212

43. 2018. Television Report: “Je třeba šlechtit nové druhy banánů, ty dnešní jsou málo odolné”, Czech TV. https://ct24.ceskatelevize.cz/veda/2537035-experti-je-treba-slechtit-nove-druhy-bananu-ty-dnesni-jsou-malo-odolne

44. 2018. J. Doležel: News Report in Czech radio “Radiožurnál”. http://www.rozhlas.cz/zaznamy/radiozurnal/#/2018-07-13/8

## 1.11 Training Workshops presented

- Regional workshop on banana Fusarium wilt TR4 prevention and diagnosis in Central Africa. Kampala, Uganda. 5-9 November 2017.
- Training on Fieldbook data collection tool, Musabase data management and BTracT**:**
  - IITA Kampala, organized by Guillaume Bauchet, Nick Morales and Margaret Karanja for WP participants (12 attendees), 1 February 2018 **(**5 days)
  - Embrapa Las Cruz das Almas, Brazil, organized by Guillaume Bauchet for Embrapa Banana breeding program (3 attendees) 2 April 2018 (5 days)
  - IITA Arusha, Tanzania, organized by Margaret Karanja and Nick Morales, specific training for BTracT (7 attendees) August 2018 (3 days)
  - IITA Sendusu, Uganda, organized by Margaret Karanja, specific training for BTracT (9 attendees) September 2018 (3 days)
- Workshop on the On-farm evaluation of NARITA hybrids in Tanzania and Uganda, Arusha, Tanzania, (15 attendees), organized by Inge van den Bergh and Pricilla Marimo, 18-20 April 2018 (5 days).
- Workshop on genomics selection in plant breeding, Arusha (36 attendees), 26-30 April 2018 (5 days).
- Scientific Writing Course Workshop, Arusha (25 attendees), 26-27 April 2018 (3 days)
- Workshop on Invasive Pests of Banana in Southern Africa: Panama disease TR4 and BBTV. Nelspruit, South Africa. 29 May 2018.
- Regional workshop on Fusarium wilt of bananas (Foc TR4) in the Caribbean: Prevention and diagnosis, methods and tools. CIRAD, Guadeloupe, 10-13 July 2018.
- Training on banana Fusarium wilt TR4 prevention and diagnosis in Mauritius. Stellenbosch, South Africa. 22 October – 2 November 2018.
- Training Workshop on seed fertility and seed set: Defining the role of environment on seed fertility in bananas and plantain, Arusha, 22 April 2018.

# 1.11.1 Training Workshop on the on-farm Evaluation of NARITA hybrids in Tanzania and Uganda

*Arusha, Tanzania – 18-20 April 2018*

## Background

Within the framework of the project on "Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa"[3], Bioversity International and partners are evaluating a set of hybrid East African Highland Bananas (EAHB), called NARITAs, for their agronomic performance and consumer acceptance in a range of expected target user environments in five regions in Uganda and Tanzania, using a Participatory Varietal Selection (PVS) methodology.

At the start of the project, a gender-differentiated baseline study was conducted in the five project regions to characterize the target user environments in terms of agro-ecological and socio-economic conditions and existing production systems. The study also assessed the target users' preferences for banana cultivars and products, and cultivar/product traits, and made a first rapid assessment of the existing seed systems.

Five on-station trials have been set up in different agro-ecological zones in Tanzania (Moshi, Mbeya and Bukoba) and Uganda (Kawanda, Mbarara) to evaluate the agronomic performance and yield characteristics of the NARITAs under the prevailing agro-ecological conditions (climate, soils, pests and diseases, abiotic constraints), and to assess their suitability for local production systems and consumer preferences.

While the baseline study provides useful insights into the current production systems and cultivar/trait preferences, and the on-station trials provide information on the performance of the new cultivars under the specific conditions of the trial sites, the current set-up does not allow for the detailed assessment of the performance of the new cultivars to a larger range of environmental conditions and production environments. On-farm trials will therefore be set up to better assess the cultivars' performance under local production and management conditions, and to capture feedback from a larger and more diverse group of farmers and other end-users.

[3] funded by the Bill and Melinda Gates Foundation (BMGF) and the CGIAR Research Program on Roots, Tubers and Bananas (RTB), and coordinated by the International Institute for Tropical Agriculture (IITA)

## Objectives

The objectives of this workshop are to:

- update each other on the progress made and current state of on-station evaluations
- learn about the varietal release procedures and data requirements in Tanzania and Uganda
- learn about methods for on-farm evaluations of new technologies, including new banana cultivars
- plan for the on-farm trials in Tanzania and Uganda

## Expected outputs

At the end of the 2-day workshop, we expect to have:

- a better understanding of the varietal release procedures and data requirements in Tanzania and Uganda
- an understanding of the principles behind and methods for crowdsourcing for the evaluation of new technologies, including new banana cultivars
- for each of the five target regions, a list of selected NARITAs to be taken for on-farm evaluation
- for each country, a list of minimum variables/traits to be evaluated during the on-farm trials
- a plan of activities and timeline for the set-up of the on-farm trials in Tanzania and Uganda

## Program

The workshop will consist of three main parts:

- a first day of presentations and open discussions, to exchange information and knowledge and share experiences
- a second day of working sessions, to develop a concrete plan of action for the on-farm trials
- a field day, to visit the on-station trial at TACRI, meet with district officers, and conduct some sensory evaluations of the NARITAs

| WEDNESDAY 18 April | | |
|---|---|---|
| | | |
| *08:00-09:00* | *Registration of participants* | *Nina* |
| 09:00-09:10 | Welcome | Cornel |
| 09:10-09:20 | Introduction to workshop | Inge |
| 09:20-09:40 | Introduction of participants | All |
| 09:40-10:00 | Overview of WP4 activities and status | Inge |
| | | |
| GETTING ON THE SAME PAGE – PRESENTATIONS AND DISCUSSIONS | | |
| 10:00-10:20 | Current knowledge of end-user trait preferences, based on literature review and results from FGD<br>Focus on information that will help:<br>• select subset of NARITAs for on-farm trials<br>• select variables to be assessed during on-farm evaluations | Pricilla |
| 10:20-10:40 | Discussion | |
| *10:40-11:00* | *Group photo* | *Nina* |
| *11:00-11:15* | *Coffee/tea break* | |
| 11:15-11:45 | Overview and first results of on-station trials | Inge + Noel |
| 11:45-12:15 | Overview and first results of sensory evaluations | Pricilla |
| 12:15-12:45 | Discussion | |
| *12:45-13:00* | *Admin matters* | *Nina* |
| *13:00-14:00* | *Lunch break* | |
| 14:00-14:30 | Varietal release in Tanzania: procedures, key actors, methods and traits to be measured, …<br>Including methods used by ARI to conduct on-farm evaluations | Ngomuo, TOSCI |
| 14:30-15:00 | Varietal release in Uganda: procedures, key actors, methods and traits to be measured, …<br>Including methods used by NARO to conduct on-farm evaluations | Robooni, NARO |
| 15:00-15:30 | Discussion | |
| *15:30-15:45* | *Coffee/tea break* | |
| 15:45-16:15 | Evaluation of crop cultivars with farmers: principles, methods, key considerations, … | Eva |
| 16:15-16:45 | Farmer experimentation, the use of triadic comparisons for the evaluation of new technologies (Tricot) and the online platform Climmob | Jacob (recorded + Skype) |
| 16:45-17:15 | Discussion | |
| 17:15-17:30 | End-of-day reflections | All |
| *17:30-19:00* | *Break* | |
| *18:30-20:30* | *Group dinner at Life Fitness Centre (walking distance from hotel)* | |

| THURSDAY 19 April | | |
|---|---|---|
| | | |
| DEVELOPING A PLAN OF ACTION – WORKING SESSIONS | | |
| **Working session 1 – Select subset of NARITAs for on-farm evaluations** | | |
| 09:00-10:30 | Decide on traits to use for selection of subset of NARITAs | All<br>Facilitator: Pricilla + Mpoki + Okurut<br>Note taker: Grace |
| | For these traits, look at available information and data:<br>• experience from the 5 sites – feedback on which NARITAs are popular among field workers, ARI staff and people from surrounding villages<br>• preliminary data from on station trials<br>• preliminary data from sensory evaluations | |
| | For each region, select subset of 10-12 NARITAs + local check | |
| *10:30-11:00* | *Coffee/tea break* | |
| **Working session 2 – Decide on methods for on-farm evaluations** | | |
| 11:00-13:00 | Decide on set up (regular vs crowdsourcing) per country | All<br>Facilitator: Inge + Eva<br>Note taker: Noel |
| | Decide on regions, number of villages per region, … and number of farmers per village | |
| | Decide on criteria for selecting farmers | |
| | Decide if/how to reward participation | |
| | Decide on list of minimum variables/traits to be evaluated during the on-farm trials | |
| | Decide on which other factors/explanatory variables to be recorded | |
| | Decide on which questions to ask about the participants | |
| | … | |
| *13:00-14:00* | *Lunch break* | |
| **Working session 3 – Develop workplans, roles and responsibilities, timelines, budgets** | | |
| 14:00-15:30 | General roles and responsibilities of project partners | All<br>Facilitator: Nina + Robooni + Cornel<br>Note taker: Daud |
| | Other key stakeholders to be engaged | |
| | Additional staff needs | |
| | Training of all project partners/stakeholders in methods and tools | |
| | Preparation of planting materials | |
| | Organization of workshop with participating farmers | |
| | Planting of on-farm trials | |
| | Timeline | |
| | Budgets | |
| *15:30-16:00* | *Coffee/tea break* | |
| 16:00-16:30 | End-of-day reflections | |

| FRIDAY 20 April | | |
|---|---|---|
| **TO THE FIELD – VISIT TO TACRI TRIAL, MEETING WITH DISTRICT OFFICERS, TASTING THE NARITAS** | | |
| 07:30 | Pick up at hotel lobby | |
| 09:30 | Arrival at TACRI field site | |
| 09:30-10:00 | Registration of all participants | Nina, Noel |
| 10:00-10:10 | Welcome | Cornel |
| 10:10-10:20 | Introduction of participants<br>Meet with field staff, district officers and extension workers | All |
| 10:20-10:30 | Introduction of BBB project: on-station trials, sensory evaluations, on-farm trials | Inge |
| 10:30-10:40 | Explaining field layout & planted genotypes | Noel, Grace |
| 10:40-10:50 | Explaining field management | Mbora Ulotu |
| 10:50-11:00 | Explaining field data collection | Maira, Kennedy |
| 11:00-12:00 | Field tour and preference ranking for different traits | Mbora Ulotu, Pricilla, Noel, Grace |
| 12:00-13:30 | Sensory evaluation exercise | Pricilla, food scientists |
| *13:30-14:30* | *Lunch* | |
| 14:30-14:45 | Group photo | Nina |
| 14:45-15:30 | Logistics (allowance & transport for district, extension agents) + gumboots distribution | Cornel, Noel, Grace |
| 15:30 | Leave for Arusha | |
| 17:30 | Arrive back at hotel | |

## Participants

| Name | Centre | Email address |
|---|---|---|
| **Inge Van den Bergh** | Bioversity International | i.vandenbergh@cgiar.org |
| **Pricilla Marimo** | Bioversity International | p.marimo@cgiar.org |
| **Noel Madalla** | Bioversity International | n.madalla@cgiar.org |
| **Cornel Massawe** | ARI Horti-Tengeru, Tanzania | massawesa@yahoo.co.uk |
| **Robooni Tumuhimbise** | NARO, Uganda | rtumuhimbise@hotmail.com |
| **Mpoki Shimwela** | ARI Maruku, Tanzania | mpokis@yahoo.com |
| **Daud Mbongo** | ARI Uyole, Tanzania | mwambongo@yahoo.co.uk |
| **Grace Kindimba** | ARI Horti-Tengeru | gracekindimba@yahoo.com |
| **Okurut Asher Wilson** | NARO, Uganda | okurutasherwilson@yahoo.com |
| **Eva Weltzien** | | eva.weltzien@gmail.com |
| **Jacob Van Etten** (by Skype) | Bioversity International | j.vanetten@cgiar.org |
| **David Brown** | Bioversity International | d.brown@cgiar.org |
| **Nina Jakobi** | Bioversity International | n.jakobi@cgiar.org |
| **Munguatosha S. Ngomuo** | TOSCI | ngomuo2004@yahoo.com |
| **Allan Brown** | IITA | a.brown@cgiar.org |

Background reading and useful links

### Varietal release guidelines

Tanzania varietal release guidelines: http://www.upov.int/edocs/tgdocs/en/tg123.pdf

Should be read in conjunction with General Introduction (document TG/1/3), and its associated TGP documents

http://www.upov.int/resource/en/dus_guidance.html

http://www.upov.int/en/publications/tg-rom/tg001/tg_1_3.pdf

http://www.upov.int/edocs/tgpdocs/en/tgp_8.pdf

http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf

http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf

http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf

Baseline survey of seed sector in Uganda, in relation to regional harmonization of seed legislation - p.21-23 Section 3: Variety evaluation, release and registration

**Participatory Varietal Evaluation**

Christinck, A., Weltzien, E. and Hoffmann V. 2005. Setting Breeding Objectives and Developing Seed Systems with Farmers: A Handbook for Practical Use in Participatory Plant Breeding Projects.

Tricot methodology: https://www.bioversityinternational.org/e-library/publications/detail/farmer-experimentation-for-climate-adaptation-with-triadic-comparisons-of-technologies-tricota-methodological-guide/

ClimMob platform: http://www.climmob.net/index.php/en/

## 1.11.2 Training Workshop on: from quantitative genetics to genomic prediction in plant breeding

**Venue**
Nelson Mandela African Institution of Science and Technology

**Dates and Course Time**
26-30 April 2018: Back to back with the annual meeting of Breeding Better Bananas ()

Days 1 and 2 /whole) + Day 5 (9:00AM – 12:00AM) Theory and Results from real data applications
Days 3 and 4 (whole) + Day 5 (2:00PM-5:00PM) Practices – hands on for running R codes fitting a variety of models using the BGLR package

**Guest Lecturers**
Dr. Jose Crossa (Biometrics and Statistics Unit, Centro Internacional de Mejoramiento de Maíz y Trigo – CIMMYT – Mexico) and Dr. Paulino Pérez-Rodríguez(Colegio de Postgraduados, Mexico)

**Co-Coordinators**
Professor Rodomiro Ortiz (Swedish University of Agricultural Sciences – SLU), rodomiro.ortiz@slu.se + Dr. Allan Brown(IITA) A.Brown@cgiar.org
Logistics: Ms. Scola Ponera (IITA) S. Ponera@cgiar.org

**History**
Previously in SLU Sweden and ICARDA Marocco

**General themes**

- Quantitative genetics in plant breeding.
- Concept on genotype-by-environment interaction (G×E) and methods for its analysis.
- Genetic models and their use in plant breeding.
- Basic concepts of association genetics and genomic-enabled prediction.

**Objectives**

- To provide some basic quantitative genetic concepts to be applied in plant breeding.
- To provide some basic statistical models and methods for genetic and genomic analyses.
- To detect and measure the genotype-by-environment interaction.
- To show practical results of association genetics and genomic prediction in a breeding context.
- To demonstrate implementations of various genetic analyses using R-packages.

**Learning outcomes**

- Refreshing some basic ideas of quantitative genetics applied to plant breeding.
- Dissecting the genotype-by-environment interaction and estimating stability of genotypes.
- Understanding the conceptual framework of selection in plant breeding.
- Learning results that clearly show how biometrical genetics, association genetics genomic-estimated prediction of breeding values WORK!

- Knowing how to run some R codes for biometrical genetics, association genetics. and genomic-estimated prediction of breeding values involving different statistical models.

**Day 1: Thursday 26th April 2018 (José Crossa)**

**Morning 9:00 -12:30 [coffee break: 10:30–11:00]**

**CONCEPTS IN QUANTITATIVE GENETICS**

- Basics of quantitative genetics. Genotypic and phenotypic values.
- Breeding value (additivity), dominance [and epitasis].
- The basic genetic model and genotypic effects among single cross.
- Phenotypic and genetic variances. Additive and dominance variances. Genetic variance from a factorial model.
- Covariance between relatives.
- Why to estimate genetic variances? Heritability concepts and use.

**BREEDER'S EQUATION**

- The breeder's equation. Response to selection. Theoretical equations. How to improve response to selection?

**Further Reading:**

Bruce Walsh (2001) Quantitative genetics in the age of genomics. Theor. Pop. Biol. 59:175–184

William G. Hill (2012) Quantitative genetics in the genomics era. Current Genomics 13:196–206

**Afternoon 14:00-17:00 [coffee break: 15:15–15:30]**

**GENOME WIDE ASSOCIATION ANALYSIS (GWAS) AND GENOMIC PREDICTION (GS)**

- Introduction and concepts of GWAS and GS.
- Single-marker regression.
- Single-marker regression, accounting for population stratification and relatedness.
- Models for variable selection.
- Principles of genomic prediction and selection (GS) as a tool to accelerate genetic gains. What genomic selection does?
- The complexity of GS data, the curse of dimensionality.
- Genomic-enabled prediction models (Ridge Regression BLUP and GBLUP).
- How GS can be implemented in plant breeding programs.

**Further Reading**:

José Crossa et al. (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics

ZeratsionAbera Desta, and Rodomiro Ortiz (2014) Genomic selection: genome-wide prediction in plant improvement. Trends Plant Sci. 19:592–601

H. P. Piepho, J. Möhring, A.E. Melchinger, and A. Büchse (2008) BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161:209–228

**Day 2: Friday 27th April 2018 (José Crossa)**

**Morning 9:00 -12:30 [coffee break: 10:30–11:00]**

**MULTI-ENVIRONMENT TRIALS AND G×E**

- Why Multi-Environment Trials in Plant Breeding?
- What is G×E?
- Dealing with G×E: Ignore it? Reduce it? Exploit it! How G×E can be study and quantified?
- Error variance, G×E variance and number of environments and number of replicates.
- Components of G×E and its influence in heritability and genetic gains.

**STATISTICAL METHODS FOR STUDYING G×E**

- Methods for assessing G×E.
- Simple Linear regression – regression on the site mean.
- Advantages and disadvantages of simple linear regression.
- Introduction to multivariate methods for assessing G×E.

**Further Reading:**

J.C. Bowman (1972) Genotype × environment interactions. Ann. Génet. Sél. Anim. 4:117–122

Walter T. Federer, and José Crossa (2012) I.4 screening experimental designs for quantitative trait loci, association mapping, genotype-by environment interaction, and other investigations. Front. Physiol. 3:156doi: 10.3389/fphys.2012.00156

**Afternoon 14:00-17:00**

**STATISTICAL METHODS FOR STUDYING G×E**

- Family of Linear-Bilinear models for assessing G×E.
- Site Regression Model (SREG).
- Random effects SREG with Factorial Regression and pedigree.
- Random effects SREG with Factorial Regression and molecular markers.
- Additive Main Effects and Multiplicative interaction Model (AMMI).
- Partial Least Squares and Factorial Regression for incorporating environmental co-variables for understanding G×E.

**Further Reading:**

Juan Burgueño et al. (2008) Using factor analytic models for joining environments and genotypes without crossover genotype × environment interaction. Crop Sci. 48:1291–1305

Hugh G. Gauch, Jr. (2006) Winning the accuracy game. Amer. Scientist 94:134–143

Rodomiro Ortiz et al. (2007) Studying the effect of environmental variables on the genotype × environment interaction of tomato. Euphytica 153:119–134

Alison B. Smith et al. (2015) Factor analytic mixed models for the provision of grower information from national crop variety testing programs. Theor. Appl. Genet. 128:55–72

**Day 3: Saturday 28th April 2018 (Paulino Pérez)**

**Morning 9:00 -12:00[coffee break: 10:30–11:00]**

- Introduction to R and fundamentals of R programming.
- Introduction and use of META-R for the basic analysis of multi-environment trials.

**Further Reading:**

Heather Merk (n/a) Introduction to R stat software application to plant breeding. http://www.extension.org/pages/60427

**Afternoon 14:00-17:00 [coffee break: 15:15–15:30]**

- Introduction and use of META-R for the basic analysis of multi-environment trials.
- Introduction and use of AGE-R for studying G×E.

**Day 4: Sunday 29th April 2018 (Paulino Pérez)**

**Morning 9:00 -12:00 [coffee break: 10:30–11:00]**

- Introduction and use of AGE-R for studying G×E.
- Use of AGD-R Software for genetic analyses, diallel analysis, and line x tester analysis.

**Afternoon 14:00-17:00 [coffee break: 15:15–15:30]**

- Software for GWAS and intro to genomic prediction

**Day 5: Monday 30th April 2018 (morning: José Crossa + afternoon: Paulino Pérez)**

**Morning 9:00 -12:00 [coffee break: 10:30–11:00]**

**GENOMIC SELECTION**

- Short introduction to GBLUP.
- How to integrate G×E into Genomics.
- Statistical models for incorporating G×E into genomic prediction.
- The Reaction Norm model for incorporating G×E.
- The Marker × Environment Model – Main effect of markers and specific effects of markers.
- Practical examples and results for models with genomic G×E.

**Afternoon 14:00-17:00 [coffee break: 15:15–15:30]**

- R forGBLUP and Ridge Regression BLUP.
- Closing

**Further Reading:**

Filippo Bassi et al. (2015) Breeding schemes for the implementation of genomic selection in wheat (*Triticum*spp.). Plant Sci. http://dx.doi.org/10.1016/j.plantsci.2015.08.021

YosephBeyene et al. (2015) Genetic gains in grain yield throughgenomic selection in eight bi-parental maizepopulations under drought stress

Shizhong Xu et al. (2014) Predicting hybrid performance in rice using genomic best linear unbiased prediction. Proc. Natl. Acad. Sci. (USA)

www.pnas.org/cgi/doi/10.1073/pnas.1413750111

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### 1.11.3 Science Writing Workshop


# Course Outline – Writing Journal Articles, and the General Principles of Scientific Writing

## Day 1: Morning

**1. Writing research papers**
1.1 Why are papers rejected?
1.2 Journal article structure
1.3 Common mistakes in different sections
1.4 Common mistakes—reporting results
Exercise: Presenting tables correctly

## Day 1: Afternoon

Exercise: Analysing and deconstructing published journal articles
**2. Writing clearly and avoiding common errors (part 1)**
2.1 Avoiding wordiness
The seven forms of wordiness
Shortening sentences and titles
Exercise: Shortening an abstract

## Day 2: Morning

Exercise: Revision game
**2. Writing clearly and avoiding common errors (part 2)**
2.2 Avoiding imprecise text
Avoiding incorrect or unclear comparisons
Dealing with ‘squinting modifiers’
Dealing with ‘dangling modifiers’
The careless use of ‘this’
Confusion following brackets or commas
Exercise: Clarifying text and shortening sentences 2

**3. Improving your style and keeping your audience interested**
3.1 Using the active rather than the passive voice
3.2 Avoiding excessive 'hedging'
3.3 Increasing emphasis – lists, punctuation and word order
3.4 Using parallelism correctly
3.5 Varying sentence length to keep your reader interested
**4. The writing cycle and making writing easy**
4.1 The writing cycle
4.2 Answering style requirements
4.3 Organising
4.4 Overcoming writer's block
4.5 Revising and editing
4.6 Peer review
4.7 Finalizing
Exercise: Planning a paper or section

## Day 2: Afternoon

**5. Editing and proofreading your own work**
5.1 Macro-editing
5.2 Micro-editing
5.3 Proofreading tips
5.4 Techniques to save you time
Exercise: Micro-editing
**6. Time-saving techniques in MS Word**
6.1 Using styles saves time
6.2 Quick table of contents
6.3 Quickly make lists of tables and figures
6.4 Check formatting
6.5 Find synonyms
6.6 Work in two parts of your document at the same time
6.7 Highlight all instances of a word or phrase
6.8 Make Autocorrect work for you
6.9 Make the custom dictionary work for you
6.10 Insert cross references quickly and easily
**7. Effective Data Presentation**
7.1 General principles of data presentation
7.2 Which method to use and why?
7.3 Tables
7.4 Statistical results: Text or tables?
7.5 Figures
Exercise: Presenting figures correctly
**Extras**
'Textual signposts'
Commonly confused meanings
Commonly confused spellings

**SCIENTIFIC WRITING COURSE PARTICIPANT LIST 26TH TO 27TH APRIL 2018**

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## 1.11.4 Training Workshop on seed fertility and seed set

## Defining the role of environment on seed fertility in bananas and plantains

to be held at the Gold Crest Hotel, Arusha,
from 9.00 – 11.00 a.m, Sunday 22 April, 2018
preceding the 2018 Annual BBB Project Meeting.

**AGENDA**

| Item | Indicative time | Title | Discussion leader |
|---|---|---|---|
| | | | |
| **1.** | 9.00 – 9.05 | Welcome | Jane G |
| | | | |
| **2.** | 9.05 – 9.45 | Presentation - setting the scene<br>Questions and discussion | Allan W, Josephine A, Veronica M |
| | | | |
| **3.** | 9.45 – 10.25 | Framework - defining the role of environment on seed fertility<br>Questions and discussion | David T |
| | | | |
| **4.** | 10.25 – 10.55 | Cytogenetics and genomic approaches to understanding impact of environment on seed fertility | Allan B, Jaroslav D |
| | | | |
| **5** | 10.55 – 11.00 | Summing up and conclusion | Jane G |
| | | | |

**Participants**
Perth, Western Australia [remote]:
*Dr Jane Gibbs [Chair], BBB Project SAG*
*Associate Professor David Turner, Honorary Research Fellow, The University of Western Australia*

On-site
*Allan Waniale [Meeting Coordinator], PhD Fellow, Makerere University, Kampala, Uganda*
*Professor Rony Swennen (IITA), BBB Project Leader*
*Dr Josephine Agogbua (IITA) WP1, Onne, Nigeria*
*Dr Delphine Amah (IITA) WP1, Onne, Nigeria*
*Veronica Massawe, Masters student, Nelson Mandela University, Arusha, Tanzania*
*Dr Allan Brown (IITA) WP1, Arusha, Tanzania*
*Dr Robooni Tumuhimbise (IITA) WP1 Kampala, Uganda*
*Dr Jaroslav Dolezel, institute of Experimental Botany, Prague, Czech Republic*

# Notes taken during the Skype meeting on "Defining the role of environment on seed fertility in bananas and plantains" held at Gold Crest Hotel, Arusha

Meeting Date and time: Sunday 22 April 2018 from 9.00 – 11.00 a.m.

The meeting was chaired by Dr. Jane Gibbs who connected remotely via Skype from Perth – Australia. The meeting started with introductions and below is a list of participants;

1. Dr. Jane Gibbs [Chair], BBB Project SAG (Via Skype from Perth, Australia)
2. Assoc. Prof. David Turner, Honorary Research Fellow, The University of Western Australia (Via Skype)
3. Allan Waniale [Meeting Coordinator], PhD Fellow, Makerere University, Kampala, Uganda
4. Prof. Rony Swennen (IITA), BBB Project PI
5. Dr. Josphine Agogbua (IITA) WP1, Onne, Nigeria
6. Dr. Delphine Amah (IITA) WP1, Onne, Nigeria
7. Veronica Massawe, Masters student, Nelson Mandela University, Arusha, Tanzania
8. Dr. Allan Brown (IITA) WP1, Arusha
9. Dr. Robooni Tuhumibise (IITA) WP1 Kampala Nigeria
10. Dr. Jaroslav Dolzel, The Czech institute of Experimental Botany
11. Eva Hribova, Bio-informatician, The Czech institute of Experimental Botany

**Power Point Presentations**

Allan W. introduced the power point presentation that was to be presented in the order of Josephine, Veronica and lastly Allan W.

**Josephine's presentation**

The biggest highlight was that seed in Onne peaked in August and February with August having the higher peak and with patterns repeating every year. It also came out clearly that parental combinations affect seed set as demonstrated by the PITAs whose seed set was highly variable.

Comments;

After Josephine's presentation Rony commented that the issue of ovule efficiency was not coming out as in discussions before the meeting. He asked whether we should consider ovule efficiency for future purposes. David responded by saying from a physiological point of view, it makes more sense to express seed set say per 10,000 ovules since bunch size and ovule number per fruit is variable from genotype to genotype. At the very least, seed set should be expressed per 100 fruits.

**Veronica's presentation**

The highlights from Veronica's presentation were that there is seasonal variation of pollen viability and counts. Wild genotypes and improved diploids generally produce more pollen counts and have higher viability.

Comments/questions;

Jane had a question on whether there is a relationship between pollen count and pollen vitality. But this question was not answered and it was to be revisited.

**Allan W's presentation**

The highlights of Allan W's presentation were that pollinating bananas with pollen germination media (PGM) increased seed set but it followed the pattern of customary pollination technique. Enhancing pollen germination did not over sterility in seed sterile banana cultivars suggesting post zygotic barriers.

Comments;

Jane and David suggested using pollen germination data using various sugars to estimate osmotic potential of pollen; this was to be considered later. They also asked whether PGM enhances pollen tube growth. It was also suggested that pollen tubes should be viewed whether they actually reach the ovules to ascertain post zygotic barriers. Jane also highlighted that from the presentation, there was a mention of water stress being responsible for seed set in edible bananas. She asked if there are manipulative experimented that could be done to answer this hypothesis. Allan W suggested growing bananas in a screen house or in drier environments for water stress. But David suggested that we could do this in the field by using plastic sheets to cover the ground between plants.

Rony made remarks that seasonal changes are more pronounced in West Africa than in East Africa. He added that West Africa has about 4 months without rain, mostly lowlands with sandy soils where East Africa has cooler temperatures and shorter dry seasons. He also talked about apparent higher seed set from natural pollen compared to hand pollination. He wondered whether this was correct and if so, is there something that we are doing wrong? Could it be the way we harvest pollen the wrong way, the way we bag flowers, stigma injuries, etc.? Rony further asked what we could be doing wrong compared to natural pollination. He also asked whether we get the right pollen quality and quantity on the stigma at the right time. Are we pollinating when the stigmas are ready (fresh and receptive) considering the time they open? What is the optimum for pollen, stigma, etc.? What about the health of the mother parent? During pollination, does the mother have adequate nutrients required for seed set, especially potassium?

**Jaroslav's presentation**

The presentation was a brief summary of the discussion he had with Allan B. He made the following remarks;

- Temperature affects the course of meiosis consequently affects pollen
- He asked about the quality of pollen in terms of karyotype; is pollen genetically perfect?
- He recommended comparison of ploidy to viability
- He also mentioned that compatibility is other crops is well documented while for the case of banana, little is known
- He stressed that we have to study pollen constitution, meiosis output and karyotypes

- Allan B supplemented that preliminary work has already been done on Mchare as a model that can be extended to other bananas (triploids)

**Rony's general remarks**

After all presentations, Jane asked Rony to make general remarks. He thanked the meeting organisers and made the following remarks;

- He said that there are so many parameters that were considered and we need to prioritize
- There are five banana breeding stations and we need to assign who should do what and when so as to avoid duplication
- We should develop a list of priorities for a future work plan
- Jane should give a feedback on the list of priorities
- The developed list parameter should be in a chronological order, Michael will give additional data on seed set in matooke
- The final list should be developed by July 1st 2018.

Rony then thanked Jane and David when then closed the meeting.

**Discussion by participants at the Gold Crest hotel after the Skype meeting**

There were brief discussions on the following issues;

- Purchase of the right microscope for pollen study work. It was resolved that a simple light microscope is adequate for pollen counts but pollen tube growth needs a florescent microscope
- There was a brief discussion about the challenge of measuring pollen size
- Genotyping and phenotyping for male fertility in arusha was also discussed
- Jaroslav was also enlightened about banana ovules and he took keen interest to study female meiosis through isolation of nuclei. Veronica is to send samples to Czech Republic for study

## Prioritization of factors influencing seed set as discussed in the meeting "defining the role of environment on seed fertility in bananas and plantains"

After the Skype meeting on April 22nd, 2018, Josephine, Veronica and I discussed about what we thought were the most likely causes of low or no seed set and the influencing factors. The factors are listed below in a chronological order and I have added my thoughts having learnt from working on the problem.

**Failure of zygote development**

From the discussion we had, we thought that this was among the top causes of low/no seed set in banana. I personally rank it to be the most important cause of low/no seed set. I think internal influencing factor is the **genotype factor**. Different genotype have different gene expressions and at different levels of expression. I think most parthenocarpic genotypes do not have the right balance of expressed genes that favour zygote development. It is most likely that the genes/hormones responsible for parthenocarpy do not favour seed set as there is an inverse relationship.

The most influencing external factors are **soil factors** and **season**. Dry seasons cause water stress and for soil factors, sandy soils drain much faster and lead to water stress. If plants are water stressed, they may change their transcriptional profile, experience slow transport of nutrients including to the bunch or both cases may happen. The pattern of seed set in parthenocarpic banana is that distal hands are the most fertile. There is also a bias of seed set at the tip of fingers and this possibly implies that some factors (possibly hormones) from leaves, stems or roots reach the proximal hands first and possibly in higher concentrations before the distal hands and particularly the fruit tips.

**Failure of fertilization**

From Josephine's presentation (slide 13), we saw that KM5 X Calcutta 4 yielded more seed compared to KM5 X Pisang Lilin cross. The pollen we put the stigma even if it has low viability should be adequate to fertilize all ovules in the fruit. For example 30% viability of 4,000 pollen grains on the stigma is 1,200 which are more than enough for an average of 300 ovules in a fruit. It is likely that the stigma pollen-interactions for some combinations do not favour pollen germination. The most influencing internal factor is therefore genotype and the external factors are time of pollination and season. Pollen viability reduces drastically after 10:00 am and dry season compound this by desiccating pollen. I think this goes hand in hand or is closely related to pollen vitality.

**Pollen number**

There are genotypes (including matooke) which hardly produce pollen and for this reason, they cannot be used as males even if they have desirable traits. Pollen number production is mainly an internal factor influenced by genotypic factors.

The above causes of low/no seed set and their influencing factors are what came out top from the discussion we had after the meeting. They have been backed by mostly my thoughts, this is open for discussion

## 1.12 Website update

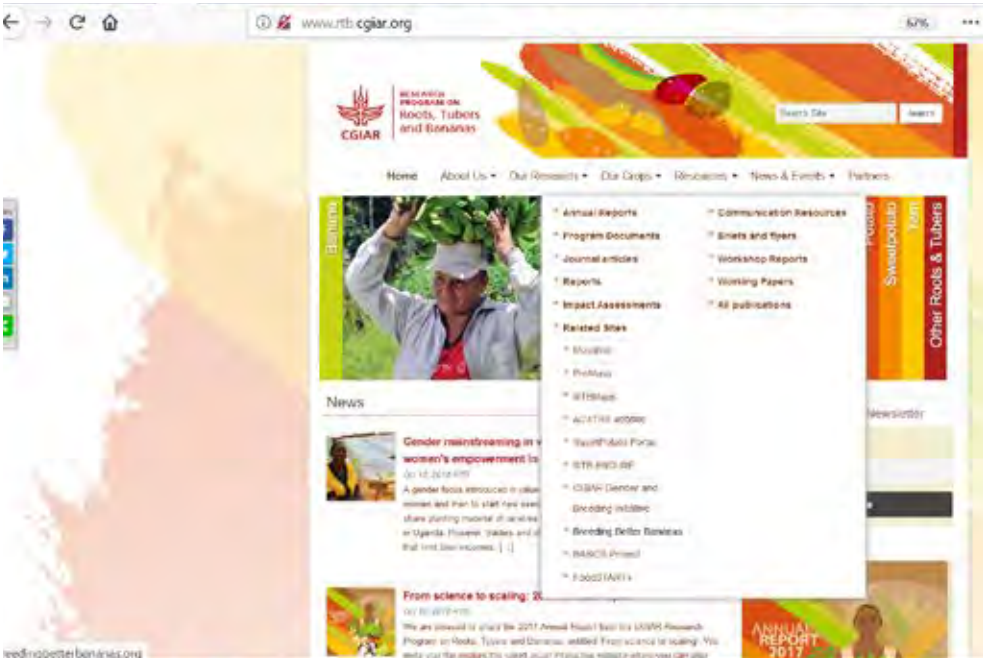
Communications on the BBB project:

**The Breeding Better Bananas website**: During this year, we have introduced new contents on our website across the different packages that portray progress and outcomes achieved in the project.

- Product profiles of Mchare and Matoke are available under WP1's section.
- Eleven epidemiology maps, with information on incidence and severity for nematodes, weevils, black Sigatoka and Fusarium, have been published under WP2's section together with technical protocols for inoculation of Fusarium wilt.
- Five tools for understanding the agricultural production systems and their socio-economic context in target regions for the introduction of new banana cultivar are available under WP4's section.
- Publications section: this new section will display a list of all the publications produced under the BBB project organized per year; links to the publication in OA through the journals or MusaLit are provided for the public.
- In addition:
    - "Our locations" is an interactive map that the visitors to the web can use to have an overview on what are the teams and partners for the BBB project and where do they work.
    - New partners and members of the project that came on board this year have been listed and included on the web.

Our website is now linked to the website of the Root, Tubers and Bananas (RTB) from the CGIAR webpage as a resource website (Figure1) and other relevant websites.

Figure 1. Screenshot of the RTB website indicating the linkage to the BBB project.



**Metrics of the web:** Over the past 12 months the BBB project had 1,389 users from which 99.6% were new users and 2,124 sessions and 4,872-page views, which represents an increase compare to the previous reporting period (2016-2017). From 1st of October 2017 to 30th September 2018 there was a total of 87.2% of new visitors and 12,8% of returning visitors. Regarding the origin, the new users are mainly coming from the United States (27.24%), followed far behind from by Tanzania (7.66%) and India (5.392%) on the top-three; for more information see Figure 2.

Figure 2. Table of origin of visitors to the BBB website by countries.

| Country | Acquisition | | | Behavior | | |
|---|---|---|---|---|---|---|
| | Users | New Users | Sessions | Bounce Rate | Pages / Session | Avg. Session Duration |
| | 1,389<br>% of Total: 100.00% (1,389) | 1,384<br>% of Total: 100.07% (1,383) | 2,124<br>% of Total: 100.00% (2,124) | 61.21%<br>Avg for View: 61.21% (0.00%) | 2.29<br>Avg for View: 2.29 (0.00%) | 00:02:30<br>Avg for View: 00:02:30 (0.00%) |
| 1. United States | 378 (26.56%) | 377 (27.24%) | 392 (18.46%) | 86.22% | 1.54 | 00:01:02 |
| 2. Tanzania | 116 (8.15%) | 106 (7.66%) | 235 (11.06%) | 50.21% | 2.77 | 00:03:46 |
| 3. India | 85 (5.97%) | 82 (5.92%) | 124 (5.84%) | 51.61% | 2.27 | 00:02:21 |
| 4. Nigeria | 66 (4.64%) | 61 (4.41%) | 188 (8.85%) | 50.53% | 2.69 | 00:04:42 |
| 5. Philippines | 59 (4.15%) | 59 (4.26%) | 60 (2.82%) | 63.33% | 1.67 | 00:01:13 |
| 6. Kenya | 54 (3.79%) | 48 (3.47%) | 189 (8.90%) | 55.03% | 2.50 | 00:03:26 |
| 7. United Kingdom | 51 (3.58%) | 51 (3.68%) | 63 (2.97%) | 58.73% | 2.17 | 00:02:38 |
| 8. Uganda | 51 (3.58%) | 41 (2.96%) | 93 (4.38%) | 49.46% | 2.87 | 00:03:12 |
| 9. France | 43 (3.02%) | 43 (3.11%) | 59 (2.78%) | 54.24% | 3.02 | 00:03:30 |
| 10. Belgium | 42 (2.95%) | 41 (2.96%) | 112 (5.27%) | 45.54% | 2.60 | 00:01:46 |

**Videos and Photography:**

- Video: A long version of the "*Journey to better Mchare: Improving Tanzania's beloved cooking banana*" portraying the efforts of the BBB project dedicated to Mchare can be found on our website (http://breedingbetterbananas.org/index.php/journey-to-a-better-mchare/).
- A shorter version of 2 min to be shared through Tweeter and social media (attached) has also been produced.
- We have a related publication on out IITA blog related to the video http://bulletin.iita.org/index.php/2018/10/06/journey-better-mchare/
- A photo gallery has been populated with new images, related to the Ontology of WP5 (Ibadan, Sendusu and Arusha Musabase. http://breedingbetterbananas.org/index.php/photo-gallery/

Photo Gallery

breedingbetterbananas.org

The CGIAR Research Program on Roots, Tubers and Bananas (RTB) is one of a new series of initiatives spearheaded by CGIAR to bring together the research synergies and resources of multiple agricultural research-for-development centers to improve efficiencies and increase impacts.

**Social Media**: On social media we've created a Tweeter official account (**@BBetterBanana**) from where we are sharing information regarding the progress of the project, together with a Tweeter Feed that it is now featuring on the website.

# Annex 1.11a. Audience overview

BreedingBetterBananas
All Web Site Data

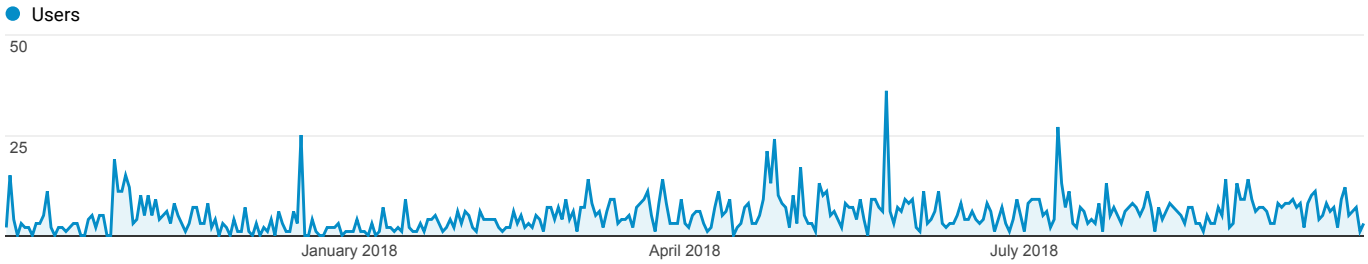
GO TO REPORT

## Audience Overview

All Users
100.00% Users

Oct 1, 2017 - Sep 30, 2018

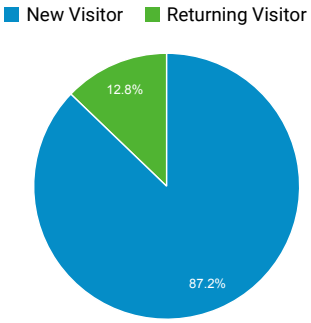
Overview




| Users | New Users | Sessions |
|---|---|---|
| 1,389 | 1,383 | 2,124 |

| Number of Sessions per User | Pageviews | Pages / Session |
|---|---|---|
| 1.53 | 4,872 | 2.29 |

| Avg. Session Duration | Bounce Rate |
|---|---|
| 00:02:30 | 61.21% |




| | Language | Users | % Users |
|---|---|---|---|
| 1. | en-us | 978 | 70.11% |
| 2. | en-gb | 151 | 10.82% |
| 3. | pt-br | 29 | 2.08% |
| 4. | fr | 26 | 1.86% |
| 5. | en | 24 | 1.72% |
| 6. | nl-nl | 17 | 1.22% |
| 7. | nl | 16 | 1.15% |
| 8. | en-au | 15 | 1.08% |
| 9. | es-es | 12 | 0.86% |
| 10. | cs | 11 | 0.79% |

# Annex 1.11b. Pages visited

BreedingBetterBananas
All Web Site Data

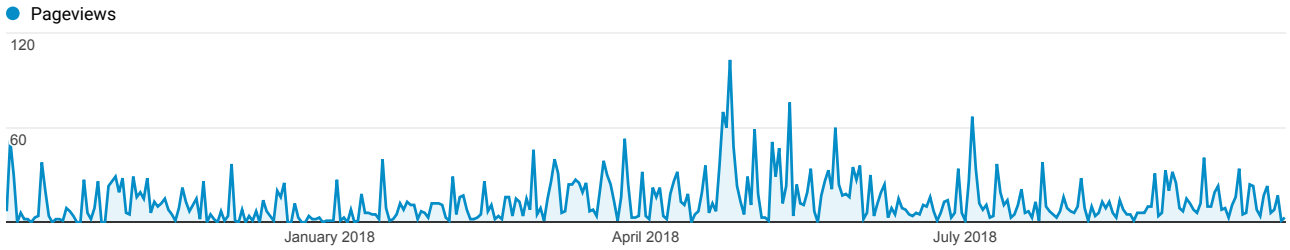
GO TO REPORT

## Pages

All Users
100.00% Pageviews

Oct 1, 2017 - Sep 30, 2018

Explorer




| Page Title | Pageviews | Unique Pageviews | Avg. Time on Page | Entrances | Bounce Rate | % Exit | Page Value |
|---|---|---|---|---|---|---|---|
| | **4,872** % of Total: 100.00% (4,872) | **3,891** % of Total: 100.00% (3,891) | **00:01:56** Avg for View: 00:01:56 (0.00%) | **2,124** % of Total: 100.00% (2,124) | **61.21%** Avg for View: 61.21% (0.00%) | **43.60%** Avg for View: 43.60% (0.00%) | **$0.00** % of Total: 0.00% ($0.00) |
| 1. \| Breeding better bananas | **1,674** (34.36%) | 1,260 (32.38%) | 00:02:03 | 1,186 (55.84%) | 48.74% | 44.44% | $0.00 (0.00%) |
| 2. Login \| | **530** (10.88%) | 414 (10.64%) | 00:01:15 | 237 (11.16%) | 89.03% | 52.26% | $0.00 (0.00%) |
| 3. Improvement of banana for smallholder farmers in the Great Lakes Region of Africa \| | **239** (4.91%) | 220 (5.65%) | 00:02:12 | 83 (3.91%) | 75.90% | 62.76% | $0.00 (0.00%) |
| 4. Objectives \| | **234** (4.80%) | 179 (4.60%) | 00:01:11 | 76 (3.58%) | 59.21% | 31.20% | $0.00 (0.00%) |
| 5. People involved \| | **226** (4.64%) | 202 (5.19%) | 00:03:30 | 92 (4.33%) | 78.26% | 62.83% | $0.00 (0.00%) |
| 6. Work Package 1: Banana Breeding Pipeline \| | **208** (4.27%) | 173 (4.45%) | 00:02:06 | 31 (1.46%) | 61.29% | 29.33% | $0.00 (0.00%) |
| 7. Work Package 2: Pest and Disease Control \| | **203** (4.17%) | 170 (4.37%) | 00:02:33 | 41 (1.93%) | 73.17% | 42.36% | $0.00 (0.00%) |
| 8. IITA leads breeding efforts in a global programme that seeks to contain serious threat to the world's bananas \| | **171** (3.51%) | 147 (3.78%) | 00:04:06 | 116 (5.46%) | 80.17% | 70.18% | $0.00 (0.00%) |
| 9. Work Package 4: Empowering End-User Evaluation \| | **159** (3.26%) | 136 (3.50%) | 00:01:51 | 33 (1.55%) | 78.79% | 42.14% | $0.00 (0.00%) |
| 10. About \| | **142** (2.91%) | 114 (2.93%) | 00:01:32 | 12 (0.56%) | 41.67% | 22.54% | $0.00 (0.00%) |

Rows 1 - 10 of 67

# Annex 1.11c. top locations

BreedingBetterBananas
All Web Site Data

GO TO REPORT

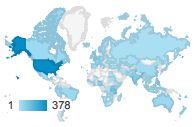
## Location

All Users
100.00% Users

Oct 1, 2017 - Sep 30, 2018

Map Overlay

Summary

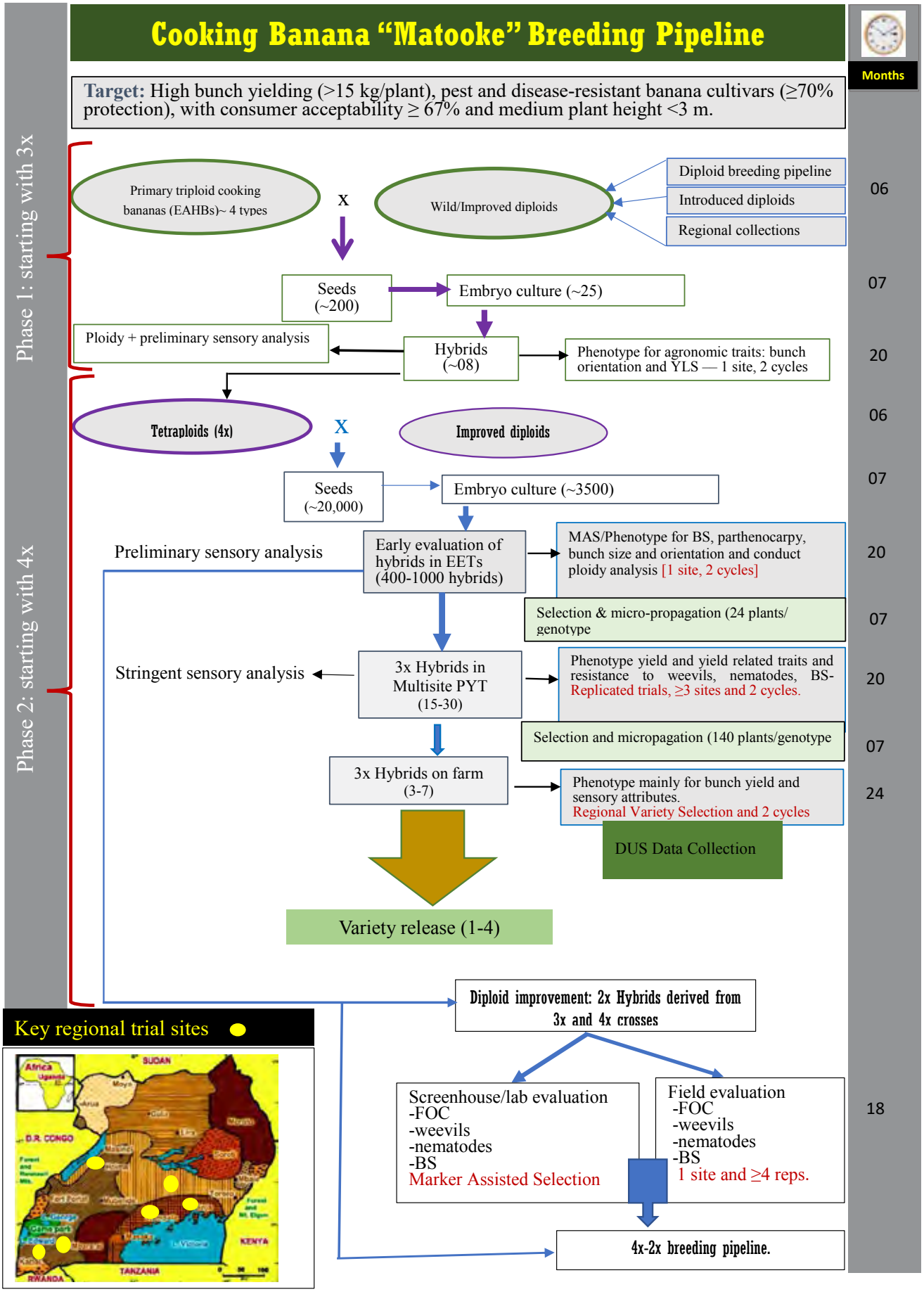



| Country | Acquisition | | | Behavior | | | Conversions | | |
|---|---|---|---|---|---|---|---|---|---|
| | Users | New Users | Sessions | Bounce Rate | Pages / Session | Avg. Session Duration | Goal Conversion Rate | Goal Completions | Goal Value |
| | **1,389** % of Total: 100.00% (1,389) | **1,384** % of Total: 100.07% (1,383) | **2,124** % of Total: 100.00% (2,124) | **61.21%** Avg for View: 61.21% (0.00%) | **2.29** Avg for View: 2.29 (0.00%) | **00:02:30** Avg for View: 00:02:30 (0.00%) | **0.00%** Avg for View: 0.00% (0.00%) | **0** % of Total: 0.00% (0) | **$0.00** % of Total: 0.00% ($0.00) |
| 1. United States | **378** (26.56%) | 377 (27.24%) | 392 (18.46%) | 86.22% | 1.54 | 00:01:02 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 2. Tanzania | **116** (8.15%) | 106 (7.66%) | 235 (11.06%) | 50.21% | 2.77 | 00:03:46 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 3. India | **85** (5.97%) | 82 (5.92%) | 124 (5.84%) | 51.61% | 2.27 | 00:02:21 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 4. Nigeria | **66** (4.64%) | 61 (4.41%) | 188 (8.85%) | 50.53% | 2.69 | 00:04:42 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 5. Philippines | **59** (4.15%) | 59 (4.26%) | 60 (2.82%) | 63.33% | 1.67 | 00:01:13 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 6. Kenya | **54** (3.79%) | 48 (3.47%) | 189 (8.90%) | 55.03% | 2.50 | 00:03:26 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 7. United Kingdom | **51** (3.58%) | 51 (3.68%) | 63 (2.97%) | 58.73% | 2.17 | 00:02:38 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 8. Uganda | **51** (3.58%) | 41 (2.96%) | 93 (4.38%) | 49.46% | 2.87 | 00:03:12 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 9. France | **43** (3.02%) | 43 (3.11%) | 59 (2.78%) | 54.24% | 3.02 | 00:03:30 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |
| 10. Belgium | **42** (2.95%) | 41 (2.96%) | 112 (5.27%) | 45.54% | 2.60 | 00:01:46 | 0.00% | 0 (0.00%) | $0.00 (0.00%) |

Rows 1 - 10 of 96

# 2. Work Package 1

## 2.1 Matooke Breeding Pipeline schematic



## 2.2 Matooke Breeding Pipeline overview

| Matooke Breeding Pipeline | | | |
|---|---|---|---|
| | | **MAP = Months after flowering** | |
| | **Time (Months)** | **Sites** | **Responsible Person(s)** |
| Tasks/activities | | | |
| **Establish and maintain pollination blocks** | Continuous | Kawanda, Sendusu | Robooni+Pollinators |
| **Make crosses (2x-2x 3x-2x, 4 x 2x) and Seed extraction** | 6 | Kawanda, Sendusu | Brigitte+Pollinators |
| **Embryo culture and weaning seedlings** | 7 | Kawanda, Sendusu | Naboth+Jane+Violet |
| **Marker Assisted Selection and Ploidy analysis for seedlings** | 2 | Kawanda/Sendusu | Brigitte+Robooni |
| Establish early evaluation trials for 2 cycles (Single site, 1 Rep) | **20** | | |
| **Assessment of plant growth parameters at flowering** | 9 MAP | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Assessment for black Sigatoka at flowering** | 9 MAP | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Assessment for yield parameters at bunch maturity of the parent crop** | 14 MAP | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Assessment for yield parameters at bunch maturity of the ratoon crop** | 20 MAP | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Preliminary assessment for fruit sensory attributes of the parent crop (fruit pulp colour, sap content and peel)** | 14 MAP | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Preliminary assessment for fruit sensory attributes of the ratoon crop (fruit pulp colour, sap content and peel)** | 20 MAP | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Selection of genotypes for advancement to Preliminary Yield Trials (PYTs)** | 11 | Kawanda, Sendusu | Namagembe, Abias,Kazigye, Robooni,Brigitte,Violet |
| **Micropropagation of sufficient plantlents for PYTs** | 7 | Kawanda, Sendusu | Naboth+Jane+Robooni +Jerome, Violet,Brigitte |
| | | | |
| Establishment of multilocation PYTs for 2 cycles (≥ 3 agroecological zones, ≥ 2 Replications) | **20** | | |
| **Assessment of plant growth parameters at flowering** | 9 MAP | Lake Victoria Crescent, South Western Farmlands, | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |

| | | | |
|---|---|---|---|
| | | Western Savannah Grasslands, Pastoral Rangelands. | |
| **Assessment for black Sigatoka at flowering** | 9 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Assessment for yield parameters at bunch maturity of the parent crop** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Assessment for yield parameters at bunch maturity of the ratoon crop** | 14MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Stringent assessment for fruit sensory attributes of the parent crop (fruit pulp colour, texture, aroma, taste)** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Stringent assessment for fruit sensory attributes of the ratoon crop (fruit pulp colour, texture, aroma, taste)** | 20 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Assessment of the parent crop for nematodes** | 9 MAP | Lake Victoria Crescent, South Western Farmlands, | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |

| | | | |
|---|---|---|---|
| | | Western Savannah Grasslands, Pastoral Rangelands. | |
| **Assessment of the ratoon crop for nematodes** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Assessment of the parent crop for weevils** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Assessment of the ratoon crop for weevils** | 20 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Selection of genotypes for advancement to on-farm trials** | 5 | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Robooni, Jerome, Kazigye, Abias,Brigitte,Violet |
| **Micropropagation of sufficient plantlents for on farm trials** | 7 | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Naboth+Jane+Violet |
| | | | |
| Establishment of multilocation on farm trials for 2 cycles (≥ 3 | **26** | | |

| | | | |
|---|---|---|---|
| agroecological zones, 2 Replications, ≥ 20 farms) | | | |
| **Assessment for simple plant growth parameters of the parent crop at flowering (number of leaves, plant type/vigour)** | 9 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Jerome, Robooni, Abias, Kazigye,Brigitte,Violet |
| **Assessment for simple plant growth parameters of the ratoon crop at flowering (number of leaves, plant type/vigour)** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Jerome, Robooni, Abias, Kazigye,Brigitte,Violet |
| **Assessment for yield parameters at bunch maturity of the parent crop (bunch yield, number of clusters)** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Jerome, Robooni, Abias, Kazigye,Brigitte,Violet |
| **Assessment for yield parameters at bunch maturity of the ratoon crop (bunch yield, number of clusters)** | 20 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Jerome, Robooni, Abias, Kazigye,Brigitte,Violet |
| **Stringent assessment for fruit sensory attributes of the parent crop (fruit pulp colour, texture, aroma, taste)** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Jerome, Robooni, Abias, Kazigye,Brigitte,Violet |
| **Stringent assessment for fruit sensory attributes of the ratoon crop (fruit pulp colour, texture, aroma, taste)** | 20 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, | Marry,Lugolobi,Robooni Jerome,Brigitte, Violet |

| | | | |
|---|---|---|---|
| | | Pastoral Rangelands. | |
| **Assessment of the parent crop for nematodes** | 9 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Josephine, Jerome, Robooni,Brigitte, Violet |
| **Assessment of the ratoon crop for nematodes** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Josephine, Jerome, Robooni,Brigitte, Violet |
| **Assessment of the ratoon crop for weevils** | 14 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Elyeza+Robooni, Jerome,Brigitte,Violet |
| **Assessment of the ratoon crop for weevils** | 15 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Elyeza+Robooni, Jerome,Brigitte,Violet |
| **Preparation and submission of application for Statutory Variety inspections and release** | 20 MAP | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, Pastoral Rangelands. | Elyeza+Robooni, Jerome,Brigitte,Violet |
| **DUS (Distictines, Uniformity and Stability) data collection and variety release** | 6 | Lake Victoria Crescent, South Western Farmlands, Western Savannah Grasslands, | Robooni+MAAIF Inspectors |

| | | | |
|---|---|---|---|
| | | Pastoral Rangelands. | |
| TOTAL BREEDING CYCLE | **81 months (6.6yrs)** | | |

## 2.3 Matooke and Mchare product profiles

| Banana PRODUCT PROFILE: Matooke | | | | |
|---|---|---|---|---|
| Region/Market segment | Trait (economic, sustainability, livelihood) and value | Target trait level | Market Priority | Selection Objective |
| *Highlands of East and Central Africa* | | | | |
| Fresh market and processing | Yield | 30% greater than Mbwazirume variety across a range of soil and management conditions | 1 | Maximize |
| | Table quality (needs regional assessment) | A general acceptability score of at least 4 (on a hedonic scale of 1 to 6), using Mbwazirume as a check (acceptability is tested after cooking as taste, aroma, colour, texture/mouth-feel) | 1 | Reach threshold |
| | Earliness: planting to harvest | 300 to 390 days | 2 | Minimize |
| | Plant stature (girth at 1m/height ratio) | A ratio of at least 0.15 | 2 | Maximize |
| | Plant height | Less than 350 cm | 2 | Minimize |
| | Suckering behavior | 75% follower sucker growth at flowering, 3-4 suckers at flowering | 2 | Optimize |
| | Resistance to black Sigatoka | INSL at flowering of 70% and above | 3 | Reach threshold |
| | Resistance to weevils | 40% resistance higher than that of the moderate resistant check (Kainja) | 2 | Maximize |
| | Resistance to *Radopholus similis and P .goodeyi* | 40% resistance higher than that of the moderate resistant check (Kainja) | 2 | Maximize |
| | Resistance to BXW | Sources of resistance to be identified | 2 | Opportunistic |
| | Bunch orientation | Pendulous score of 1 or 2 | 1 | Opportunistic |
| | Drought tolerance (water productivity)- Needs regional assessment. | Tools to be developed | 3 | Reach threshold |
| | High ProVitA content | Average –Carotene (≥20 µg/ g dry weight) | 2 | Opportunistic |
| | Fusarium | Comparable to resistant check (Calcutta 4) | 1 | Maximize |
| | Resistance to BBTV | Sources of resistance to be identified | 3 | Opportunistic |

| MCHARE PRODUCT PROFILE | | | | |
|---|---|---|---|---|
| Region/Market segment | Trait (economic, sustainability, livelihood) and value | Target trait level | Market Priority | Selection Objective |
| Arusha/Kilimanjaro | | | | |
| Fresh market and processing | Yield | 15% greater than Huti White Bell | 1 | Maximize |
| | Table quality (palatability) | Texture comparable to Mchare laini. Need metrics | 1 | maximize |
| | Bunch compactness | Comparable to Huti White | 2 | maximize |
| | Earliness: planting to harvest (production cycle) | <365 day | 2 | Minimize |
| | Plant stature (girth at 1m/height ratio) | A ratio of at least 0.15 | 2 | Maximize |
| | Plant height | <2.75 m | 1 | Minimize |
| | Suckering behavior | >50% follower sucker growth at harvest | 2 | Maximize |
| | Average Fruit weight | >150g – 200g | 1 | Optimize |
| | Storage duration of fruits (shelf life) | >5 days from harvest until fruit begins to yellows under natural conditions | 2 | Maximize |
| | Resistance to black Sigatoka | INSL at flowering of 70% and above | 2 | Reach threshold |
| | Resistance to weevils | Resistance higher than that of the susceptible check (Huti White) | 2 | Maximize |
| | Resistance to Radopholus similis and P.goodeyi | Resistance higher than that of the susceptible check (Huti White) | 2 | Maximize |
| | Resistance to Fusarium | Comaprable to resistant check (Calcutta 4) | 1 | Maximize |
| | Resistance to BBTV | Sources of resistance to be identified | 3 | Opportunistic |
| | Bunch orientation | Pendulous score of 1 or 2 | 1 | Opportunistic |
| | Fruit parthenocapy and fertility | Parthenocarpic fruit development without seed production | 1 | Maximize |
| | Drought tolerance (water productivity) | Tools to be developed | 3 | Reach threshold |
| | High pVAC content | Average total carotenoid content of 12 ug/g fresh weight at the green stage | 2 | Opportunistic |

## 2.4 Summary of progress in Matooke and Mchare breeding for Years 2-3-4

| Parameter | Target | Actual overall numbers (after 2 years) | Actual overall numbers (after 3 years) | Actual overall numbers (after 4 years) | Expected results for the last year/ General remarks |
|---|---|---|---|---|---|
| **Seed increase in Matooke and Mchare** | 15-20% | 3x-2x = 1,650<br>4x-2x = 113,153<br>Mchare-2x =1508 | 3x-2x = 2680<br>4x-2x = 170,236<br>Mchare-2x =16737 | 3x-2x = 4,918<br>4x-2x = 220,186<br>Mchare-2x = 2984<br><br>The cumulative number of embryos cultured:<br>3x -2x = 2894<br>4x - 2x = 149,341 | Will be achieved |
| **Improved diploids integrated into the Matooke and Mchare breeding pipeline** | 70 | 30 improved diploids are being characterized and already integrated in the NARO-IITA breeding pipeline.<br><br>20 parthenocarpic diploids received from EMBRAPA are under multiplication. | 32 improved diploids are being characterized and already integrated in the NARO-IITA breeding pipeline.<br><br>28 improved diploids have been identified as potential selections from EETs<br><br>20 parthenocarpic diploids received from EMBRAPA were multiplied in vitro and are to be planted at IITA-Sendusu in October 2017.<br><br>62 *banksii* diploids received from ITC and multiplied *in vitro* at NARL. They are weaned for planting at IITA-Sendusu. | 22 diploids resulting from 2x-2x crosses, with a bunch weight of over 5kg, high pollen quantity, and resistance to black Sigatoka have been selected for further evaluation for resistance to weevils and nematodes.<br><br>Another set of more than 100 diploid resulting from 4x-2x and 3x-2x are under evaluation.<br><br>22 parthenocarpic diploids sourced from EMBRAPA are under field characterization.<br><br>62 diploids of *banksii* background sourced from ITC are under field characterization. | Considering the available number of improved diploids being characterized (22) and yet to be characterized (100+22), it most likely that the total number of diploids to be integrated in the NARO-IITA breeding pipeline will exceed 70. |

| | | | | | |
|---|---|---|---|---|---|
| **Matooke hybrid under evaluation in the EET (early evaluation trial)** | 12000 | 3008 hybrids from 4x-2x and 127 hybrids from 3x-2x crosses are in EETs. | 6343 hybrids from 4x-2x crosses<br><br>193 hybrids from 3x-2x are under evaluation in the EETs.<br><br>Over 1000 seedlings generated from 4x-4x crosses from May-October 2017 are in the screen houses at IITA and NARO.<br><br>Over 10,000 hybrid seeds generated during July to September 2017 are undergoing embryo rescue and more seeds are being generated. | 10,210 hybrids from 4x-2x crosses are in EETs.<br><br>328 hybrids from 3x-2x crosses are in EETs. | At this rate we expect to reach the project target. |
| **Matooke hybrids (beyond NARITA 1-26) tested in PYT (preliminary yield trial)** | 95 | 25 potential hybrid selections for advancement to PYTs are already identified from an EET of 930 genotypes. This is an estimated rate of success of selection of 2.7%. | Of the 190 hybrids selected from EETs, 105 are under *in vitro* multiplication for PYT to be planted by April 2018. | We have selected 216 Matooke hybrids from EET for advancement to PYT. 92 were multiplied in vitro and ready for a multilocation preliminary yield trial (once it starts raining) in Sendusu, Mbarara and Hoima. The other 124 Matooke hybrids are multiplied for the second wave in testing. | The target of 95 hybrids to be tested in PYTs is surpassed. |
| **Development of Mchare hybrids** | 2400 seeds for embryo rescue | | 1,650 Mchare seeds are so far generated. | 2042 crosses were made.<br>A total of 542 Mchare hybrids are under evaluation in EETs in Tanzania and Uganda | We had reached the target as we promised seeds but deliver now hybrids for evaluation. |

## 2.5 Sixty-one banana genotypes selected from the second and third early evaluation trials of 957 genotypes established during 2016

| SN. | Genotype Code | PHT | GTH | NSL | YLS | NoS | DTM | BWT | HDS | FL | FC | NF | Remark‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 15/10K-4 | 285 | 39 | 10 | 9 | 3 | 147 | 10 | 11 | 14 | 11 | 97 | **** |
| 2 | 15/10K-5 | 279 | 42 | 11 | 10 | 4 | 152 | 12 | 10 | 13 | 9 | 115 | **** |
| 3 | TC59K-1 | 302 | 44 | 10 | 10 | 3 | 133 | 14 | 12 | 15 | 9 | 105 | **** |
| 4 | TC59K-3 | 286 | 42 | 9 | 8 | 5 | 145 | 13 | 12 | 14 | 10 | 93 | *** |
| 5 | 15/10K-11 | 297 | 47 | 10 | 9 | 6 | 147 | 12 | 10 | 12 | 13 | 96 | **** |
| 6 | 15/40K-5 | 308 | 48 | 12 | 11 | 6 | 120 | 13 | 11 | 16 | 12 | 101 | *** |
| 7 | 10/967K-10 | 265 | 38 | 11 | 9 | 7 | 125 | 10 | 9 | 17 | 11 | 87 | **** |
| 8 | 10/967K-8 | 288 | 43 | 13 | 10 | 3 | 141 | 13 | 10 | 13 | 13 | 111 | **** |
| 9 | TC200120K-26 | 296 | 42 | 10 | 8 | 3 | 138 | 11 | 9 | 12 | 13 | 98 | **** |
| 10 | TC200120K-30 | 312 | 39 | 9 | 9 | 4 | 126 | 9 | 8 | 15 | 10 | 89 | **** |
| 11 | TC200120K-35 | 315 | 46 | 11 | 10 | 5 | 161 | 8 | 11 | 13 | 9 | 109 | **** |
| 12 | TC67K-11 | 274 | 37 | 12 | 12 | 5 | 149 | 14 | 10 | 14 | 10 | 98 | **** |
| 13 | TC20013-1 | 289 | 41 | 15 | 13 | 6 | 145 | 16 | 12 | 14 | 12 | 118 | *** |
| 14 | TC20018K-3 | 210 | 30 | 8 | 6 | 6 | 167 | 6 | 5 | 12 | 11 | 59 | *** |
| 15 | 15/26K-11 | 160 | 30 | 6 | 5 | 3 | 153 | 9 | 6 | 12 | 9 | 75 | *** |
| 16 | 15/18K-1 | 260 | 43 | 9 | 7 | 5 | 134 | 15 | 5 | 13 | 11 | 114 | **** |
| 17 | 10/973K-1 | 250 | 35 | 14 | 10 | 6 | 126 | 12 | 6 | 15 | 11 | 108 | **** |
| 18 | TC20018K-18 | 260 | 40 | 12 | 9 | 7 | 167 | 12 | 6 | 14 | 12 | 89 | *** |
| 19 | 10/900K-3 | 240 | 38 | 13 | 10 | 3 | 155 | 10 | 7 | 13 | 10 | 93 | **** |
| 20 | 10/900K-11 | 240 | 38 | 12 | 6 | 2 | - | 8 | 5 | 12 | 9 | 69 | **** |
| 21 | TC20018K-25 | 280 | 39 | 13 | 11 | 3 | 133 | 8 | 5 | 13 | 12 | 50 | **** |
| 22 | TC20018K-27 | 260 | 45 | 15 | 11 | 8 | 160 | 11 | 7 | 15 | 11 | 124 | **** |
| 23 | TC20018K-33 | 260 | 40 | 15 | 10 | 4 | 155 | 6 | 4 | 14 | 12 | 49 | **** |
| 24 | TC20018K-34 | 270 | 32 | 10 | 7 | 3 | - | 10 | 6 | 15 | 11 | 95 | **** |
| 25 | TC20018K-35 | 250 | 32 | 14 | 12 | 5 | 133 | 6 | 6 | 11 | 9 | 54 | **** |
| 26 | 10/931K-15 | 190 | 41 | 7 | 6 | 8 | 195 | 7 | 7 | 11 | 9 | 97 | **** |

| SN. | Genotype Code | PHT | GTH | NSL | YLS | NoS | DTM | BWT | HDS | FL | FC | NF | Remark‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | 10/931K-17 | 190 | 36 | 10 | 7 | 8 | 202 | 9 | 7 | 13 | 11 | 93 | **** |
| 28 | 10/789K-1 | 170 | 35 | 9 | 7 | 5 | 125 | 8 | 5 | 12 | 10 | 46 | *** |
| 29 | 10/789K-2 | 180 | 31 | 6 | 9 | 2 | 134 | 7 | 7 | 17 | 8 | 69 | **** |
| 30 | 15/45K-1 | 220 | 32 | 10 | 8 | 3 | 176 | 8 | 5 | 16 | 12 | 56 | **** |
| 31 | 10/845K-3 | 231 | 31 | 7 | 9 | 2 | 162 | 6 | 4 | 12 | 11 | 43 | **** |
| 32 | 10/963K-15 | 280 | 43 | 11 | 9 | 7 | 153 | 15 | 7 | 13 | 11 | 180 | *** |
| 33 | 10/963K-18 | 230 | 35 | 7 | 6 | 2 | 151 | 10 | 7 | 13 | 9 | 92 | **** |
| 34 | TC29K-11 | 260 | 35 | 12 | 10 | 4 | 148 | 16 | 8 | 14 | 12 | 145 | **** |
| 35 | 15/18K-20 | 270 | 33 | 8 | 6 | 2 | 160 | 7 | 5 | 12 | 11 | 72 | **** |
| 36 | 10/973K-12 | 210 | 39 | 8 | 7 | 4 | 161 | 7 | 5 | 11 | 10 | 63 | **** |
| 37 | 10/973K-13 | 260 | 32 | 11 | 9 | 4 | 160 | 8 | 6 | 13 | 11 | 86 | **** |
| 38 | TC22K-8 | 240 | 35 | 12 | 8 | 7 | 161 | 8 | 5 | 11 | 10 | 53 | **** |
| 39 | 10/973K-19 | 260 | 35 | 12 | 10 | 4 | 148 | 9 | 6 | 13 | 9 | 83 | **** |
| 40 | 10/817K-2 | 220 | 42 | 12 | 10 | 3 | 167 | 10 | 6 | 13 | 12 | 79 | ***** |
| 41 | 10/931K-30 | 230 | 37 | 12 | 10 | 7 | 155 | 8 | 6 | 12 | 10 | 94 | **** |
| 42 | 10/963K-31 | 240 | 40 | 10 | 8 | 4 | 167 | 8 | 5 | 11 | 9 | 66 | **** |
| 43 | 15/26K-31 | 250 | 33 | 10 | 9 | 4 | 100 | 8 | 6 | 12 | 11 | 83 | **** |
| 44 | 15/18K-32 | 200 | 30 | 11 | 9 | 2 | 173 | 9 | 6 | 13 | 11 | 75 | **** |
| 45 | 10/973K-24 | 220 | 35 | 10 | 10 | 3 | 147 | 9 | 6 | 13 | 12 | 95 | **** |
| 46 | 10/900K-17 | 260 | 35 | 8 | 6 | 2 | 162 | 7 | 6 | 12 | 10 | 75 | **** |
| 47 | 10/922K-7 | 190 | 35 | 12 | 9 | 4 | 162 | 8 | 6 | 12 | 9 | 87 | **** |
| 48 | 10/922K-9 | 220 | 23 | 8 | 6 | 3 | 163 | 8 | 6 | 11 | 9 | 77 | **** |
| 49 | 10/922K-11 | 160 | 30 | 14 | 11 | 3 | 148 | 7 | 5 | 14 | 10 | 63 | *** |
| 50 | 10/963K-42 | 230 | 35 | 14 | 10 | 4 | 160 | 12 | 6 | 13 | 12 | 97 | **** |
| 51 | 10/859K-2 | 120 | 30 | 12 | 10 | 4 | 156 | 6 | 4 | 12 | 10 | 44 | *** |
| 52 | 10/859K-3 | 220 | 35 | 11 | 11 | 5 | 176 | 6 | 5 | 11 | 9 | 49 | *** |
| 53 | 10/922K-12 | 230 | 32 | 11 | 10 | 4 | 161 | 15 | 7 | 15 | 12 | 95 | **** |
| 54 | 10/837K-7 | 160 | 30 | 14 | 12 | 2 | 148 | 8 | 6 | 11 | 10 | 57 | **** |
| 55 | 10/863K-1 | 230 | 35 | 11 | 9 | 3 | 146 | 10 | 7 | 12 | 10 | 94 | **** |
| 56 | 10/863K-3 | 220 | 33 | 11 | 10 | 3 | 145 | 9 | 7 | 14 | 11 | 84 | *** |

| SN. | Genotype Code | PHT | GTH | NSL | YLS | NoS | DTM | BWT | HDS | FL | FC | NF | Remark‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 57 | 10/969K-12 | 210 | 29 | 8 | 6 | 2 | 167 | 5 | 5 | 8 | 9 | 63 | *** |
| 58 | 10/863K-16 | 260 | 39 | 8 | 6 | 7 | 154 | 16 | 8 | 20 | 10 | 104 | **** |
| 59 | 10/842K-1 | 230 | 35 | 12 | 9 | 7 | 148 | 8 | 7 | 12 | 11 | 97 | *** |
| 60 | 10/982K-6 | 245 | 36 | 10 | 9 | 2 | 139 | 9 | 7 | 13 | 10 | 93 | *** |
| 61 | 10/842K-14 | 290 | 40 | 10 | 7 | 4 | 141 | 10 | 12 | 11 | 11 | - | *** |
| **62** | **Mbwazirume-Check** | **263** | **49** | **11.0** | **9** | **3** | **102** | **13** | **6** | **16** | **12.7** | **82** | ***** |
| **Mean** | | **242.3** | **36.8** | **10.7** | **8.8** | **4.2** | **151** | **9.7** | **7** | **13.1** | **10.6** | **86.2** | |
| **Min.** | | **120.0** | **23.0** | **6.0** | **5.0** | **2.0** | **100.0** | **5.0** | **4.0** | **8.0** | **8.0** | **180.0** | |
| **Max.** | | **315.0** | **49.0** | **15.0** | **13.0** | **8.0** | **202.0** | **16.0** | **12.0** | **20.0** | **13.0** | **26.4** | |
| **S.D.** | | **41.8** | **5.2** | **1.8** | **1.8** | **1.8** | **17.2** | **28** | **2.2** | **1.9** | **1.2** | **32.3** | |
| **C.V (%)** | | **17.2** | **14.4** | **20.7** | **20.6** | **41.8** | **13.5** | **29.7** | **31.4** | **14.4** | **11.4** | **28.5** | |

*HT=Plant height (cm), GTH= plant girth (cm), NSL= number of green standing leaves, YLS= youngest leaf spotted at flowering, NoS= number of suckers, DTM=days to bunch maturity, BWT= bunch weight (kg), HDS= number of hands, FL= fruit length, FC= fruit circumference, NF= number of finger/bunch.*

***Remark‡ =*** *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*

## 2.6 Hybrids selected for joint NARO–IITA Multi-location PYT

Ninety-two banana hybrids selected from EETs at NARO (37 hybrids) and IITA (55 hybrids) were multiplied *in vitro* at IITA-Sendusu and are ready for planting. Fields at three diverse sites (Sendusu, Mbarara and Hoima) are ready for planting. Experimental design (10 x10 Triple Lattice) is also finalized. Planting is expected as soon as it rains.

**Matooke hybrids from IITA and NARO ready planting in PYTs in Sendusu, Mbarara and Hoima**

| Sn. | Genotype | Origin | Female parent | Male parent |
|---|---|---|---|---|
| 1 | 27879S-2 | Sendusu | 1438k-1 | Cv. Rose |
| 2 | 28682S-16 | Sendusu | 917K-2 | Malaccensis 250 |
| 3 | 28682S-17 | Sendusu | 917K-2 | Malaccensis 250 |
| 4 | 29673S-23 | Sendusu | 917K-2 | Cv. Rose |
| 5 | 29792S-2 | Sendusu | 917K-2 | Cv. Rose |
| 6 | 29792S-6 | Sendusu | 917K-2 | Cv. Rose |
| 7 | 29809S-16 | Sendusu | 5610S-1 | 917K-2 |
| 8 | 29809S-6 | Sendusu | 5610S-1 | 917K-2 |
| 9 | 31123S-10 | Sendusu | 1438k-1 | SH 3217 |
| 10 | 31580S-2 | Sendusu | 917K-2 | SH 3362 |
| 11 | 31949S-3 | Sendusu | 917K-2 | SH 3217 |
| 12 | 32131S-1 | Sendusu | 376K-7 | Malaccensis 250 |
| 13 | 32171S-10 | Sendusu | 917k-2 | SH 3362 |
| 14 | 32171S-2 | Sendusu | 917k-2 | SH 3362 |
| 15 | 32173S-1 | Sendusu | 917k-2 | SH 3142 |
| 16 | 32197S-10 | Sendusu | 917k-2 | Cv. Rose |
| 17 | 32219S-2 | Sendusu | 917k-2 | SH 3217 |
| 18 | 32219S-9 | Sendusu | 917k-2 | SH 3217 |
| 19 | 32290S-1 | Sendusu | 1201K-1 | Cv. Rose |
| 20 | 32410S-7 | Sendusu | 401k-1 | SH 3362 |
| 21 | 32455S-30 | Sendusu | Nfuuka | Cv. Rose |
| 22 | 32730S-1 | Sendusu | 1201K-1 | Cv. Rose |
| 23 | 32730S-24 | Sendusu | 1201K-1 | Cv. Rose |
| 24 | 32764S-1 | Sendusu | 917k-2 | SH 3362 |
| 25 | 32764S-2 | Sendusu | 917k-2 | SH 3362 |
| 26 | 32932S-1 | Sendusu | 917k-2 | 9128-3 |
| 27 | 33372S-1 | Sendusu | 917k-2 | 9128-3 |
| 28 | 33388S-5 | Sendusu | 917k-2 | SH 3142 |
| 29 | 34597S-2 | Sendusu | 1201k-1 | SH 3217 |
| 30 | 34607S-45 | Sendusu | 376k-7 | SH 3217 |
| 31 | 34895S-122 | Sendusu | 1201K-1 | SH 3217 |
| 32 | 35192S-19 | Sendusu | 917k-2 | 9128-3 |
| 33 | 35536S-32 | Sendusu | Tereza | 7197-2 |
| 34 | 35140S-19 | Sendusu | 660k-1 | SH 3217 |
| 35 | 35409S-21 | Sendusu | 660k-1 | SH 3217 |
| 36 | 36505S-47 | Sendusu | 1201K-1 | SH 3362 |
| 37 | 33857S-29 | Sendusu | 1201k-1 | Cv. Rose |
| 38 | 35947s-99 | Sendusu | 1438k-1 | SH 3362 |
| 39 | 34147S-24 | Sendusu | 376k-7 | SH 3362 |
| 40 | 35065S-10 | Sendusu | 222k-1 | 9128-3 |
| 41 | 35827S-88 | Sendusu | 1201k-1 | 7197-2 |
| 42 | 35836S-32 | Sendusu | 1201k-1 | 5265-1 |

| Sn. | Genotype | Origin | Female parent | Male parent |
|---|---|---|---|---|
| 47 | 35502S-74 | Sendusu | 401k-1 | SH 3217 |
| 48 | 35502S-99 | Sendusu | 401k-1 | SH 3217 |
| 49 | 33709S-11 | Sendusu | 917K-2 | Cv. Rose |
| 50 | 34186S-3 | Sendusu | 917k-2 | 5265-1 |
| 51 | 34285S-127 | Sendusu | 917k-2 | Kokopo |
| 52 | 34536S-3 | Sendusu | 1201k-1 | SH 3217 |
| 53 | 34536S-33 | Sendusu | 1201k-1 | SH 3217 |
| 54 | 33398S-9 | Sendusu | 1201K-1 | SH 3362 |
| 55 | 33455S-23 | Sendusu | 917k-2 | Malaccensis 250 |
| 56 | 10/669-32 | Kawanda | 1411K-1 | 402 |
| 57 | 10/671-2 | Kawanda | 376K | 402 |
| 58 | 10/671-7 | Kawanda | 376K | 402 |
| 59 | 10/672-2 | Kawanda | 401K | 402 |
| 60 | 10/669-21 | Kawanda | 1411K-1 | 402 |
| 61 | 10/579-3 | Kawanda | 1411K-1 | 2905 |
| 62 | 10/672-5 | Kawanda | 401K | 402 |
| 63 | 10/601-2 | Kawanda | 1154K-1 | 402 |
| 64 | 10/671-3 | Kawanda | 376K | 402 |
| 65 | 10/772-19 | Kawanda | 401K | 402 |
| 66 | 10/671-5 | Kawanda | 376K | 402 |
| 67 | 10/574-18 | Kawanda | 199K-1 | 402 |
| 68 | 10/843-20 | Kawanda | 222K-1 | 402 |
| 69 | 10/595-1 | Kawanda | 199K-3 | 402 |
| 70 | 10/669-38 | Kawanda | 1411K-1 | 402 |
| 71 | 10/672-8 | Kawanda | 401K | 402 |
| 72 | 10/669-3 | Kawanda | 1411K-1 | 402 |
| 73 | 10/579-1 | Kawanda | 1411K-1 | 2905 |
| 74 | 10/579-4 | Kawanda | 1411K-1 | 2905 |
| 75 | 10/700-14 | Kawanda | 660K | 716 |
| 76 | 10/843-13 | Kawanda | 222K-1 | 402 |
| 77 | 10/669-62 | Kawanda | 1411K-1 | 402 |
| 78 | 10/601-1 | Kawanda | 1154K-1 | 402 |
| 79 | 10/689-7 | Kawanda | 660K | 2905 |
| 80 | 10/569-15 | Kawanda | 365K-1 | 402 |
| 81 | 10/687-12 | Kawanda | 660K | 716 |
| 82 | 10/669-24 | Kawanda | 1411K-1 | 402 |
| 83 | 10/585-8 | Kawanda | 401K-1 | 402 |
| 84 | 10/672-1 | Kawanda | 401K | 402 |
| 85 | 10/843-4 | Kawanda | 222K-1 | 402 |
| 86 | 10/579-2 | Kawanda | 1411K-1 | 2905 |
| 87 | 10/585-6 | Kawanda | 401K-1 | 402 |
| 88 | 10/601-7 | Kawanda | 1154K-1 | 402 |

| | | | | |
|---|---|---|---|---|
| 43 | 35827S-3 | Sendusu | 1201k-1 | 7197-2 |
| 44 | 32969S-3 | Sendusu | 376k-7 | SH 3217 |
| 45 | 33875S-6 | Sendusu | 660k-1 | 5610S-1 |
| 46 | 35350S-1 | Sendusu | 660k-1 | SH 3217 |

| | | | | |
|---|---|---|---|---|
| 89 | 10/689-5 | Kawanda | 660K | 2905 |
| 90 | 10/700-20 | Kawanda | 660K | 716 |
| 91 | 10/700-4 | Kawanda | 660K | 716 |
| 92 | 10/702-2 | Kawanda | 199K-3 | 2905 |

## 2.7 Improved diploids sourced from EMBRAPA being characterized in Uganda

Twenty-two improved diploids sourced from EMBRAPA were planted in a field trial at IITA-Sendusu to evaluate their agronomic performance and resistance to Foc race 1, black Sigatoka, weevils and nematodes. The same diploids were sent to Arusha and Ibadan in Year 3 where they are currently under *in vitro* multiplication. The ploidy of these genotypes is yet to be confirmed. Their evaluation for Foc Race 1 resistance will be done using the most prevalent VCG in Uganda. The plants have started flowering and data collection on plant vigor, suckering behavior and resistance to black Sigatoka is ongoing. Yield data will be collected at bunch harvest.

Improved diploids from EMBRAPA planted at Sendusu for field evaluation

| Genotype | Pedigree |
|---|---|
| BMPG 043/001016-01 | Borneo x Guyod |
| BMPG 044/003004-02 | Calcutta 4 x Madang |
| BMPG 045/003023-03 | Calcutta 4 x S/Nº2 |
| BMPG 046/003037-02 | Calcutta 4 x Galeo |
| BMPG 048/013004-06 | Malaccensis x Madang |
| BMPG 050/028003-01 | Tuugia x Calcutta 4 |
| BMPG 051/041054-04 | 003004-01 (Calcutta 4 x Madang) x 001004-01 (Borneo x Madang) |
| BMPG 052/041054-08 | 003004-01 (Calcutta 4 x Madang) x 001004-01 (Borneo x Madang) |
| BMPG 055/042023-06 | M53 [(Malaccensis – Kedah x Banksii- Samoa) x (Banksii - Samoa)] x Cultivar sem Nome Nº 2 |
| BMPG 056/042049-04 | M53 [(Malaccensis – Kedah x Banksii - Samoa) x (Banksii - Samoa)] x M48 |
| BMPG 057/042052-03 | M53 [(Malaccensis – Kedah x Banksii - Samoa) x (Banksii - Samoa)] x Kumburgh |
| BMPG 060/042079-13 | M53 [(Malaccensis – Kedah x Banksii- Samoa) x (Banksii - Samoa)] x 028003-01 (Tuugia x Calcutta 4 |
| BMPG 061/042085-02 | M53 [(Malaccensis – Kedah x Banksii- Samoa) x (Banksii - Samoa)] x 015003-01 (Madu x Calcutta 4) |
| BMPG 062/050012-02 | M61 x Lidi |
| BMPG 063/058054-03 | 003005–01 (Calcutta 4 x Pahang) x 001004–01 (Borneo x Madang) |
| BMPG 064/073041-01 | Khai x 003004–01 (Calcutta 4 x Madang) |
| BMPG 065/073041-01 | Khai x 003004–01 (Calcutta 4 x Madang) |
| BMPG 071/091079-03 | 01016-01 (Borneo x Guyod) x 028003 (Tuugia x Calcutta 4) |
| BMPG 072/091094-04 | 001016–01 (Borneo x Guyod) x SH3263 |
| BMPG 075/TH-0301 | Terrinha x Calcutta 4 |
| BMPG 099/017041-01 | Jari Buaya x 003004–01 (Calcutta 4 x Madang) |
| BMPG 100/086079-12 | 003037–02 (Calcutta 4 x Galeo) x 028003–01 (Tuugia x Calcutta 4) |
| BMPG 066/086079-09 | 003037–02 (Calcutta 4 x Galeo) x 028003–01 (Tuugia x Calcutta 4) |

## 2.8 Twenty-two diploid hybrids selected from early evaluation trials at NARO-Kawanda for further evaluation for agronomic traits, pollen fertility and resistance to black Sigatoka, weevils and nematodes

| No | Picture | Diploid name/ code | Pedigree | Plant height (cm) | BWT (Kg) | #Clusters | Response to black Sigatoka | Pollen quantity |
|---|---|---|---|---|---|---|---|---|
| 1 |  | DC111-40 | 3142K-2 x 201017K | 230 | 7.8 | 8.0 | Resistant | Medium |
| 2 |  | DC111-43 | 3142K-2 x 201017K | 150 | 5.4 | 7.0 | Resistant | Medium |
| 3 |  | DC111-44 | 3142K-2 x 201017K | 230 | 6.6 | 7 | HR | High |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4. |  | DC111-35 | 3142K-2 x 201017K | 160 | 4.5 | 6.0 | Resistant | Medium |
| 5 |  | DC111-22 | 3142K-2 x 201017K | 200 | 6.5 | 7.0 | Resistant | Medium |
| 6 |  | DC111-23 | 3142K-2 x 201017K | 205 | 5.2 | 6.0 | Resistant | Medium |
| 7 |  | DC111-24 | 3142K-2 x 201017K | 210 | 5.6 | 6 | Highly Resistant | Medium |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 |  | DC111-25 | 3142K-2 x 201017K | 220 | 9.8 | 9.0 | Highly Resistant | Medium |
| 9 |  | AG93-3 | 716x2905 | 240 | 7.1 | 8.0 | Highly Resistant | High |
| 11 |  | AG93-8 | 716x2905 | 260 | 6.8 | 8.0 | Highly Resistant | High |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 |  AG50-2 | 2905 x 201087K-4 | 210 | 8.3 | 9.0 | Resistant | Medium |
| 13 |  AG50-3 | 2905 x 201087K-4 | 245 | 7.1 | 8.0 | Highly Resistant | Medium |
| 14 |  AG50-4 | 2905 x 201087K-4 | 235 | 9.5 | 10 | Highly Resistant | Medium |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 16 |  | AG127-5 | 716x2905 | | 6.3 | 7.0 | Highly Resistant | High |
| 17 |  | AG02-8 | TMB2x8075-7 xTMB2x8075-7 | 280 | 7.2 | 8.0 | Highly Resistant | High |
| 18 |  | 14/991-2 | 28K-2 x TMB2x8075-7 | 235 | 6.2 | 7 | Highly Resistant | High |
| 19 |  | 14/991-6 | 28K-2 x TMB2x8075-7 | 170 | 7.5 | 8 | Resistant | High |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 20 |  | 14/991-8 | 28K-2 x TMB2x8075-7 | 230 | 9.3 | 10 | Resistant | Medium |
| 21 |  | 14/939-3 | 716 x 81K-2 | 240 | 7.1 | 9.0 | Highly Resistant | High |
| 22 |  | 14/991-11 | 28K-2 x TMB2x8075-7 | 235 | 6.2 | 8.0 | Highly Resistant | High |

## 2.9 Ploidy analysis of some EET hybrids

The International Institute of Tropical Agriculture in Uganda and NARO analyzed some of their hybrids in EETs in preparation for the harmonization of protocols for establishing trials as agreed on by the two institutions. Ploidy analysis was envisioned enable appropriate evaluation of genotypes of the same ploidy and purpose. During the fourth year of this project, IITA has analyzed 2117 hybrids (Fig. 1) while NARO, has analyzed 1288 (Fig. 2).

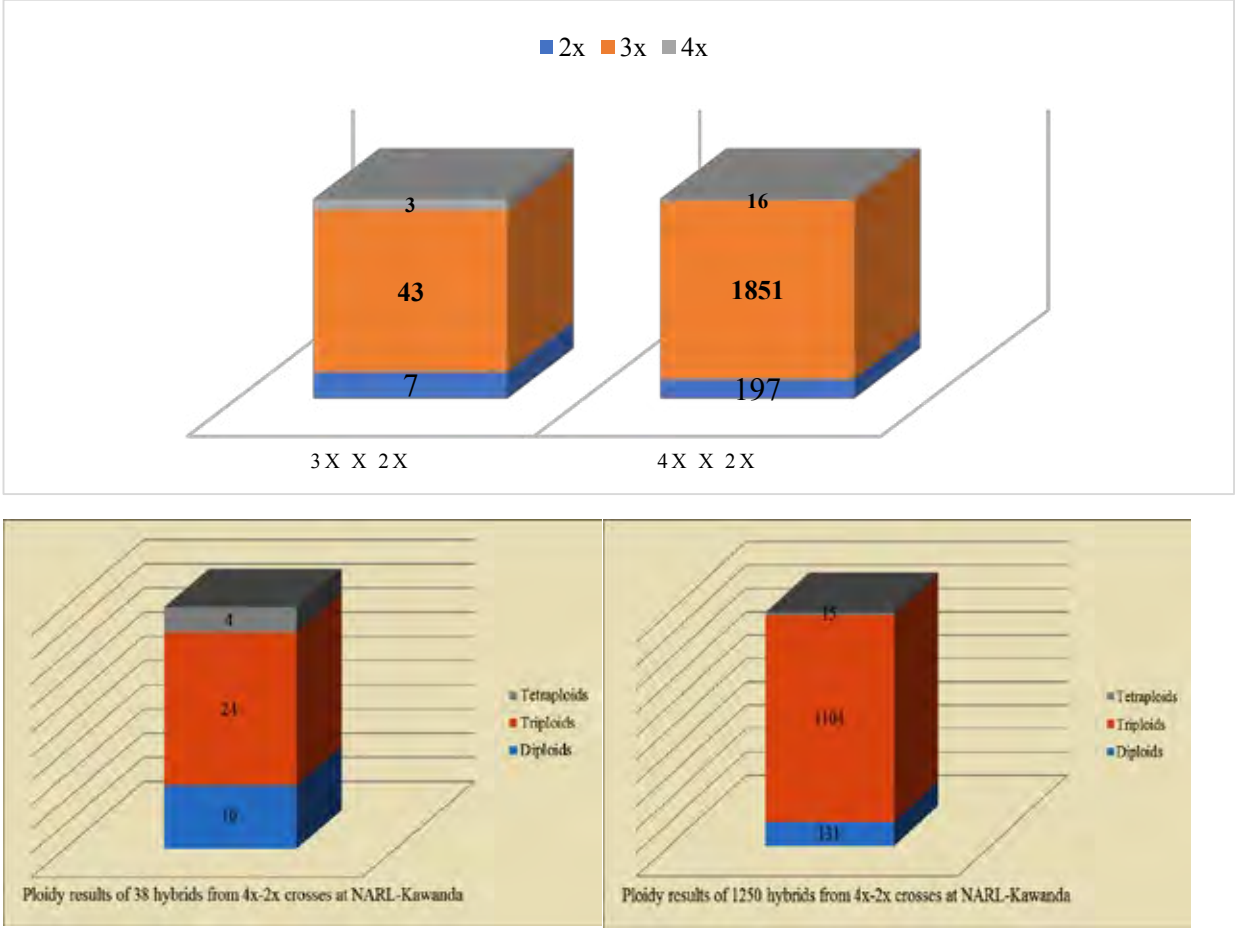



**Fig. 1**: Ploidy results of 3x-2x and 4x-2x crosses done at IITA-Sendusu (left) and Ploidy results of 3x-2x and 4x-2x crosses done at NARL-Kawanda (right)

Contrary to the common belief that crossing 3x-2x gives more tetraploids than triploids and diploids, these results revealed that 3x-2x crosses generate more triploid hybrids than tetraploids and diploids. This implies that focusing more attention on making many 3x-2x crosses could result in generation of many triploid hybrids, thus shortening the breeding cycle through avoiding the step of making 4x-2x crosses. Besides, one would get more “tookeness” in making direct crosses of 3x-2x. Moreover, the resulting diploids could be more useful in generating 3x hybrids with more of matooke background.

## 2.10 Development of triploids from triploids crossed with diploids

Tereza (3x) x 8075-7 (2x)

Primary triploid:
28776S-2 (3x)

Tereza (3x) x 7197-2 (2x)

Primary triploid:
25356S-1 (3x)

## 2.11 Pollen germination by sugars and nectar

Mean pollen germination percentages after 3 hour incubation of pollen growth medium prepared from different sugars at varying concentrations

| Concentration | Glucose | Sucrose | Fructose | Glucose + Fructose | Glucose + Fructose + Sucrose |
|---|---|---|---|---|---|
| 1% | 47.2ab | 25.5de | 33.9c | 41.1b | 31.9cd |
| 3% | 48.9a | 22.4ef | 22.3ef | 30.3cd | 28.7cde |
| 5% | 41.4b | 13.6ghi | 15.9fgh | 13.1ghi | 16.9fg |
| 10% | 13.7ghi | 4.8jkl | 5.3jkl | 10.7ghij | 14.4ghi |
| 15% | 13.0ghi | 2.4l | 3.5kl | 3.0l | 9.9hijk |
| 20% | 5.6jkl | 1.3l | 5.2jkl | 2.3l | 7.8ijkl |
| **Mean** | **28.3A** | **11.7B** | **14.3B** | **16.8B** | **18.3B** |

Means with different letters are statistically different at P < 0.001

Mean pollen germination percentages after 3 hours of incubation of pollen growth medium prepared from 3% glucose and diluted banana nectar of improved diploid TMB2X8075-7 and EAHB cv Tereza on ten banana genotypes with 2 repetitions

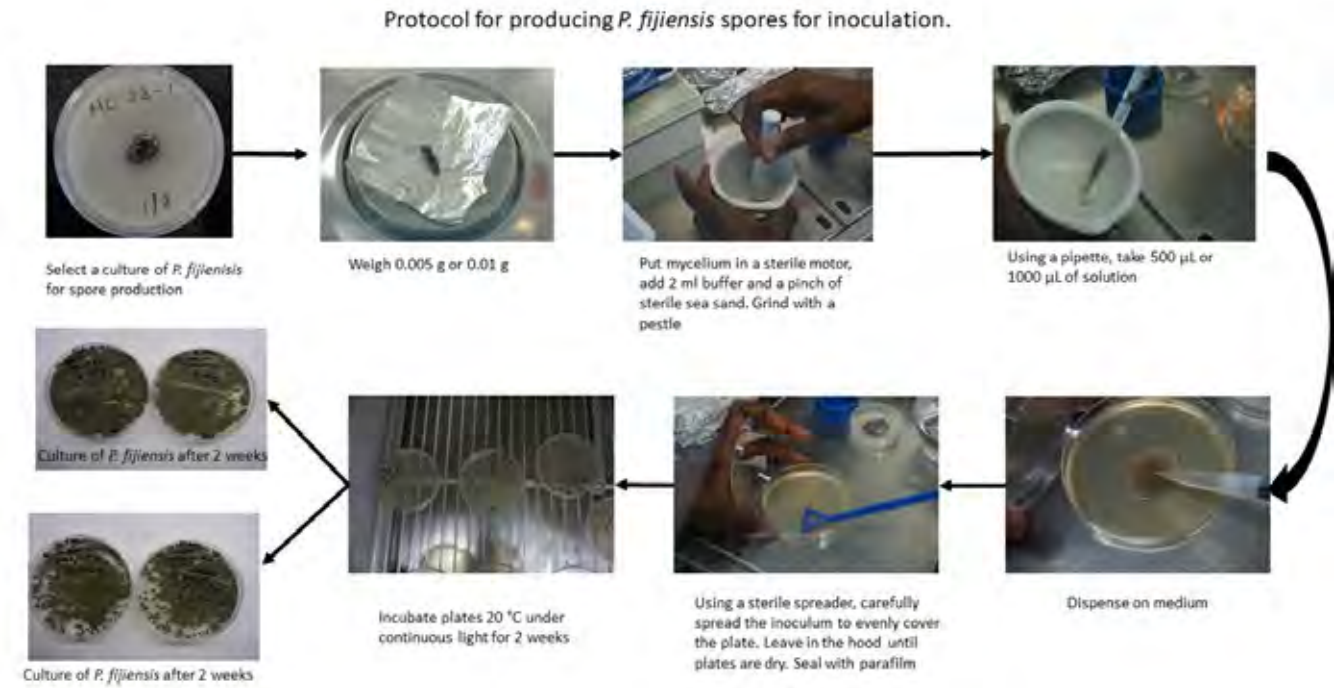
| Genotype | Genome / Ploidy | Use | 3% Glucose | Nectar - 8075 | Nectar – Tereza | Genotype mean |
|---|---|---|---|---|---|---|
| Calcutta 4 | AA | Wild | 39.5 | 37.2 | 24.5 | 33.7bc |
| Zebrina GF | AA | Breeding | 46.3 | 44.6 | 27.4 | 39.4ab |
| TMB2X8075-7 | AA | Breeding | 57.2 | 56.9 | 21.7 | 45.3a |
| 1119 | AA | Breeding | 31.7 | 45.6 | 37.8 | 38.4ab |
| Enzirabahima | AAA | Matooke | 26.1 | 20.7 | 29.9 | 25.5cd |
| Tereza | AAA | Matooke | 22.3 | 13.3 | 23.2 | 19.6d |
| Namwezi | AAA | Matooke | 23.9 | 22.8 | 16.3 | 21.0d |
| 365K-1 | AAAA | Breeding | 53.7 | 34.5 | 14.1 | 34.1bc |
| 376K-1 | AAAA | Breeding | 45.3 | 26.8 | 21.6 | 31.2bc |
| 401K-1 | AAAA | Breeding | 53.0 | 31.6 | 22.7 | 35.8b |
| **Mean** | | | **39.9A** | **33.4AB** | **23.9B** | |

## 2.12 Some statistics related to pictures uploaded onto MusaBase

| | Total no. pictures | Total no. cultivars | Duplicate cultivars | Average number of characteristics per cultivar | Total size of pictures | Cultivars with ITC codes |
|---|---|---|---|---|---|---|
| All | 2690 | 185 | 8 | | 459Mb | 127 |
| Arusha | 867 | 58 | 0 | 15 | 163Mb | 54 |
| Ibadan | 914 | 66 | 0 | 14 | 96Mb | 56 |
| Sendusu | 909 | 69 | 0 | 13 | 201Mb | 17 |

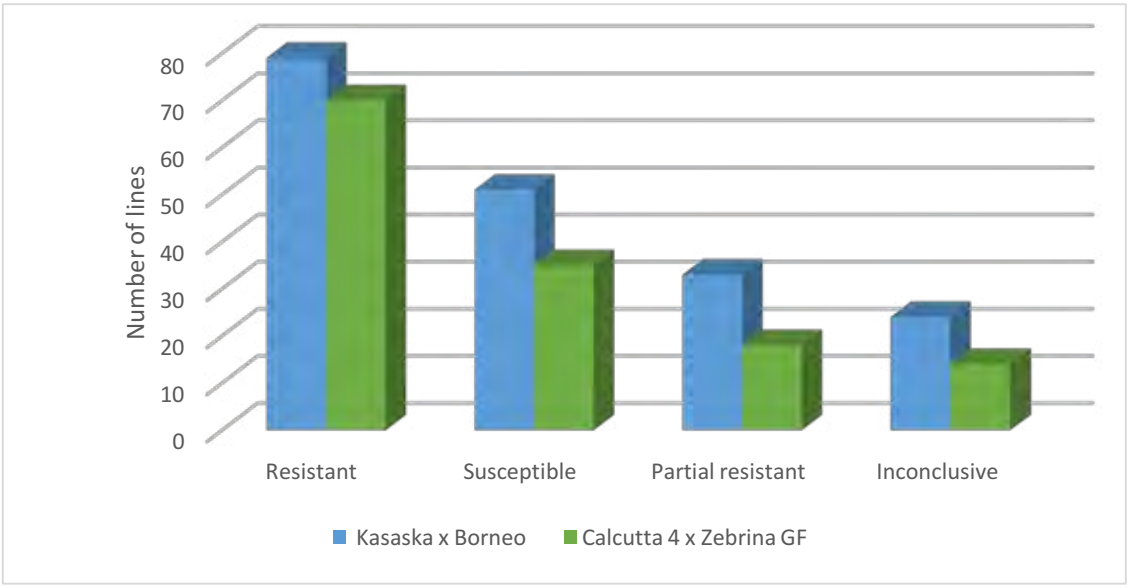
# 3. Work Package 2

## 3.1 Protocol for producing *Pseudocercospora fijiensis* spores for inoculation



# 4. Work Package 3

## 4.1 Host response to *Radopholus similis* for Kasaska x Borneo and Calcutta 4 x Zebrina GF hybrids



## 4.2 Summary of current phenotyping progress

**Summary of current phenotyping progress on a training population of 307 hybrids and parents from fields under different management in Sendusu and Mbarara, Uganda, and from 200 genotypes selected from early evaluation trials at Sendusu**

| Field | Cycle 1 | | Cycle 2 | | Cycle 3 | |
|---|---|---|---|---|---|---|
| | Flowering | Harvesting | Flowering | Harvesting | Flowering | Harvesting |
| GS Training Population LIM - Sendusu | 100% | 98% | 94% | 89% | 73% | 68% |
| GS Training Population HIM - Sendusu | 100% | 96% | 97% | 96% | 93% | 87% |
| GS Training Population HIM - Mbarara | 100% | 96% | 91% | 85% | 72% | 65% |
| Validation (200 genotypes, Sendusu) | 100% | 100% | 80% | 63% | 43% | 18% |

LIM – low input management
HIM – high input management

## 4.3 Comparison of genomic prediction models

**Comparison of average correlation for five-fold cross validations between the predicted and observed phenotypes across models fitted with data from low input management (LIM), high input management fields (HIM) as published in Nyine et al. (2018) and a combination of the two fields with data from cycles 1, 2 and 3 (LIM_HIM_C123)**

| Trait category | Traits | BRR | | | Bayes A | | | BayesB | | | RKHS_M | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LIM | HIM | LIM_HIM_C123 | LIM | HIM | LIM_HIM_C123 | LIM | HIM | LIM_HIM_C123 | LIM | HIM | LIM_HIM_C123 |
| Plant stature | Plant height | 0.54 | 0.46 | 0.20 | 0.54 | 0.45 | 0.53 | 0.54 | 0.44 | 0.49 | 0.54 | 0.45 | 0.49 |
| | Plant girth | 0.60 | 0.52 | 0.54 | 0.60 | 0.51 | 0.52 | 0.60 | 0.52 | 0.50 | 0.60 | 0.51 | 0.50 |
| Suckering behaviour | Total suckers | 0.16 | 0.17 | **0.33** | 0.17 | 0.20 | **0.39** | 0.16 | 0.19 | **0.31** | 0.17 | 0.18 | **0.39** |
| | Height of tallest sucker at flowering | 0.28 | 0.18 | **0.41** | 0.27 | 0.18 | **0.38** | 0.27 | 0.20 | **0.37** | 0.28 | 0.19 | **0.33** |
| | Height of tallest sucker at harvesting | 0.27 | 0.26 | **0.43** | 0.28 | 0.25 | **0.41** | 0.28 | 0.24 | **0.38** | 0.26 | 0.26 | **0.38** |
| Disease resistance | Number of standing leaves at flowering | 0.36 | 0.42 | **0.45** | 0.37 | 0.42 | 0.29 | 0.43 | 0.40 | **0.48** | 0.37 | 0.41 | **0.44** |
| | Index of non-spotted leaves | 0.35 | 0.42 | **0.54** | 0.34 | 0.43 | **0.56** | 0.34 | 0.43 | **0.55** | 0.35 | 0.42 | **0.57** |
| Yield | Days to fruit maturity | 0.44 | 0.42 | **0.51** | 0.44 | 0.42 | 0.47 | 0.44 | 0.42 | **0.50** | 0.44 | 0.42 | **0.51** |
| | Bunch weight | 0.63 | 0.61 | **0.64** | 0.62 | 0.62 | 0.60 | 0.64 | 0.62 | 0.61 | 0.61 | 0.61 | 0.60 |
| | Number of hands | 0.60 | 0.62 | 0.58 | 0.59 | 0.63 | 0.58 | 0.60 | 0.62 | 0.58 | 0.59 | 0.62 | 0.58 |
| | Number of fruits | 0.47 | 0.51 | **0.55** | 0.47 | 0.52 | **0.55** | 0.47 | 0.52 | **0.55** | 0.45 | 0.51 | **0.55** |
| Fruit filling | Fruit length | 0.66 | 0.65 | 0.65 | 0.66 | 0.66 | **0.66** | 0.66 | 0.65 | 0.65 | 0.66 | 0.65 | **0.65** |
| | Fruit circumference | 0.67 | 0.66 | 0.65 | 0.67 | 0.66 | **0.67** | 0.70 | 0.69 | 0.64 | 0.65 | 0.66 | 0.64 |
| | Fruit diameter | 0.67 | 0.63 | 0.63 | 0.66 | 0.67 | 0.65 | 0.70 | 0.71 | 0.64 | 0.65 | 0.67 | 0.63 |
| | Pulp diameter | 0.67 | 0.68 | 0.54 | 0.66 | 0.68 | 0.66 | 0.70 | 0.72 | 0.63 | 0.65 | 0.67 | 0.65 |

**Bold:** prediction accuracy improved by the addition of cycle 3 data and the combination of the two fields
Underlined: the best prediction considering the new analysis (addition of cycle 3 data and combination of the two fields, taking into account GxE

## 4.4 Genotyping IITA banana landraces and hybrids in support of banana breeding

Materials genotyped:

1) 'Kokopo (ITC1243) x Monyet (ITC1179)' population from Sendusu/Uganda – 172 individuals
2) 'Calcutta4 (ITC0249) x Zebrina GF (ITC0966)' population from Sendusu/Uganda – 73 individuals
3) Mchare/Muraru/Mlali accessions from Arusha/Tanzania – 32 individuals
4) 'Paliama x Borneo' population from Arusha/Tanzania – 70 individuals
5) 'Calcutta4 (ITC0249) x Pisang Lilin' population from Sendusu/Uganda – 131 individuals
6) Samples received from Delphine Amah (Ibadan/Nigeria)– 18 individuals
7) 'Kasaska x Borneo' population from Sendusu/Uganda (weevil resistance) – 189 individuals

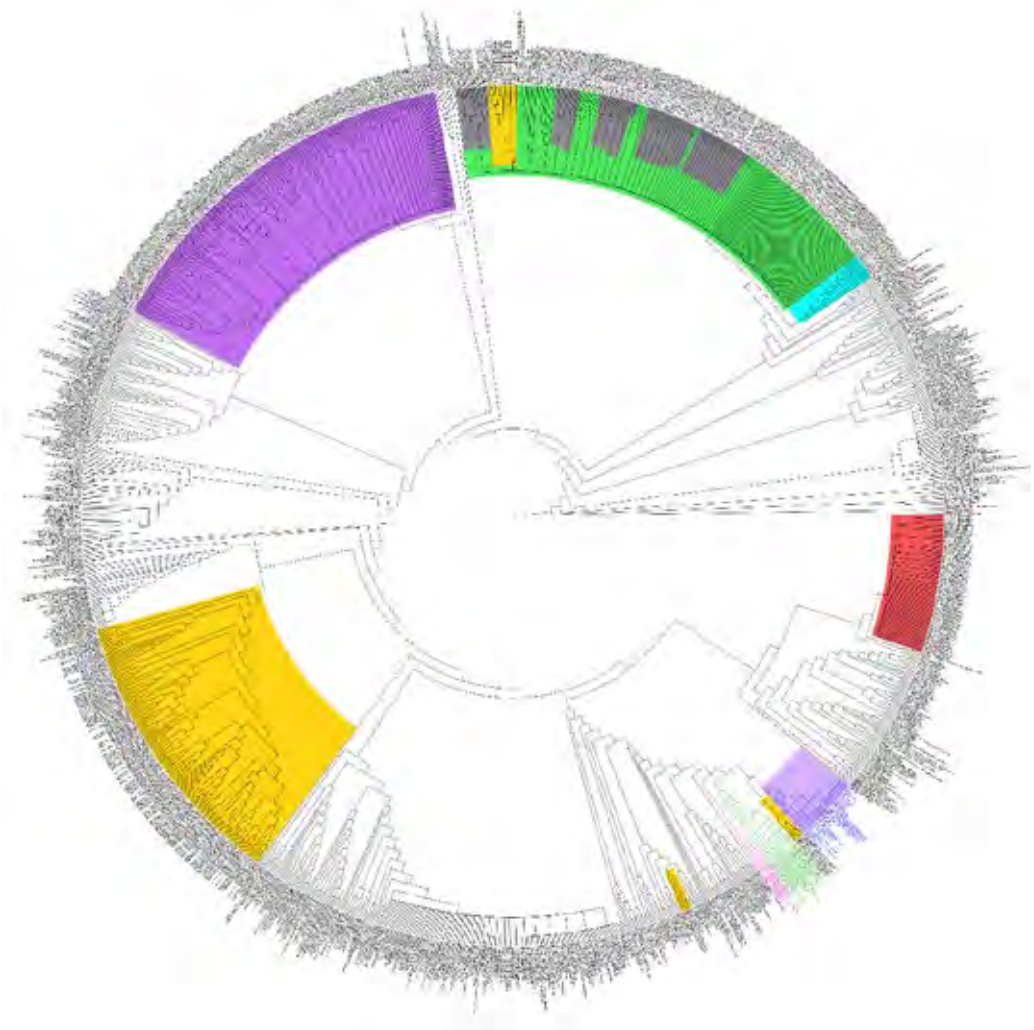


Figure: Neighbor-Joining tree constructed from SSR data of the core set and the analyzed accessions

Description of the clades:

1) The clade as well as taxons of Mchare/Muraru/Mlali accessions are labeled in blue
2) Clade of Gros Michel cultivars is labeled in pink and clade of Cavendish cultivars is labeled in green
3) Clade of Kokopo x Monyet population is highlighted in yellow
4) Clade of Calcutta4 x Zebrina GF population is highlighted in green
5) Clade of Paliama x Borneo population is highlighted in red
6) Clade of Calcutta4 x Pisang Lilin population is highlighted in grey
7) Clade of Kasaska x Borneo population is highlighted in violet
8) Clade of samples received from Delphine Amah is highlighted in light blue

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

| Type of material | Location | Number of genotypes |
|---|---|---|
| NARITA hybrids | Sendusu | 23 |
| PYT | Sendusu | 97 |
| Heterosis | Sendusu | 31 |
| 2X, 3X, 4X PARENTS | Sendusu | 18 |
| Collection | Sendusu | 72 |
| PITAs & BITAs | Sendusu | 36 |
| Cacutta 4 X Zebrina GF | Sendusu | 157 |
| BS experiment | Sendusu | 9 |
| Calcutta 4 x P. Lilin | Sendusu | 349 |
| Training population | Sendusu | 231 |
| EET 22 | Sendusu | 294 |
| EET 23 | Sendusu | 533 |
| EET 24 | Sendusu | 141 |
| EET 25 | Sendusu | 157 |
| EET 26 | Sendusu | 372 |
| EET 27 | Sendusu | 444 |
| EET 28 | Sendusu | 265 |
| EET 13 | Kawanda | 255 |
| EET 15 | Kawanda | 164 |
| Paliama x Borneo | Arusha | 170 |
| GWAS panel/collection/pollination | Arusha | 21 |
| Other (diversity study, controls for Foc, nematode screening) | Arusha | 16 |
| 2x EMBRAPA | Sendusu | 23 |
| ITC banksii's | Sendusu | 34 |
| New 4x Sendusu | Sendusu | 5 |
| **Total** | | **3917** |

# 5. Work Package 4
## 5.1 Protocol for Sensory Evaluations

*Protocol for the sensory evaluations of NARITAs by farmers in the 5 field sites*

Marimo P, Nowakunda K

**ntroduction:** When new crop varieties are developed, it is imperative to assess their acceptability by end users before release. One method of assessing end user acceptability is through sensory evaluations (also referred to as organoleptic or acceptability tests). During these evaluations, end users characterize the traits/attributes/characteristics that they like and dislike about the new varieties and products from these varieties. Comparisons are often made with the traditional local varieties which can give an indication of the potential for consumer acceptability of the new hybrids. Evaluations provide valuable information that can help inform future banana breeding programs (Dadzie and Orchard 2007).

NARO and IITA jointly developed 27 banana hybrids for food and juice (referred to as NARITAs). The NARITAs are currently planted in 5 research sites in different agro-ecological zones in Uganda and Tanzania (Mbarara and Kawanda in Uganda; Kilimanjaro, Maruku and Mitalula in Tanzania). Consumer acceptability tests with farmers will be conducted in the 5 sites to assess sensory parameters and perceptions of the NARITAs before baby trials and subsequent varietal release. This document highlights the procedures that will be used to conduct the evaluations. The protocol is based on a comprehensive literature review and discussions with NARO, IITA and ARI partners who have experience conducting sensory evaluations.

**ites:** Evaluations will be conducted in all the 5 sites where on station trials for NARITAs are established Kawanda, Mbarara, Maruku, Mitalula and Kilimanjaro). The sites are in different agroecological zones in Uganda and Tanzania. A pilot will be conducted at the Kawanda field site before implementing the protocol in the other sites.

**Number of NARITAS in each of the sites:** The total number of NARITAs that will be evaluated in each of the sites is shown in the table and range from 17-21.

| | Tot no. | NARITAs (shaded in blue are in all sites) | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| anda* | 17 | 2 | 4 | 6 | 7 | 8 | | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | | | 21 | | 23 | 24 | | | | |
| rara* | 17 | 2 | 4 | 6 | 7 | 8 | | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | | | 21 | | 23 | 24 | | | | |
| ba | 19 | 2 | 4 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | | 18 | 19 | 20 | 21 | 22 | 23 | | | | | 27 |
| lula | 21 | 2 | 4 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | | 18 | 19 | 20 | 21 | 22 | 23 | | 25 | | | |
| anjaro | 21 | 2 | 4 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | | 18 | 19 | 20 | 21 | 22 | 23 | | 25 | 26 | | |

19 has one plant each in Kawanda and Mbarara so will not be evaluated

**Number of farmers:** 80-120 (to cater for attrition). Ideally 30 older women, 30 older men, 30 youth women and 30 youth men. We want to ensure that at least 60 of the farmers evaluate **all** the NARITA hybrids in a site.

**Sampling method:** Random selection of male and female farmers (including youth) in the villages surrounding the field trial site will be done. Given (1) the logistical challenges of arranging to bring farmers from the baseline surveys on station as some are quite far, (2) the different maturity times for the cultivars 3) that we want to ensure males, females and youth participate freely and do not spend too many days away from home and (4) the fact that during baby trials sensory evaluations will be conducted with numerous farmers from the baseline villages, it was decided that for this initial stage its best to do

evaluations with farmers living within close proximity of the trials who can be called anytime and are not too far from home. Also, the assumption is that for sensory tests, ratings by farmers from the baseline villages and those in areas near the sites will be the same. As mentioned before, a participating farmer should test all the cultivars on site otherwise we will not be able to do meaningful comparisons during data analysis or provide a convincing justification that errors are minimized if different farmers participate all the time. The target is to find a period where at least 10 NARITA cultivars are mature at the same time so that less time is spent in a site. The ideal plan is to do evaluations in a space of 1- 2 weeks (5 or 3 times each week). Extension agents, data collectors and other technical staff in the study areas will help with mobilization and/or assessments.

Youths from the surrounding villages will be recruited to assist in the activities (as data collectors, enumerators). This is one way of ensuring the farmers and the communities participating are comfortable and feel that sense of ownership. This is also part of capacity building in the communities we are working with. The youths will be trained before embarking on the activities. Opinions of youth are also essential as they are the future decision makers.

**Sensory evaluation process:** Participants will evaluate a total of 5 varieties per day (4 NARITAs and one local check). This means that the same farmer will need to attend 6 sessions to evaluate all varieties in sites like Mitalula! We have to be sensitive about this because this means farmers will need to trade off their time and leave their work to come and do the evaluations hence a small token of appreciation will be provided. Bunches will be harvested and carried to the various localities were the participants live so that they feel more comfortable and will be at ease. This will also make the logistical preparations easier.

**Products that will be evaluated in the different sites:** The 5 sites are in different agroecological zones with unique and/or shared staple food products. Evaluations will be conducted using the main staple product in the region. The proposed products based on discussions during the project meeting in Arusha are as follows:

| | | |
|---|---|---|
| Kawanda | - | Steamed matooke |
| Mbarara | - | Steamed matooke |
| Bukoba | - | Boiled fingers |
| Mbeya | - | Fried fingers or roasted fingers |
| Kilimanjaro | - | Fried fingers or boiled fingers |

**Harvesting, Preparation, Serving, Evaluation tool:**

Harvesting: Harvesting of 'mature' bunches will be done 1 day before evaluations. This is to simulate what normally happens especially when taking bunches to the market.

**Definition of maturity – Maturity at harvest time is an important factor that affects the quality perception and rate of quality during post-harvest handling. Visual morphological indicators that farmers also use will be employed to ensure that mature bunches are harvested and cooked. These will include a combination of the following: vertical lines on the fruits become less pronounced/marked, fingers lose their angularity and fruits become fuller in size, visible crack one of the fingers, stylar ends become drier, finger on one of the hands shows visual signs of ripening or yellowing.

Preparation: A group of participants chosen by the farmers themselves and/or volunteers from the participating group will do the preparation. The preparers will follow the same procedure they normally use to prepare the products in their homes (there will however be standardization of some process to ensure uniformity and reduce bias e.g. ensuring that all samples are cooked in one saucepan (for steamed matooke), preparers use the same type of knife etc.). the cooking process is highlighted in Appendix A1). Preparers will not participate in the sensory evaluations but will have a separate questionnaire where they will rate traits related to preparation and processing such as ease of peeling, peel colour, amount of sap, cooking time etc. Preparers will be trained and assisted by the enumerators.

Serving: *Monadic* presentation where samples are served one at a time and all questions answered before presenting the next will be used. This will give participants enough time to critically assess each of the provided samples. It will be emphasised that the samples are independent of each other. The experimental design that will be followed is a balanced incomplete block design (BIBD) with a reference sample. BIBDs are recommended for sample presentation in conventional descriptive methods when the total number of samples is greater than the number that participants can evaluate in one session before sensory fatigue sets in. Instead of presenting all the ***t*** samples in one session (block), subsets will be presented in ***b*** smaller blocks, each containing ***k***<***t*** samples (where ***k*** = number of samples evaluated in every block). Errors/biases to be wary of and should be controlled for during analysis include order effects (since samples will be presented to each participant in the same order) and carry over effects (from previous treatment). In some sites the order in which samples will be presented to participants per session will be randomized and statistical tests will be done to assess if there are statistically significant differences with/without randomization.

Evaluation tool: A five-point hedonic scale will be used to access acceptability (1 = very bad, 2 = bad, 3 = fair, 4 = good, 5 = very good). The sensory analyses will involve an examination of different characteristics of the product using one's 5 senses either in combination or individually (see Fig 1). The attributes that panellists will evaluate include: colour, aroma, texture in hand, texture in mouth, taste, and overall acceptability. A 'mood -o- meter'/ visual representation of scores with facial images where the mouth is the only expressive facial feature will be used to aid panellists.

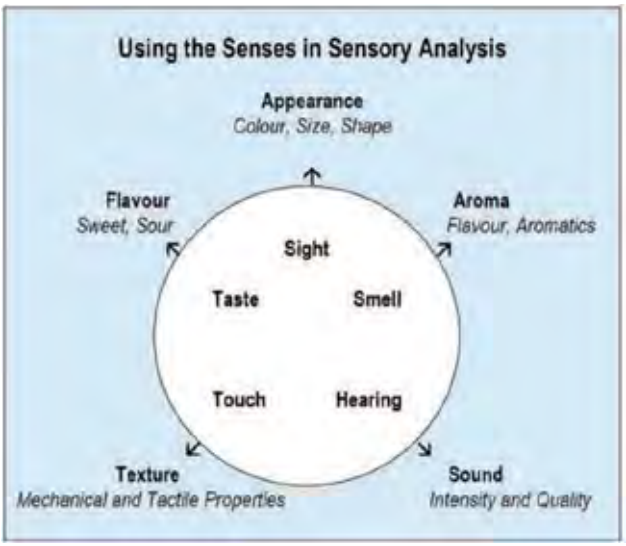



*Figure 1: Adapted from (PDST 2017)*

**Consent:** Before starting any activities, informed consent will be sought from all participants explaining to them their rights as research participants in the local language. As a portion of the study sample may be illiterate, verbal consent for survey participation will be obtained by enumerators and documented. Participants will be given as much time as they want to consider whether or not to participate in the study. Additionally, the protocol will allow time for the respondent to consider and ask the enumerator questions, and the consent form will emphasize that the respondent can opt not to participate without penalty.

**Focus group discussions (either on 1st or last day):** In each site, 4 focus group discussions (FGDs) will be conducted with: female youth; male youth (<35 years) and older women older men (>35years). Each group should ideally have 30 participants. Participants will be asked to individually rank the most important consumption attributes for the product they will be testing. This is important information for breeding programs especially when assessing which consumption traits to maintain, reach threshold or maximize in the breeding product profile. The FGD will also include a general discussion on: consumption attributes that farmers perceive to be important for the particular product they will be testing, improved banana varieties and their expectations in terms of consumption attributes.

**Translation:** ALL documents will be translated into the native language. Communication will be done in the local language.

**APPENDIX:**

### A1. Cooking process

| Site | Product | Cooking process |
|---|---|---|
| Kawanda & Mbarara | Mashed matooke | 2 people will peel each variety and assess characteristics<br>Process: Peel fingers, wrap in banana leaves, steam, mash, simmer, serve.<br>Matooke will be steamed for 2 hours, mashed, returned on fire and simmered for another hour then served<br><<enumerators should keep track of time>> |
| Mbarara and Bukoba | Boiled fingers | 2 people will peel each variety<br>Process: Peel fingers, wash, boil.<br>Fingers will be boiled for xx hours. First day will be used to gauge the average time it takes to cook however cooking time differs by variety. <<enumerators should keep track of cooking time for each variety>> |
| Mitalula | Fried fingers or roasted fingers | Need to finalize and get more info on protocol for preparation method …. |
| Kilimanjaro | Fried fingers or boiled fingers | Need to finalize and get more info on protocol for preparation method …. |

**A2. Tentative dates**: Considering maturity and Kephas', Robooni's schedule, other activities at hand. Ideally it is essential to do a pilot exercise in Kawanda and/or Mbarara with Kephas before embarking on the other sites. The plan is to stay in each site for 2 weeks

| Site | Proposed dates |
|---|---|
| Kawanda | Pilot/Pretesting - 28 Jun and 3 Jul (evaluation dates to be determined later) |
| Maruku | 25-31 Jul |
| Mbarara | 15-24 Jul |
| Kawanda | 10-16 Sep |
| Mitalula | 15-26 Oct |
| Kilimanjaro | 27 Oct – 9 Nov |

### A3. Capacity in the different sites

| Site | Extent of capacity building |
|---|---|
| Kawanda | Minimal (already have experience conducting evaluations with banana) |
| Mbarara | Minimal (already have experience conducting evaluations with banana) |
| Maruku | Minimal (already have experience conducting evaluations with banana) |
| Mitalula | Major |
| Kilimanjaro | Major |

### A4. Staff who will travel to the different sites

| | |
|---|---|
| Kawanda | Pricilla, Daudi, Charity, Lilian, Wilber |
| Mbarara | Pricilla, Okurut, Kephas, Wilber, Ibrahim, Lilian, Charity |
| Maruku | Pricilla, Okurut, Daudi |
| Mitalula | Pricilla, Okurut, Daudi (not certain) Ashraf (not certain) |
| Kilimanjaro | Pricilla, Okurut, Daudi (not certain) Ashraf (not certain) |

**Okurut should be there in all sites because he knows the different NARITAs and can differentiate between them. He also has experience conducting sensory evaluations.
**Daudi, Lilian and Charity are interns at Bioversity; Wilber and Ibrahim are data collectors at the Kawanda site

Protocol for Sensory Evaluations

**A5. Program/Activities** – **should be as participatory at all levels to ensure there is a sense of ownership!**

Sample of Day 1 and Day 2 activities

1pdf. Annex 5.2 Protocol Preference analysis

**Day 1**

1. Introductions
2. Introduce objectives activities (Kephas, Robooni, site managers)
3. Explanation of consent form
4. Signing consent form and collection of demographic information
5. FGD

**Day 2 (**and subsequent days)

1. Reiterate objectives of the sensory activities
2. Explanation of the evaluation tool, definitions of the consumption attributes that will be assessed
3. Conduct evaluations (can be 2 sessions per day i.e. 60 panelists in the morning and 60 in the afternoon) Enumerators will conduct the evaluations together with participants. Need to budget for around 10-12 enumerators.
4. Collect questionnaires
5. Discussion
6. Snacks, token of appreciation
7. END OF SESSION

**A6: Challenges:**

- Order and carry over effects (from comparing with previous sample(s))- randomization should cater for this
- The type and amount of fire is not easy to control as this depends on the size of firewood, how dry the firewood is, wind conditions etc.
- In the case of matooke, leaves from Sukali Ndizi and Kayinja varieties are preferred for wrapping, however fresh leaves may not always be. It is also difficult to standardize the age of the leaves and not possible to get leaves from the same plant and/or field

# 5.2 Protocol for Sensory Evaluations

## PREFERENCE ANALYSIS

The objective of the preference analysis (PA) exercise is to allow male and female farmers to participate in the selection of the most preferred NARITA hybrids to be taken for further on-farm testing, and to obtain a better understanding of the traits that are important to them in the selection of new banana varieties.

During the PA exercise, two types of data will be generated: (1) a quantitative preference score for each variety; and (2) a qualitative assessment of traits that farmers look for (or avoid) in the selection of new banana varieties. The method described below is adapted from (Paris et al., 2011) and (Christinck, Weltzien, & Hoffmann, 2005).

## PARTICIPANTS SELECTION AND MOBILIZATION

- The PA exercise will be done at the five sites where the on-station trials are established (Kawanda, Mbarara, Maruku, Mitalula and Kilimanjaro). The sites are in different agroecological zones in Uganda and Tanzania.
- Male and female farmers will be recruited from the surrounding villages – see protocol for sensory evaluations for more information on sampling and mobilization of participants.
  Note: It is important that the number of visitors is sufficiently high so that the preference analysis can give reliable results. We are targeting 60 farmers per site.
- Given that banana is a perennial crop and harvest occurs throughout the year with periods of low and high production, it is advised that the exercise be done during the peak season where most of varieties to be scored will have mature bunches. Even then, it is expected that not all NARITAs will have bunches available on the same day, and more than 1 field day (most likely 2-3 days) will thus need to be organized in each site.

## MATERIALS

- A4 envelope and label for each cultivar (NARITA + local check(s))
  *# envelopes = # NARITA hybrids + # local checks*
- Voting ballots:
  - 3 scores: LIKE, DON'T LIKE, DON'T KNOW
  - 2 different colors for men and women

  Voting ballots need to be prepared in sufficient numbers, so that every visitor can give any of the three scores to every cultivar:
  *# voting ballots, per score per color = # participants (male or female) x # cultivars to be scored*
  For example: if 15 cultivars will be scored, and there are 25 male and 30 female participants, you will need (25 x 15) = 375 LIKE, 375 DON'T LIKE and 375 DON'T KNOW voting ballots in the "male colour" and (30 x 15) = 450 LIKE, 450 DON'T LIKE and 450 DON'T KNOW voting ballots in the "female colour".

1


  - [if applicable/desired: In case we would want to differentiate the responses of different types of visitors: mark the papers with easily recognizable symbols for each type of visitor, e.g. plain papers for farmers, striped papers for extension workers, dotted papers for traders, diagonally striped papers for seed providers, … (or different shapes of ballot papers (round, square, triangle, …)]
- Small envelopes to containing the voting ballots
  *# small envelopes = # of participants*
- At least 5 flip chart pieces of paper per group, to write down what is discussed during the FGD
- Sticky tape to tape the flip charts on to a wall or other vertical surface that the whole group can see
- Markers of different colours to write on the flip charts
- A clipboard for the note taker
- Lined A4 paper for taking notes
- Pens to take notes
- A printed copy of Section A: Exercise information
- A printed copy of Section B: Introduction and individual informed consent
- A printed copy of Section C: Roster of participants
- A stapler to staple Sections A, B, and C, and the notes together
- Sticky labels to write the exercise code on and stick this to each of the flip chart pages used by the facilitator, and to the pile of stapled papers collated by the note taker
- Rope
- Stakes
- Snacks and drinks for all participants

## GETTING THE FIELD / SPACE READY

### FOR THE PREFERENCE ANALYSIS

- In the morning, before the exercise starts, identify for each NARITA hybrid and the local check a representative plant that has a bunch near harvesting stage. Make sure that the plants that the group will look at are easily accessible.
- Clearly label the selected plants. Use a stake or a colored label. Do not put the real names on the labels, but put a code (not the QR code, but a code that is easy to use by the participants, and does not reveal whether the plant is a test genotype or a local check); example A, B, C, D, etc. Remember to record the correct genotype of each code. Attach an envelope to each labeled plant, and put the same code on the envelope.
- Note: there is no need to include the resistant/susceptible checks – we only need to rate the NARITAs and the local check(s).

### FOR THE FGD

- Make sure there is a shaded place, where flip charts can be put up.
- Hang 2 sheets of flip chart paper next to one another on a wall (horizontally / landscape). Make sure that the papers are firmly secured to the surface of the wall with tape.
- Off to one side, hang another sheet of flip chart paper. This sheet will be used for generating lists that group members will need to refer to or keeping other relevant information that remains to be discussed (as a 'parking space' for issues to return to).

## SECTION A: EXERCISE INFORMATION

**A1. Date of field day (dd/mm/yyyy) _____/_____/_____**

**A2. Start time (hh:mm) _____**

**A3. End time (hh:mm) _____**

**A4. Name of enumerator 1 (facilitator) _____**

**A5. Name of enumerator 2 (note taker) _____**

**A6. Name of enumerator 3 (if applicable) _____**

**A7. Country _____**

**A8. Field site _____**

## SECTION B: INTRODUCTION AND INDIVIDUAL CONSENT

***~**~*** *Please read this script to each and every participant as you greet them when they arrive, before you add their details to the roster of participants (Section C).*

"We are _____, _____ and _____, and we work on behalf of Bioversity International on a project where new matooke/ndizi (banana) hybrids, produced by NARO and IITA, are being tested for their performance in different regions of Uganda and Tanzania. If any of the varieties perform well, they will be recommended for official release and made available to farmers.

Thank you for coming today to participate in the **preference analysis** exercise, where we will walk through the field to assess the physical characteristics/traits of the new banana hybrids and rate which ones you like or don't like. We will then have a discussion about the traits that are important to you in the selection of new banana varieties.

We would like to record what is said today so that we can make notes later, and we may take some photographs. Also, as some sensitive information might be shared within the group that could cause disharmony in the wider community, we ask you not share any information discussed here outside of this group.

Your participation today is entirely voluntary and you are free to leave now before we start, or at any time during the discussion."

**~~*****~~**

**B1. Do you have any questions for us?**

**B2. Having heard all of this information, are you still happy to participate in the seasonal calendar exercise?**

**~~*****~~**

"Thank you.

Now I'd like to get a few details about you before we start."

## SECTION C: REGISTER OF PARTICIPANTS

***~**~*** *All participants must consent to be interviewed if they wish to take part in the exercise (C1). If a participant does not consent to being photographed (C2), please inform the supervisor and ensure that no photographs are taken during the exercise.*

| | |
|---|---|
| **C1. Does the participant consent to participate in the exercise?** (*Yes/No*) | |
| **C2. Does the participant consent to being photographed?** (*Yes/No*) | |
| **C3. Name of participant** | |
| **C4. Village that participant comes from** | |
| **C5. Age of participant** (*Years*) | |
| **C6. Gender of participant** (*See code #1*) | |
| **C7. Marital status of participant** (*See code #2*) | |
| **C8. Level of education of participant** (*See code #3*) | |
| **C9. Main occupation of participant?** (*See code #4*) | |
| **C10. Role in banana production** | |
| **C11. Phone number** | |

**Codes:**

**#1:** 1 = woman; 2 = man

**#2:** 1 = single, 2 = cohabitating, 3 = married (monogamous), 4 = married (polygamous), 5 = divorced, 6 = widowed, 7 = don't know, 8 = other, please specify.

**#3:** 1 = no formal education, 2 = nursery, 3 = primary, 4 = secondary, 5 = post-secondary, 6 = don't know, 7 = other, please specify.

**#4:** 1 = agriculture, 2 = livestock, 3 = business, self-employed, 4 = construction, bricklaying, 5 = transportation, 6 = timber, charcoal, wood products, 7 = non-timber forest products, 8 = housework, 9 = salaried professional, 10 = casual temporary labour, 11 = studying, 12 = no occupation (adult), 13 = don't know, 14 = other, please specify

## SECTION D: PREFERENCE ANALYSIS

"Let us start by introducing ourselves - please say your name and any other information that you want to share about yourself."

**D1. Does anyone have any questions or comments before we begin?**

**~**~** *Address any issues before starting the exercise.*

**~~~***~~~**

"Now, let us begin the preference analysis exercise.
We will first walk through the field, and look at the varieties to be scored today. You will be able to observe the plants and discuss their appearance with other participants. You will receive 3 types of voting ballots (LIKE, DON'T LIKE and DON'T KNOW). After having gone through the field, you will be asked to give each variety a vote, by placing the relevant voting ballot in the envelope placed near that plant. After all votes have been cast, we will discuss the results in group."

**~**~** *Give each participant the same number of ballots of each category (LIKE, DON'T LIKE, DON'T KNOW) as there are cultivars to be scored; use the correct color code, and symbol, for each participant*

**~**~** *Walk with the participants through the field, and invite them to carefully observe the plants that have been labelled.*

**~**~** *Provide a short description of each variety to be scored, taking great care to state only the factual information (e.g. length of crop cycle). Avoid influencing the participants' opinion by not providing any information that is subjective (e.g. "the crop cycle is too long"). Give the participants enough time to observe and familiarize themselves with the varieties to be scored.*

**~**~** *After the guided tour through the field, let the participants walk through the field again, on their own or in small groups. The participants cast their votes by placing one ballot in each envelop – i.e. participants give a vote for each cultivar: LIKE, DON'T LIKE, DON'T KNOW.*

**~~~***~~~**

"Ok, now that you have all cast your votes, we invite you take a drink and a snack. We will in the meantime count the votes for the different varieties."

**~**~** *Collect the envelopes with the ballot papers.*

**~**~** *For each variety, count the votes in each category (LIKE, DON'T LIKE, DON'T KNOW) and by gender. Enter this information in the form below.*

***~**~** *Calculate a preference score (PS) for each cultivar, by using the following formula:*

*PS (%) = (n1*1+ n2*0.5 + n3*0) * 100/(n1+n2+n3)*

*with n1 = the number of LIKE ballots*
*n2 = the number of DON'T KNOW ballots*
*n3 = the number of DON'T LIKE ballots*

**Form - Preference analysis of banana varieties.**

| Country: | | | | Field site: | | | | | Date: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety | Count of LIKE, DON'T LIKE, DON'T KNOW votes | | | | | | | | | Preference score | | |
| | Males (N=…) | | | Female (N=…) | | | Total (N=…) | | | Males (N=…) | Female (N=…) | Total (N=…) |
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |
| I | | | | | | | | | | | | |

**~~~*****~~~**

"Now, let's take a look at the results of the voting exercise."

**D2. Which variety received the highest score? The second highest score?**

**D3. Which variety received the lowest score? The second lowest score?**

***~**~** *Report the results back to the group of visiting farmers: name the varieties that received the highest PS, and varieties that received the lowest PS.*

**~~~*****~~~**

"Next, we'll discuss these results and identify the traits that you use to assess whether or not a certain banana variety meets your needs. For this exercise, we'll split up in two groups: one group with the women and one group with the men."

***~**~** *Split the group into two sub-groups by gender, and take each group to the space where the FGD will be held.*

**~~~*****~~~**

## SECTION E: FOCUS GROUP DISCUSSION

"Now, let's first look at the variety that received the highest score by your group."

**D4. What did you like about the highest ranked variety?**

**~**~** *Facilitator writes down all traits, and trait status, that are mentioned.*

"Let's do the same for the variety that received the second highest score by your group."

**D5. What did you like about the second highest ranked variety?**

**~~~***~~~**

"Now, let's first look at the variety that received the lowest score by your group."

**D6. What didn't you like about the lowest ranked variety?**

**~**~** *Facilitator writes down all traits, and trait status, that are mentioned.*

"Let's do the same for the variety that received the second lowest score by your group."

**D7. What did you like about the second lowest ranked variety?**

**~~~***~~~**

"Now from the list of desired traits, let's identify which ones are the most important."

**D8. Which are the three most important traits that you consider when selecting a new banana variety, in order of importance?**

**~**~** *Facilitator records the consensus response on the flip chart.*

**D9. For these three most important traits, can you provide a short description of what they mean to you? How is the trait characterized? Why is it important?**

**~~~***~~~**

"The preference analysis exercise is now complete. Thank you for being so generous with sharing your time, views, and experiences!"

## REFERENCES

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# 6. Work Package 5

## 6.1 Summary of the WP3 trials uploaded on MusaBase

| Category | Trial uploaded | Data uploaded |
|---|---|---|
| **Training population field 1 (Sendusu)** | Yes | Yes |
| **Training population field 2 (Sendusu)** | Yes | Yes |
| **Training population (Mbarara)** | Yes | No |
| **Calcutta 4 x P. Lilin** | Yes | No |
| **Calcutta 4 x Zebrina G.F.** | Yes | No |
| **Monyet x Kokopo Screening 1 for Weevil resistance** | Yes | No |
| **Monyet x Kokopo Screening 2 for Weevil resistance** | Yes | No |
| **Calcutta 4 x Zebrina G.F. Screening for nematode resistance - 1** | Yes | No |
| **Calcutta 4 x Zebrina G.F. Screening for nematode resistance - 2** | Yes | No |
| **Calcutta 4 x Zebrina G.F. Screening for nematode resistance - 3** | Yes | No |
| **Calcutta 4 x Zebrina G.F. Screening for nematode resistance - 4** | Yes | No |
| **Calcutta 4 x Zebrina G.F. Screening for nematode resistance - 5** | Yes | No |
| **Calcutta 4 x Zebrina G.F. Screening for nematode resistance - 6** | Yes | No |
| **Kasaska x Borneo (field)** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 1** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 2** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 3** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 4** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 5** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 6** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 7** | Yes | No |
| **Kasaska x Borneo Nematode Resistance trial Experiment 8** | Yes | No |
| **Paliama x Borneo Foc R1_1** | Yes | Yes |
| **Paliama x Borneo Foc R1_2** | Yes | Yes |
| **Paliama x Borneo Foc R1_3** | Yes | Yes |
| **Paliama x Borneo Foc R1_4** | Yes | Yes |
| **Malaccensis x Malaccensis Foc R1_1** | Yes | Yes |
| **Validation population trial (made up of 22 &23)** | Yes | No |

# 7. Student Research Progress Reports

## WP1 - PhD Research Progress Report (2017-2018)

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

**Name of PhD Student:** **BATTE MICHAEL**

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

**Timeline of study:** 1st October 2017 to 31st March 2018

**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 one years (1995-2015)
- Determining grandparent heterobeltiosis of NARITA hybrids
- Mapping resistance to banana weevils
- Mapping resistance to banana nematodes (*Radopholus similis*)
- Identifying traits for banana ideotype

**Achievements**

Highlight significant achievements – e.g. in bullets

- The research article[4] "Suitability of existing *Musa* morphological descriptors to characterize the East African highland 'Matooke' bananas" was published in the Genetic Resources and Crop Evolution Journal. DOI:10.1007/s10722-017-0562-9

  - 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.
- Manuscript "Crossbreeding East African highland bananas: lessons learnt relevant to botany of the crop after 21 years of genetic enhancement**"** submitted to Frontiers in Plant Science journal.

  - From this study it was found out that pollination success in East African highland bananas was not affected by month of the year when pollination was done. Two diploid male parents; *Musa acuminata* subsp. *malaccensis* accession 250 and cultivar 'Rose' outperformed Calcutta 4 and therefore recommended for use in screening banana

[4]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

groups for female fertility. Most female fertile triploid and tetraploid parents identified according to available records.

- All NARITA and promising secondary triploid hybrids exhibited high grandparent heterobeltiosis for bunch weight. There was no correlation between genetic distances and heterobeltiosis in East African highland bananas.
- F1 population (Monyet × Kokopo) was found to be segregating for banana weevil resistance.
- F1 population (Calcutta 4 × Zebrina GF) was found to be segregating for resistance to *Radopholus similis*.
- Results from path analysis revealed that: finger length, finger circumference, number of fingers on bunch, number of hands on bunch and plant cycle had a direct positive effect on the bunch weight. However, index of no-spotted leaves, days to fruit filling, days to maturity and plant stature had an indirect effect on bunch weight.

**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 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 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's 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 18th September 2017 in the journal *Genetic Resources and Crop Evolution* (open access at DOI:10.1007/s10722-017-0562-9)

**Objective / Study 2. Review of breeding East African highland bananas for the first twenty one years (1995-2015)**

The manuscript "Crossbreeding East African highland bananas: lessons learnt relevant to botany of the crop after 21 years of genetic enhancement**"** has been submitted to Frontiers in Plant science journal. Some of the highlights of this paper are: Month of the year had no significant effect (P=0.5) at 95% level of confidence, on pollination success (Fig. 1), implying that pollinations in the EAHB should be done throughout the year. *Musa acuminata* subsp. *malaccensis* accession 250 had the highest pollination success - 66.8% (Fig. 2) when used as a male, followed by cultivar 'Rose' (66.6%). These two accessions outperformed 'Calcutta 4' (18.4%) which was regarded as the best male-fertile parent. Therefore they should be used as male parents to screen banana accessions for female fertility. Pollination success results further revealed that tetraploids were more female fertile than triploids (Fig. 3). More female fertile EAHB were from 'Nakabululu' clone set followed by 'Nfuuka' clone set (Fig. 3). Cultivar 'Nakabululu' had the highest pollination success (34.3%) and average of 1.5 seeds per pollinated bunch, followed by 'Nakawere' with pollination success of 31.6% and average of 1.4 seeds per pollinated bunch. However, these two cultivars are not part of the current crossing scheme. It is therefore recommended that these two cultivars should be incorporated in the current crossing scheme so as to increase the chances of seed set. Likewise, genotype 1201K-1 exhibited the highest pollination success (48.4%) and average of 29 seeds per pollinated bunch, followed by 917K-2 with pollination success of 48.2% and average of 39.2 seeds per pollinated bunch, 660K-1 having a pollination success of 43.3% and average of 14.8 seeds per pollinated bunch and 222K-1 having a pollination success of 40.9% and average of 16.6 seeds per pollinated bunch (Fig. 3). These tetraploid parents were the most female fertile and therefore recommended to be given higher preference in crossing. Germination success was highest in $2x \times 4x$ crosses -36% (Fig. 4), followed by $2x \times 2x$ (22%). $3x \times 2x$ crosses had higher germination success (11.1%) than $4x \times 2x$ crosses (7.4%), despite the fact that most of the hybrids in field are from $4x \times 2x$ cross. The possible explanation for this phenomenon is that the $3x \times 2x$ crosses produce few seeds such that even with the higher germination success, they cannot outcompete the $4x \times 2x$ crosses which produce high number of seeds.

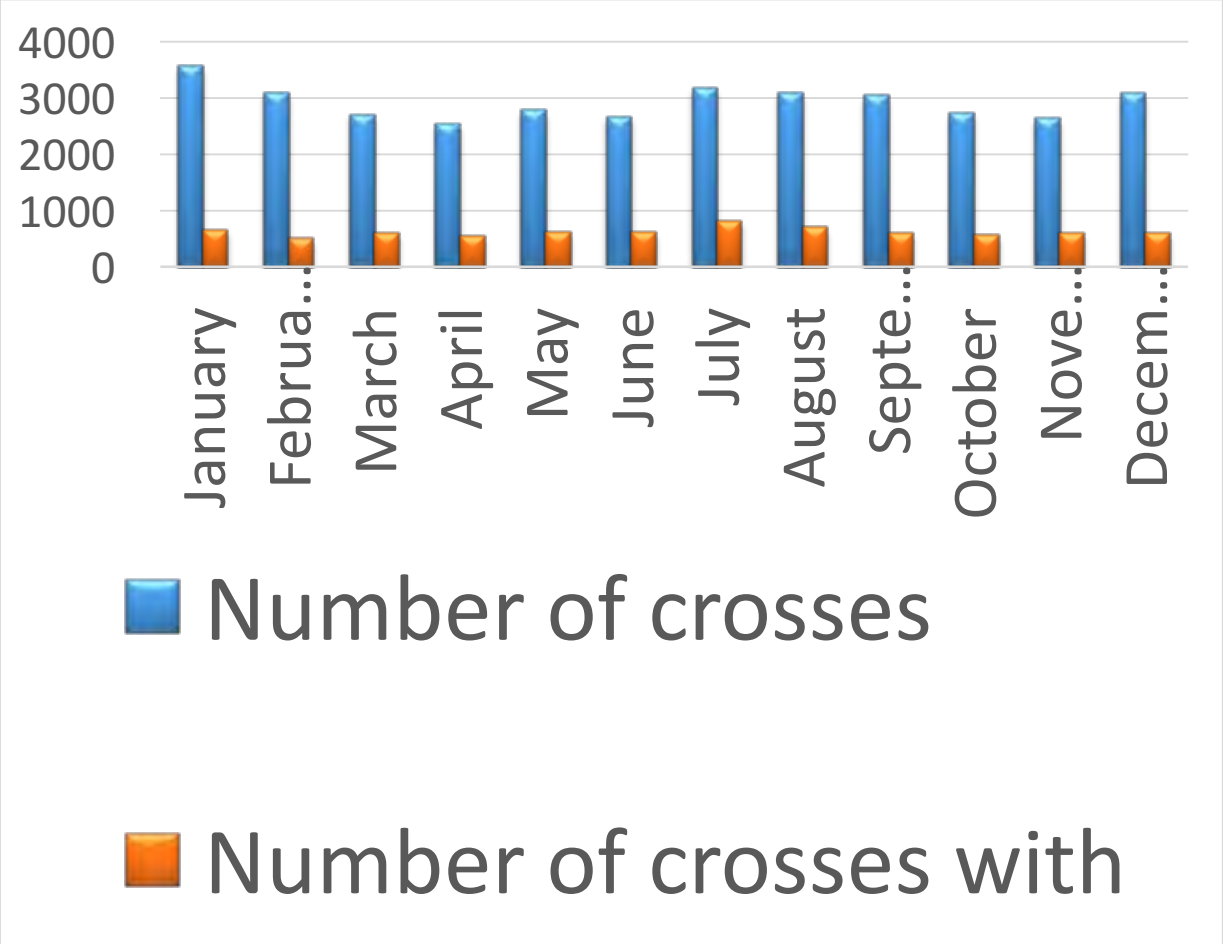


**Fig.1. Total number of crosses and crosses with seed per month from 1995 to 2015**

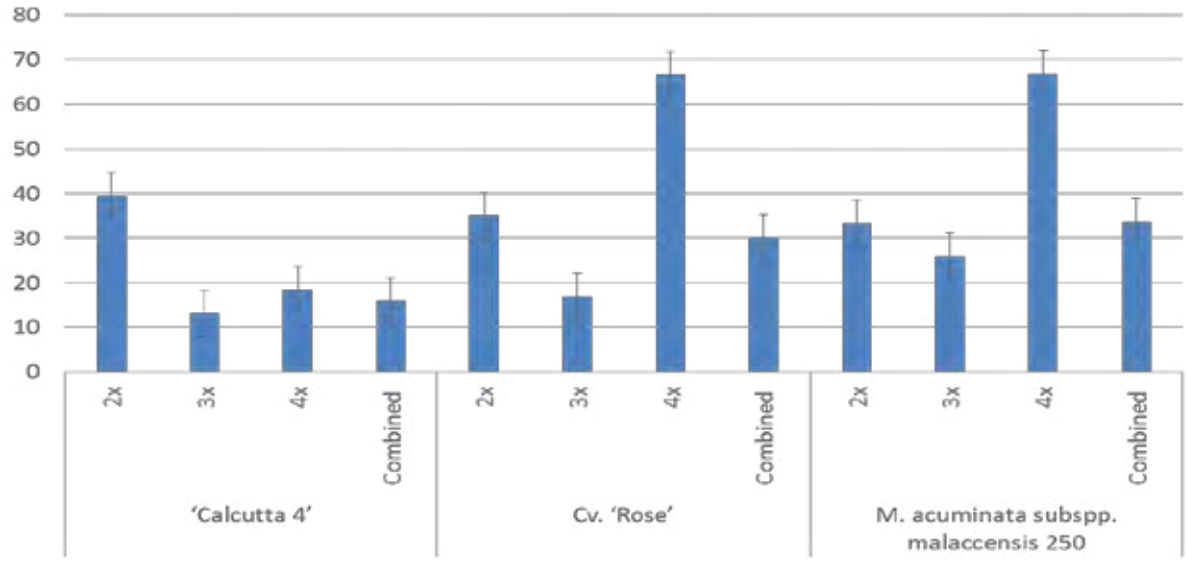


**Fig. 2. Pollination success (%) in 2x parents**

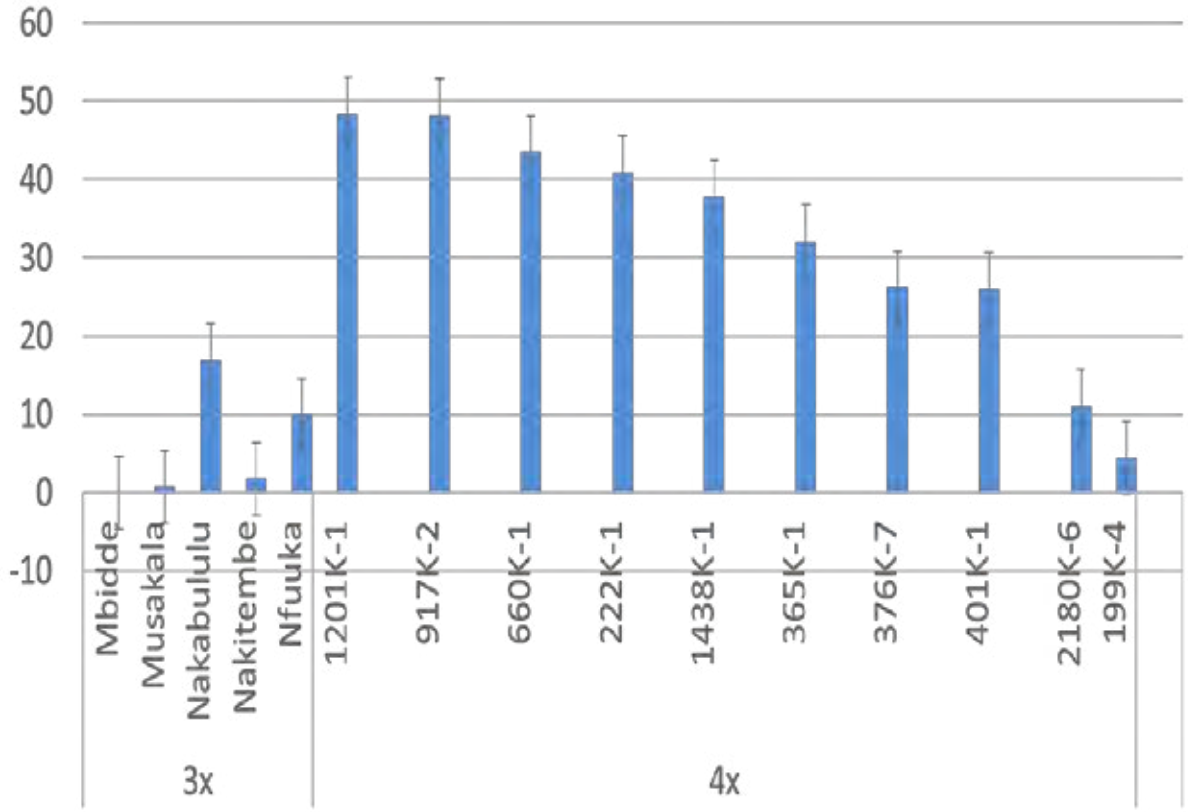


**Fig. 3. Pollination success (%) of 3x and 4x parents**

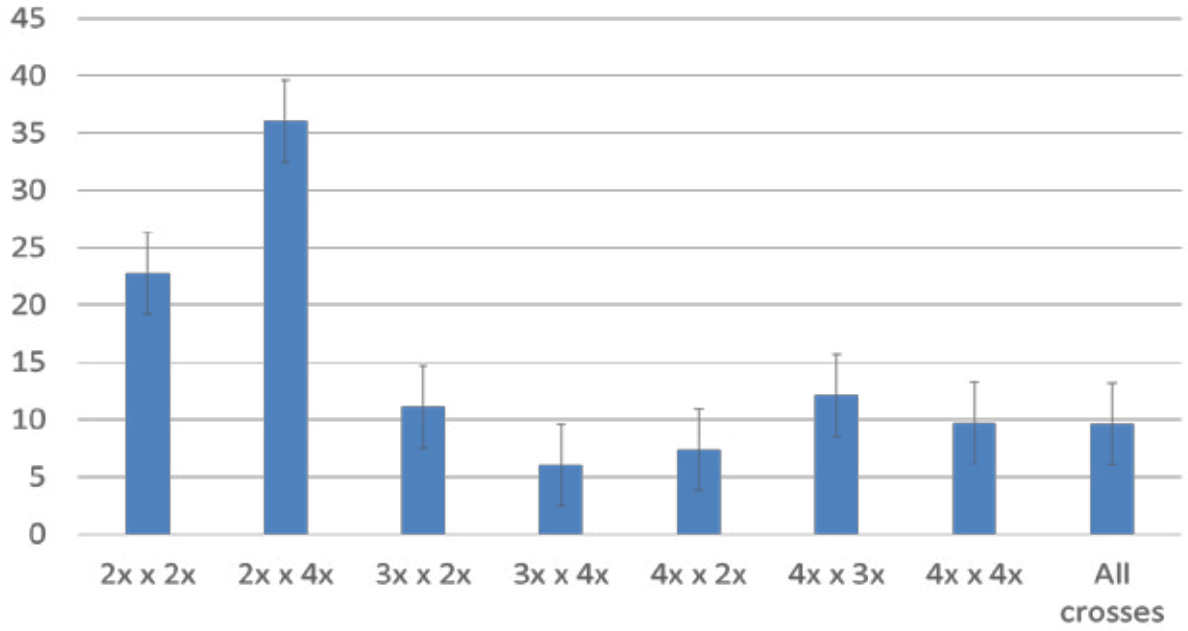


**Fig. 4. Seed germination success (%)**

- **Objective / Study 3. Determining grandparent heterobeltiosis of NARITA hybrids**

Twenty six officially named NARITA hybrids and 8 other promising secondary triploids, their parents, grandparents and local 'Matooke' cultivar checks (Table 1) were planted in the field at Namulonge/Sendusu following a 7 × 8 rectangular lattice design with two replications.

Bunch weight (kg) of each individual bunch at harvest was measured. Yield potential (t ha $^{-1}$ yr $^{-1)}$ was calculated as:

YLD = BW × 365 × 1667/ (DH×1000)

Where, YLD= Yield potential (t ha $^{-1}$ yr $^{-1)}$ , BW= bunch weight (kg), DH= days to harvest.

The mean bunch weights and standard errors were calculated and used to determine heterobeltiosis using the formula:

Heterobeltiosis (%) = [("NARITA" mean bunch weight - "3*x* Grandmother" mean bunch weight)/"3*x* Grandmother" mean bunch weight] × 100

Means of grandmothers were used to calculate heterobeltiosis of hybrids instead of their parents because the direct parents are not suitable for food, hence the type of heterobeltiosis calculated is grandparent heterobeltiosis.

**Genotyping using SSR**

In order to determine the effect of genetic distance on heterobeltiosis in banana, we genotyped the advanced hybrids (NARITAs and other hybrids), their parents and grandparents using simple sequence repeat (SSR) markers. Fresh young cigar leaf samples were collected from the field in Uganda and shipped under cold chain to the Institute of Experimental Botany, Olomouc, Czech Republic. Leaf samples were lyophilized in falcon tubes and stored at room temperature. Approximately 20mg of lyophilized tissue was crushed into powder in 2ml Eppendorf tubes using a tissuelyzer. DNA was extracted from tissue powder using NucleoSpin Plant II kit, Macherey-Nagel, Germany, following the manufacturer's instructions. The concentration and quality of DNA was assessed by NanoDrop ND-1000 spectrophotometer. The working concentration of DNA was adjusted to ~10ng/µl. Genotyping was done using 19 informative *Musa* SSR primers following the protocol of Christelová et al. (2011). Two independent rounds of PCR were performed followed by fragment analysis. Alleles for each sample were inspected in GeneMarker v1.75 (Softgenetics, State College, PA, USA) and manually scored for presence (1) or absence (0) only when concordance of alleles between PCR runs was observed. In case a sample showed inconsistence in allele sizes between two PCR runs, a third PCR run was performed to confirm the alleles. Squared Euclidean distances between genotypes were calculated using R software v3.4 (R core team 2018) using dist function provided in the package ape. Clustering was done with function hclust based on ward.D2 method (Ward 1963; Murtagh and Legendre 2014). The genetic distances between the NARITA hybrids and their female grandparents are presented in Table 2. Also the bunch weights and grandparent heterobeltiosis of the NARITA hybrids are presented in Table 2. A cladogram showing the clustering of the NARITAS hybrids, parents and grandparents (Fig.5) shows that the female grandparents, male grandparents, NARITAs, female parents and male parents clustered separately.

All the 23 NARITAs available at Sendusu and with known pedigrees together with other 8 promising secondary triploid hybrids showed heterobeltiosis for bunch weight, compared to their grandmothers (3*x* 'Matooke'), despite the heterozygosity of the parents. NARITA 23 had the highest bunch weight (29.3 Kg), followed by NARITA 17 (29.0 kg), NARITA (28.6kg) and lastly NARITA 19 (11.1 kg) (Table 2). NARITA 17 had the highest yield potential (35.6 t ha $^{-1}$ yr $^{-1}$), followed by NARITA 23 (35.0 t ha $^{-1}$ yr $^{-1}$ ), NARITA 18 (34.4 t ha $^{-1}$ yr $^{-1}$ ) and lastly NARITA 19 (14.7 t ha $^{-1}$ yr $^{-1}$ ).NARITA 17 had the highest heterobeltiosis of 248.7% (Table 2) (Fig. 6), followed by 26666S-1 (229.3%), NARITA 9 (201.2%) and NARITA 19 had the lowest heterobeltiosis: 1.2%. NARITA 7, the only released NARITA hybrid cultivar in Uganda so far, had a heterobeltiosis of 77.2%. The factors behind heterobeltiosis in banana are yet to be defined. Nonetheless, heterobeltiosis shows the potential to produce high yielding banana hybrids in relatively few crossbreeding cycles. Exploiting heterobeltiosis in banana breeding

will contribute to selecting breeding material with high compatibility, thus increasing banana breeding efficiency. Since bananas are vegetatively propagated, the effect of heterobeltiosis is easily fixed in the hybrids and will not be lost over time after release and further commercialization of these hybrids.

There was no significant correlation between the Euclidean distances and the heterobeltiosis scores. The Euclidean distances were almost uniform for all NARITAs and promising secondary triploid hybrids (Table 2)

**Table 1. NARITA hybrids and other promising secondary triploid hybrids with parents and female grand parents**

| N0. | Genotype | Female parent | Male parent | Female Grand mother |
|---|---|---|---|---|
| **1** | NARITA 17 | 1438K-1 | 9719-7 | Entukura |
| **2** | 26666S-1 | 917K-2 | SH3362 | Enzirabahima |
| **3** | NARITA 9 | 917K-2 | SH3217 | Enzirabahima |
| **4** | NARITA 22 | 917K-2 | 9128-3 | Enzirabahima |
| **5** | 26874S-5 | 917K-2 | 5610S-1 | Enzirabahima |
| **6** | 26787S-1 | 917K-2 | 9128-3 | Enzirabahima |
| **7** | NARITA 23 | Kazirakwe | 7197-2 | Kazirakwe |
| **8** | NARITA 14 | 917K-2 | 7197-2 | Enzirabahima |
| **9** | NARITA 8 | 917K-2 | SH3217 | Enzirabahima |
| **10** | 26337S-11B | 1201K-1 | SH3217 | Nakawere |
| **11** | NARITA 4 | 660K-1 | 9128-3 | Enzirabahima |
| **12** | NARITA 3 | 917K-2 | SH3362 | Enzirabahima |
| **13** | NARITA 1 | 917K-2 | 9128-3 | Enzirabahima |
| **14** | NARITA 2 | 401K-1 | 9128-3 | Entukura |
| **15** | NARITA 10 | 917K-2 | SH3217 | Enzirabahima |
| **16** | 25974S-19 | 917K-2 | SH3362 | Enzirabahima |
| **17** | NARITA 5 | 917K-2 | SH3217 | Enzirabahima |
| **18** | 29792S-14 | 917K-2 | CV.Rose | Enzirabahima |
| **19** | 26316S-7 | 1201K-1 | SH3362 | Nakawere |
| **20** | NARITA 16 | 917K-2 | SH3362 | Enzirabahima |
| **21** | NARITA 13 | 1201K-1 | SH3362 | Nakawere |
| **22** | NARITA 21 | 1201K-1 | 7197-2 | Nakawere |
| **23** | NARITA 15 | 660K-1 | 9128-3 | Enzirabahima |
| **24** | NARITA 18 | 365K-1 | 660K-1 | Kabucuragye |
| **25** | NARITA 7 | 1201K-1 | SH3217 | Nakawere |
| **26** | NARITA 20 | Entukura | 365K-1 | Entukura |
| **27** | NARITA 12 | 1201K-1 | 9128-3 | Nakawere |
| **28** | NARITA 11 | 1201K-1 | 9128-3 | Nakawere |
| **29** | 29285S-20 | 1201K-1 | CV.Rose | Nakawere |
| **30** | NARITA 6 | 222K-1 | 9128-3 | Nfuuka |
| **31** | NARITA 19 | 1201K-1 | 8075-7 | Nakawere |
| **32** | NARITA 24 | Unknown | Unknown | Unknown |
| **33** | NARITA 25 | Unknown | Unknown | Unknown |
| **34** | NARITA 26 | Unknown | Unknown | Unknown |

**Table 2. Mean bunch weight ± standard error, genetic distances, yield potential ± standard error and grandparent heterobeltiosis for NARITA and other promising secondary triploid hybrids**

| N0. | Genotype | Bunch weight ± SE (Kg) | Genetic distance | Yield potential ± SE (t ha $^{-1}$ yr $^{-1}$) | Heterobeltiosis (%) |
|---|---|---|---|---|---|
| **1** | NARITA 17 | 29.0 ± 1.5 | 5.3 | 35.6 ± 2.0 | 248.7 |
| **2** | 26666S-1 | 26.1 ± 1.8 | 5.2 | 28.9 ± 1.2 | 229.3 |
| **3** | NARITA 9 | 23.8 ± 2.2 | 6.2 | 31.3 ± 3.1 | 201.2 |
| **4** | NARITA 22 | 23.3 ± 1.9 | 6.2 | 31.8 ± 2.3 | 194.4 |
| **5** | 26874S-5 | 22.6 ± 2.3 | 5.4 | 27.5 ± 2.8 | 186.0 |
| **6** | 26787S-1 | 22.6 ± 1.7 | - | 31.1 ± 2.2 | 185.1 |
| **7** | NARITA 23 | 29.3 ± 2.5 | 6.1 | 35.0 ± 2.1 | 172.9 |
| **8** | NARITA 14 | 20.7 ± 2.0 | 5.5 | 27.4 ± 3.1 | 161.9 |
| **9** | NARITA 8 | 20.3 ± 1.8 | 5.7 | 22.2 ± 1.9 | 157.0 |
| **10** | 26337S-11B | 27.6 ± 2.6 | 5.4 | 29.2 ± 2.2 | 151.8 |
| **11** | NARITA 4 | 18.7 ± 1.7 | 5.4 | 23.1 ± 2.1 | 136.4 |
| **12** | NARITA 3 | 18.7 ± 1.6 | 4.8 | 20.7 ± 1.9 | 136.2 |
| **13** | NARITA 1 | 18.5 ± 1.7 | 4.9 | 20.1 ± 2.0 | 133.8 |
| **14** | NARITA 2 | 17.8 ± 1.2 | 5.1 | 24.5 ± 1.9 | 114.4 |
| **15** | NARITA 10 | 16.7 ± 2.0 | 6.3 | 21.0 ± 2.5 | 111.2 |
| **16** | 25974S-19 | 16.7 ± 1.0 | 5.7 | 24.7 ± 1.1 | 111.0 |
| **17** | NARITA 5 | 15.6 ± 1.2 | 4.8 | 17.4 ± 1.5 | 97.1 |
| **18** | 29792S-14 | 15.6 ± 2.0 | - | 23.0 ± 3.1 | 97.0 |
| **19** | 26316S-7 | 21.4 ± 0.8 | 5.4 | 33.6 ± 1.0 | 95.9 |
| **20** | NARITA 16 | 15.5 ± 1.7 | 6.0 | 17.6 ± 1.4 | 95.9 |
| **21** | NARITA 13 | 21.0 ± 1.6 | 5.7 | 30.7 ± 2.5 | 91.9 |
| **22** | NARITA 21 | 20.2 ± 3.1 | 5.6 | 27.0 ± 3.8 | 84.3 |
| **23** | NARITA 15 | 14.5 ± 0.9 | 6.2 | 18.2 ± 1.2 | 83.7 |
| **24** | NARITA 18 | 28.6 ± 2.0 | 5.7 | 34.4 ± 1.7 | 83.4 |
| **25** | NARITA 7 | 19.4 ± 2.1 | 5.7 | 19.6 ± 1.7 | 77.2 |
| **26** | NARITA 20 | 14.3 ± 2.9 | 5.7 | 19.5 ± 4.2 | 71.8 |
| **27** | NARITA 12 | 18.1 ± 1.1 | 5.4 | 24.2 ± 1.5 | 65.0 |
| **28** | NARITA 11 | 16.6 ± 0.9 | 5.4 | 23.0 ± 1.6 | 51.8 |
| **29** | 29285S-20 | 16.2 ± 1.7 | 6.0 | 22.9 ± 2.7 | 47.9 |
| **30** | NARITA 6 | 18.5 ± 1.2 | 6.0 | 23.2 ± 1.5 | 31.0 |
| **31** | NARITA 19 | 11.1 ± 1.2 | 5.6 | 14.7 ± 1.5 | 1.2 |

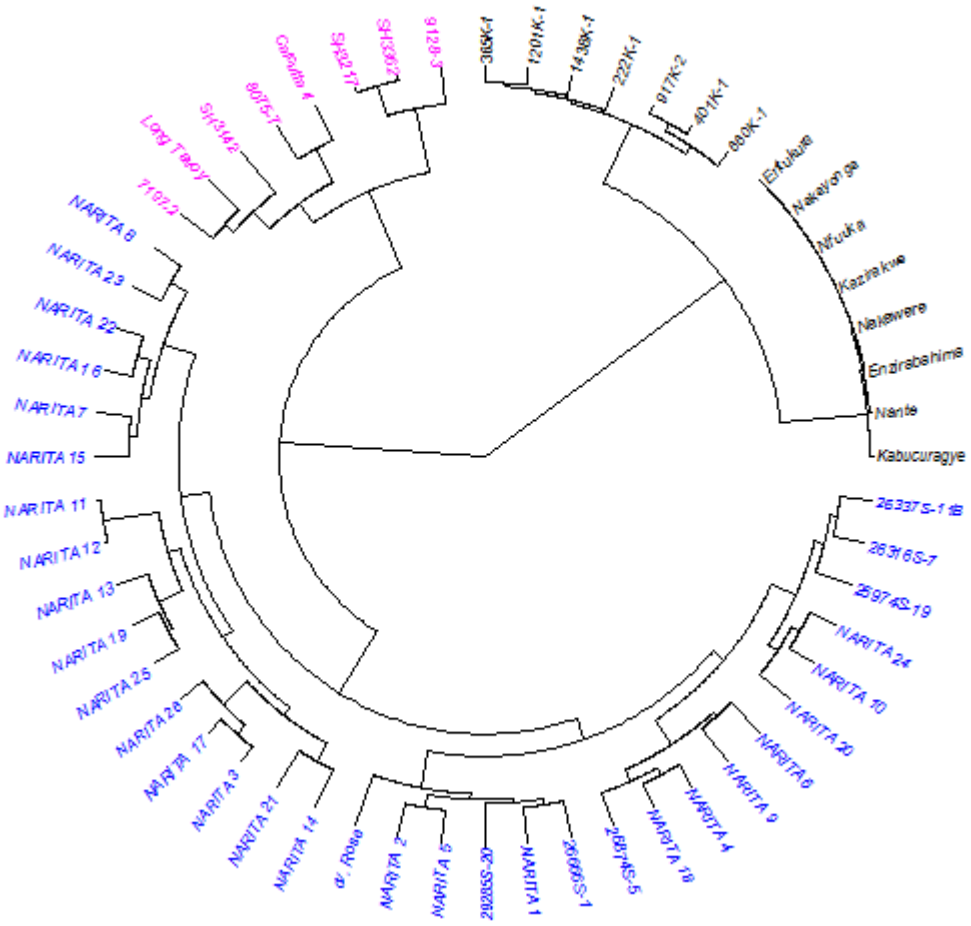


**Fig. 5. A cladogram showing clustering of NARITAs and promising secondary triploids, their parents, and grandparents using genotypic data generated from 19 *Musa* SSR primers.**


**Fig. 6. Heterobeltiosis on bunch size, comparing NARITA 17 with its progenitor landrace – 'Entukura'**

**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, with a few modifications. Suckers after undergoing hot water treatment were used in this experiment. Three suckers per test genotype were planted in a completely randomised design replicated twice. Parents and resistant checks (Calcutta 4, Km5) and susceptible checks ('Nakyetengu', 'Kabucuragye') were also included in the experiment. The corms were sectioned crosswise at collar area (upper cross-section). Percentage weevil damage was assessed separately for the cortex (outer) and central cylinder (inner) at this position. Another transverse section was made halfway the remaining corm (lower cross-section). Percentage weevil damage was assessed separately for the cortex (outer) and central cylinder (inner) at this position. The total cross-section damage (XT) was calculated by getting the average of the cross-section damages for the outer (cortex) and inner (central cylinder) at both the upper and lower cross-sections of the corm.

Four experiments have been set up with varying numbers of genotypes depending on the available suckers for planting. Experiments 1, 2 and 3 with 42, 29 and 36 F1 genotypes were harvested and data for (107 genotypes) were collected, cleaned and analysed using R-software. Experiment 4 (32 genotypes) is ongoing. Genotyping of this population using a SNP chip will be done as soon as the chip is ready.

Results from Dunnet's test revealed 55 resistant genotypes, 23 susceptible genotypes and 29 intermediates.

The following linear model was used in the analysis:

$Yij = \mu + Ti + Rj + eij$

Where Yij= $j^{th}$ observation of the $i^{th}$ treatment, μ= Grand mean of the observations made on a certain trait, Ti= Treatment factor, Rj= Replication and eij= Residual error.
Analysis of variance (ANOVA) for checks and parents which were represented in all the 3 experiments was done (Table 3).

ANOVA results from Table 3 showed that the accessions were significantly different in performance, the experiments were also significantly different, and the interaction between accessions and experiments was significant. Because of this, data for each experiment were analyzed separately.

The accessions in experiment 1 and 2 were significantly different in resistance to banana weevils (Table 4 and Table 5 respectively). However, the accessions in experiment 3 were not significantly different from each other in terms of resistance to banana weevils (Table 6). This may be due to the random selection of suckers for planting based on what is available. Since the susceptible and resistant checks performed as expected, this population qualifies to be regarded as segregating for nematode resistance

Broad sense heritability (H) (Table 7) was determined using the variance component formula:

H= $\frac{\delta G}{\delta P}$ where (δP=δe+δG) and δe is Residual error mean square

δe + RδG is Genotype expected mean square

$$\delta G(\text{Genetic variance component}) = \frac{\text{MsGenotype} - \text{Mserror}}{\text{R}}$$

$$\text{R} = \text{number of replications}$$

**Table 3. Analysis of variance table for checks and parents**

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **Accession** | 5 | 132.801 | 26.5602 | 138.8739 | 5.198e-12 *** |
| **Rep** | 1 | 0.072 | 0.0723 | 0.3779 | 0.547917 |
| **Expt.** | 2 | 6.780 | 3.3898 | 17.7239 | 0.000112 *** |
| **Accession: Expt.** | 10 | 17.491 | 1.7491 | 9.1454 | 9.906e-05 *** |
| **Rep: Expt.** | 2 | 0.420 | 0.2102 | 1.0991 | 0.358561 |
| **Residuals** | 15 | 2.869 | 0.1913 | | |
| **Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1** | | | | | |

**Table 4. Analysis of variance table for cross sectional damage of genotypes from Experiment 1**

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **Accession** | 43 | 165.334 | 3.8450 | 5.5121 | 6.663e-08 *** |
| **Rep** | 1 | 2.680 | 2.6796 | 3.8415 | 0.0565 |
| **Residuals** | 43 | 29.995 | 0.6976 | | |
| **Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1** | | | | | |

**Table 5. Analysis of variance table for total cross sectional damage of genotypes from Experiment 2**

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **Accession** | 30 | 103.463 | 3.4488 | 4.9282 | 1.799e-05 *** |
| **Rep** | 1 | 0.028 | 0.0281 | 0.0402 | 0.8425 |
| **Residuals** | 30 | 20.994 | 0.6998 | | |
| **Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1** | | | | | |

**Table 6. Analysis of variance table for total cross sectional damage of genotypes from Experiment 3**

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **Accession** | 37 | 70.223 | 1.8979 | 1.4550 | 0.1362 |
| **Rep** | 1 | 1.626 | 1.6259 | 1.2464 | 0.2721 |
| **Residuals** | 34 | 44.350 | 1.3044 | | |

**Table 7. Heritability values for banana for banana weevil resistance**

| PARAMETER | HERITABILITY VALUES | | | |
|---|---|---|---|---|
| | Expt. 1 | Expt. 2 | Expt. 3 | COMBINED Expts. |
| **Total cross sectional damage** | 69% | 66% | 23% | 55% |

**Table 8. Chi square test between observed ratios and different expected ratios**

| Trait | Observed number | Total | Tested ratio | Expected resistant | Expected intermediates | Expected suscepitibles | Chi calculated | Chi probability | Significate level |
|---|---|---|---|---|---|---|---|---|---|
| **Total cross sectional damage** | Resistant (55)<br>Intermediates (29)<br>Susptible(23) | 107 | 9:4:3 | 60.19 | 26.75 | 20.06 | 1.0665 | 0.5867 | ns |
| | | 107 | 12:3:1 | 80.25 | 20.06 | 6.69 | 51.717 | 5.887e-12 | *** |
| | | 107 | 9:6:1 | 60.19 | 40.13 | 6.69 | 43.322 | 3.915e-10 | *** |

Ho: Observed and expected ratios are the same
HA: Observed ratio is not the same as the expected ratio
Chi square tests (Table 8) revealed that the available data for F1 population (Monyet x Kokopo) segregates according to 9:4:3 ratio. This points out to a possibility of recessive epistasis, characterized by complete dominance at both gene pairs; however, when one gene is homozygous recessive, it hides the phenotype of the other gene.

**Objective/Study 5. Mapping resistance to banana nematodes (*Radopholus similis*)**

An F1 population of Calcutta 4 × Zebrina GF is being phenotyped using the cup method, 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.

Phenotypic data for 111 F1 genotypes (Calcutta 4 × Zebrina GF) population were collected, cleaned and analysed using R- software. Forty three more F1 genotypes are still undergoing screening.

Genotyping of this population using a SNP chip will be done as soon as the chip is ready.

Analysis of variance for checks and parents across different experiments (Table 9) revealed that genotypes were significantly different, replicates were also significantly different, and experiments were significantly different. For both total nematode counts and percentage necrosis. However the experiment by genotype interaction was not significant for total nematode counts, but it was significant for percentage necrosis. Therefore the percentage necrosis data were dropped for further calculations and used total nematode count data. Due to absence of interaction between genotypes and experiment, all the data from the 7 different experiments were combined and analyzed together.

Results from Dunnet's test revealed 14 resistant genotypes, 58 susceptible genotypes and 39 partially resistant. Both traits were positively correlated with a correlation coefficient of 0.589 and $P < 0.001$. This indicates that the greater the number of nematodes, the higher the root damage.

Broad sense heritability (Table 10) for nematode resistance was estimated to be 0.58 and 0.41 for total nematode counts and percentage necrosis respectively. This value is high enough for the trait to be fixed through cross breeding.

**Table 9. Analysis of variance for checks and parents which were in all the experiments**

| | | MS | |
|---|---|---|---|
| **Source** | df | TNC | % Necrosis |
| **Rep** | 2 | 3.711*** | 0.125* |
| **Exp** | 6 | 2.226*** | 0.236*** |
| **Geno** | 3 | 47.292*** | 2.742*** |
| **Rep x Geno** | 6 | 0.158 | 0.048 |
| **Exp x Geno** | 12 | 0.550 | 0.093** |
| **Rep x Exp** | 10 | 0.444 | 0.047 |
| **Rep x Exp x Geno** | 9 | 0.228 | 0.095* |
| **Residual** | 110 | 0.335 | 0.039 |
| **Total** | 158 | 1.351 | 0.107 |
| **CV (%)** | | 29.520 | 41.760 |
| **SED** | | 0.523 | 0.179 |

SED: Standard error of the mean, CV: Coefficient of variation, Exp: Experiment, Rep: Replication, TNC Total nematode count, *, **, ***: significant at 0.05, 0.01 and 0.001 respectively.

**Table 10. Broad sense heritability estimation**

| Variance components | TNC | Necrosis |
|---|---|---|
| **Vg(F1)** | 0.27 | 0.01 |
| **Ve** | 0.60 | 0.05 |
| **Vp (Vg+Ve/r)** | 0.47 | 0.03 |
| **$H^2$(Vg/Vp)** | 0.58 | 0.41 |

TNC: Total Nematodes Count, Vg: Genotypic variance component, Ve: Environmental variance component, Vp: Phenotypic variance component, $H^2$: Heritability

**Table 11. Chi square test between observed ratios and different expected ratios**

| Tested ratio | Expected values | observed values | Chi square calculated/ $X^2 = \sum (O-E)^2/E$ | chi-square probability | Significant |
|---|---|---|---|---|---|
| **1:2:1** | 28:55:28 | 14:39:58 | 36.081 | < 0.001 | *** |
| **9:6:1** | 62:42:7 | 58:39:14 | 4.007 | 0.057 | ns |
| **9:3:4** | 62:21:28 | 58:14:39 | 13.587 | 0.005 | ** |
| **12:3:1** | 83:21:7 | 58:39:14 | 22.584 | < 0.001 | *** |

Ho: Observed and expected ratios are the same
HA: Observed ratio is not the same as the expected ratio
The segregation pattern conformed to a ratio of 9:6:1 (Table 11), implying polymeric gene action. This trait could be controlled by two dominant genes with additive effects. The presence of one gene produce identical phenotype but when both are present, the phenotype is enhanced.

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

Fifty six banana genotypes including hybrids, their parents and grandparents were planted in the field at Namulonge/Sendusu following a 7 x 8 rectangular lattice design with two replications. The following data were recorded over three cycles: planting date, cycle, date of flowering, height of plant at flowering, number of standing leaves at flowering (NSL), youngest leaf with at least 10 necrotic spots at flowering, Plant girth at 100cm from the ground, height of tallest sucker at flowering, number of suckers (peeper, sword, maiden) at flowering, harvest date, bunch weight, number of hands on a bunch, number of fingers on the bunch, finger length, finger circumference. The plant stature was computed as the ratio of plant girth at 100 cm to plant height at flowering. The number of days to flowering, number of days from planting to harvest (maturity period) and number of days from flowering to harvest (fruit filling period) were computed. The planting dates for second and third cycles were obtained by recording the dates of sucker emergency from the soil. The index of non-spotted leaves (INSL) was computed using the formula below:

$$INSL = \frac{(YLS-1)}{NSL} X100$$

In situations where the YLS was 0, the above formula was modified in such a way that, YLS = NSL+1

Path analysis was performed using IBM SPSS version 23 (IBM corporation, 2015) on the agronomic parameter to investigate the traits which contribute significantly to bunch weight, which is a measure of yield. Bunch weight was the dependent variable while, cycle, number of hands on a bunch (NOHOB), number of fingers on the bunch (NOFB), finger length (FL), finger circumference (FC), plant stature, number of days to flowering (DTF), number of days from planting to harvest (DTM), number of days from flowering to harvest (DTFF) and index of non-spotted leaves (INSL) were the independent variables. Path coefficients for direct effects and for indirect effects were calculated. Error for the dependent variable was also calculated from the formula: $\sqrt{(1-R^2)}$.

Finger length (FL), finger circumference (FC), number of hands on a bunch (NOHOB), number of fingers on the bunch (NOFB) and plant cycle were found to have a direct effect on bunch weight (Table 12) (Fig.7). However, index of no-spotted leaves, days to fruit filling, days to maturity and plant stature had an indirect effect on bunch weight (Fig. 7)

Number of days from planting to harvest (DTM), number of days from flowering to harvest (DTFF), index of non-spotted leaves (INSL) and plant stature had an indirect effect to bunch weight through number of fingers on the bunch (NOFB) (Table 13).

Also, it was revealed that number of days from planting to harvest (DTM), number of days from flowering to harvest (DTFF), index of non-spotted leaves (INSL) and plant stature had an indirect effect to bunch weight through plant cycle (CYCLE) (Table 14).

Number of days from planting to harvest (DTM) was also shown to have an indirect effect on bunch weight through finger length (FL) (Table 15) and through number of hands on a bunch (NOHOB) (Table 16)

**Table 12. Path coefficients for components of bunch weight with direct effects**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Tolerance | VIF |
| **1** | (Constant) | -18.182 | 0.936 | | -19.431 | 0 | | |
| | FC | 0.236 | 0.035 | 0.14 | 6.636 | 0 | 0.909 | 1.1 |
| | FL | 1.119 | 0.05 | 0.489 | 22.502 | 0 | 0.857 | 1.166 |
| | NOFB | 0.051 | 0.003 | 0.373 | 16.573 | 0 | 0.797 | 1.255 |
| | NOHOB | 0.077 | 0.023 | 0.069 | 3.355 | 0.001 | 0.946 | 1.057 |
| | Cycle | 1.693 | 0.225 | 0.165 | 7.543 | 0 | 0.844 | 1.185 |

a. Dependent Variable: BW (Kg)

Standardised coefficients (Beta) are the path coefficients.

Error BW = 1 - 0.637= 0.363

**Table 13. Path coefficients for growth characteristics with indirect effects to bunch weight through number of fingers on the bunch**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Tolerance | VIF |
| **1** | (Constant) | 39.694 | 10.861 | | 3.655 | 0 | | |
| | DTM | 0.153 | 0.017 | 0.282 | 8.941 | 0 | 0.982 | 1.018 |
| | DTFF | 0.119 | 0.031 | 0.12 | 3.816 | 0 | 0.98 | 1.02 |
| | STATURE | -7.968 | 2.525 | -0.141 | -3.156 | 0.002 | 0.486 | 2.059 |
| | INSL | 0.392 | 0.084 | 0.21 | 4.676 | 0 | 0.485 | 2.062 |

a. Dependent Variable: NOFB

Standardised coefficients (Beta) are the path coefficients

Error NOFB = 1 - 0.127 = 0.873

**Table 14. Path coefficients for growth characteristics with indirect effects on bunch weight through plant cycle**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Tolerance | VIF |
| **1** | (Constant) | 0.54 | 0.143 | | 3.788 | 0 | | |
| | DTM | 0.002 | 0 | 0.342 | 10.909 | 0 | 0.982 | 1.018 |
| | DTFF | -0.001 | 0 | -0.093 | -2.96 | 0.003 | 0.98 | 1.02 |
| | STATURE | -0.143 | 0.033 | -0.192 | -4.312 | 0 | 0.486 | 2.059 |
| | INSL | 0.005 | 0.001 | 0.2 | 4.485 | 0 | 0.485 | 2.062 |

a. Dependent Variable: Cycle

Standardised coefficients (Beta) are the path coefficients; Error Cycle = 1 – 0.137 = 0.863

**Table 15. Path coefficient for growth characteristic with indirect effect to bunch weight through finger length**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Tolerance | VIF |
| **1** | (Constant) | 14.708 | 0.531 | | 27.687 | 0 | | |
| | DTM | 0.007 | 0.001 | 0.206 | 6.307 | 0 | 1 | 1 |

a. Dependent Variable: FL

Standardised coefficients (Beta) are the path coefficients

Error Finger length = 1 – 0.042 = 0.958

**Table 16. Path coefficient for growth characteristic with indirect effect to bunch weight through number of hands on a bunch.**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Tolerance | VIF |
| **1** | (Constant) | 5.744 | 1.105 | | 5.197 | 0 | | |
| | DTM | 0.008 | 0.002 | 0.121 | 3.651 | 0 | 1 | 1 |

a. Dependent Variable: NOHOB

Standardised coefficients (Beta) are the path coefficients

Error NOHOB = 1 – 0.015 = 0.985

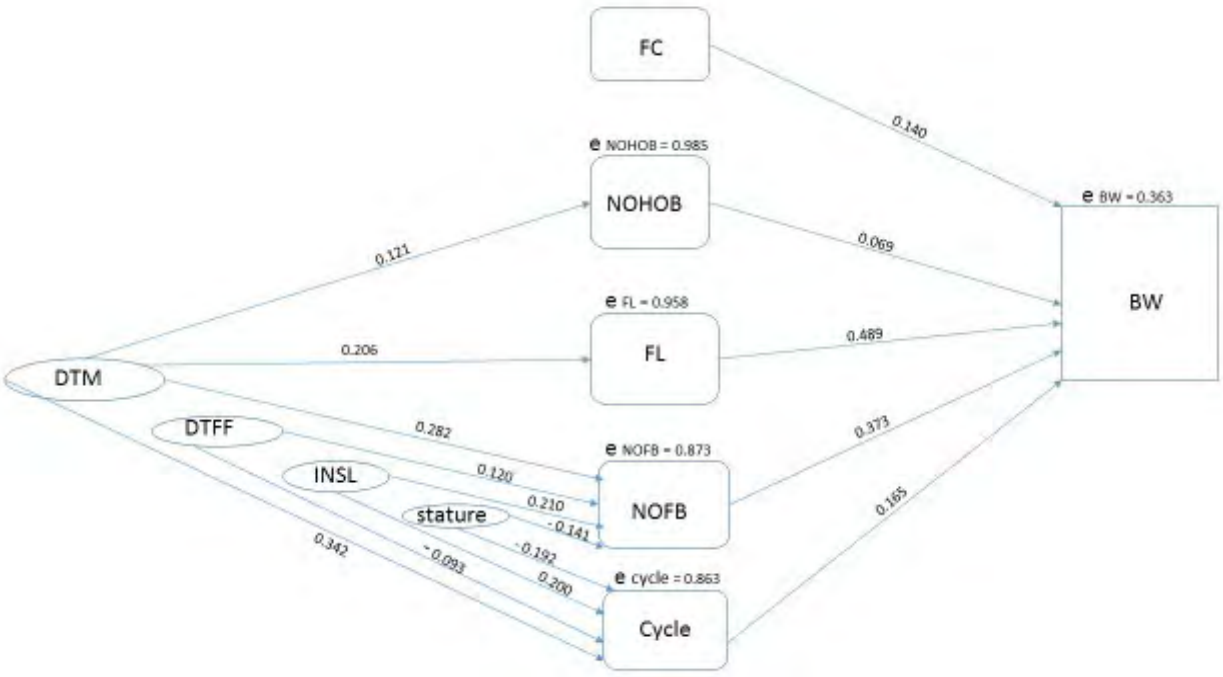


**Fig. 7. Path diagram showing direct and indirect effects of bunch components and growth characteristics on bunch weight. BW = Bunch weight (Kg); FC = Finger circumference; NOHOB = Number of hands on bunch; FL= Finger length; NOFB = Number of fingers on bunch; Cycle = Plant cycle; DTM = Days to maturity; DTFF = Days to fruit filling; INSL = Index of non-spotted leaves; Stature = Plant stature at flowering; e = error of dependent variable.**

**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
- Mapping quantitative trait loci accounting for resistance to *Radopholus similis*
- Mapping quantitative trait loci accounting for resistance to banana weevil
- Write manuscripts for each study objective

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## WP1 - PhD Research Progress Report (2017-2018)

**TITLE:** Genomic selection to accelerate banana breeding: Genotyping by sequencing of banana hybrids

**Name of Student:** **MOSES NYINE**

**Supervisor:** Prof. Jaroslav Doležel, Prof. Rony Swennen, Dr. Brigitte Uwinama and Dr. Allan Brown

**Timeline of study:** 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
4. population and select the best genomic prediction model for each trait, or a group of traits.
5. To determine the predictive ability of models with a training population grown under two different field management practices (Genotype × Environment interaction).
6. 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) .
7. To determine the effect of accounting for allelic dosage on the predictive ability of the best genomic prediction model for each trait.
8. 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.
9. To determine the accuracy of selection achieved based on GEBV relative to phenotypic data within the training population.

**Achievements**

- PhD thesis was submitted and defended on July 12, 2018 and diploma received on July 23, 2018.
- Gave a talk on "the benefits, challenges and prospects of genomic prediction in polyploid banana" on January 16, 2018 at PAG XXVI, San Diego, California.
- 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 have been published and can be accessed from the link: https://doi:10.3835/plantgenome2017.10.0090
- 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

**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 are 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, 52 primer pairs were designed from sequences in that region and screened with a set of eight genotypes including four good and four poor fruit filling hybrids. None of the screened primers were polymorphic for the region and could not distinguish the genotypes. However, the SNP markers and those in linkage disequilibrium were used in the banana SNP chip being developed. Genome wide association study of the yield component traits in the training population using phenotype data from three fields and three cycles is ongoing and will be summarized into a manuscript.
Validation population of 200 genotypes was selected from early evaluation trials and is being phenotyped at Sendusu. Some NARITA hybrids pollinated produced some seed ranging from 0-78 but regeneration of hybrids from those seeds was very poor. Crosses involving NARITAs were temporarily suspended as the field was re-established. Systematic crosses involving NARITA hybrids have to be made to evaluate their potential for further improvement.

**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. These results were used to select SNPs to include in the banana SNP chip. Finally, it is important that the fertility of triploid hybrids with high GEBV is tested to ensure progressive breeding in bananas but this will depend on the priorities of the breeders.

# WP1 - PhD Research Progress Report (2017-2018)

**TITLE:** Floral Biology and Crossability Studies for Improving Matooke and Mchare Banana (*Musa* ssp.) Breeding in East Africa

**Name of Student:** **ALLAN WANIALE**

**Supervisor:** Assoc. Prof. Settumba B. MUKASA (Makerere University) and Prof. Rony SWENNEN (IITA Supervisor)

**Timeline of study:** May 1, 2015 to April 30, 2019

**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 2nd May, 2017
- Increased seed set by 41.5 % in seed fertile matooke – Enzirabahima, 56.7 % in Mshale and 135.9 % in Nshonowa
- Determined that glucose is a better energy source for pollen viability test and *in vivo* germination
- First paper submitted to *Acta Horticulturae* for review, second and third are being written, they will be submitted by December 2018

**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 an entry when banana flowers are most receptive for successful controlled pollination. A photographic catalogue has been finalized for all flower developmental stages including pre-emergence, post-emergence, anthesis and post anthesis. It was 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 more 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*. Results (Table 1) have shown that glucose works better than sucrose, fructose or diluted banana nectar for viability test of banana pollen. Results indicated that use of 3% glucose pollen germination media was able to germinate more pollen in a short time compared to no stigma treatment (Fig 1 A&B). Results also revealed that 3% glucose pollen germination media was able to germinate pollen of a distant banana relative on banana stigmas (Fig 1 C&D). This implies that banana crosses that have pollen and stigma incompatibility can easily be dealt with by using 3% glucose pollen germination media. Pollen germination media was prepared by weighing;

- 0.01g $H_3BO_3$ (Boric acid),
- 0.25g $MgSO_4.7H_2O$ (Magnesium Sulphate),
- 0.25g $KNO_3$ (Potassium Nitrate) and,
- 0.4g $Ca(NO_3)_2$ (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 (1:9) was also compared to 3% glucose pollen germination media and results revealed that glucose was better than diluted nectar (Table 2).

**Table 1.** Mean pollen germination percentages after 3 hour incubation of PGM prepared from different sugars at varying concentrations

| Concentration | Glucose | Sucrose | Fructose | Glucose + Fructose | Glucose + Fructose + Sucrose |
|---|---|---|---|---|---|
| **1%** | 47.2ab | 25.5de | 33.9c | 41.1b | 31.9cd |
| **3%** | 48.9a | 22.4ef | 22.3ef | 30.3cd | 28.7cde |
| **5%** | 41.4b | 13.6ghi | 15.9fgh | 13.1ghi | 16.9fg |
| **10%** | 13.7ghi | 4.8jkl | 5.3jkl | 10.7ghij | 14.4ghi |
| **15%** | 13.0ghi | 2.4l | 3.5kl | 3.0l | 9.9hijk |
| **20%** | 5.6jkl | 1.3l | 5.2jkl | 2.3l | 7.8ijkl |
| Mean | **28.3A** | **11.7B** | **14.3B** | **16.8B** | **18.3B** |

Means with different letters are statistically different at P < 0.001

**Table 2.** Mean pollen germination percentages after 3 hours of incubation of PGM prepared from 3% glucose and diluted banana nectar of improved diploid TMB2X8075-7 and EAHB Tereza on ten banana genotypes with 2 repetitions

| Genotype | Genome / Ploidy | Use | 3% Glucose | Nectar - 8075 | Nectar – Tereza | Genotype mean |
|---|---|---|---|---|---|---|
| **Calcutta 4** | AA | Wild | 39.5 | 37.2 | 24.5 | 33.7bc |
| **Zebrina GF** | AA | Breeding | 46.3 | 44.6 | 27.4 | 39.4ab |
| **TMB2X8075-7** | AA | Breeding | 57.2 | 56.9 | 21.7 | 45.3a |
| **1119** | AA | Breeding | 31.7 | 45.6 | 37.8 | 38.4ab |
| **Enzirabahima** | AAA | Matooke | 26.1 | 20.7 | 29.9 | 25.5cd |
| **Tereza** | AAA | Matooke | 22.3 | 13.3 | 23.2 | 19.6d |
| **Namwezi** | AAA | Matooke | 23.9 | 22.8 | 16.3 | 21.0d |
| **365K-1** | AAAA | Breeding | 53.7 | 34.5 | 14.1 | 34.1bc |
| **376K-1** | AAAA | Breeding | 45.3 | 26.8 | 21.6 | 31.2bc |
| **401K-1** | AAAA | Breeding | 53.0 | 31.6 | 22.7 | 35.8b |
| Mean | | | **39.9A** | **33.4AB** | **23.9B** | |

Means with different letters are statistically different at P < 0.001

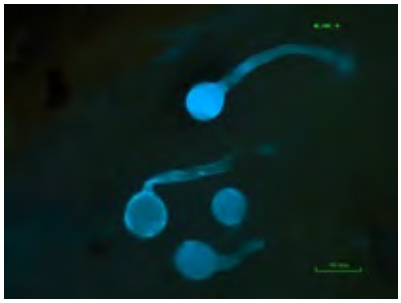

Fig 1-A. Scanty Calcutta 4 pollen on untreated Mbwazirume stigma

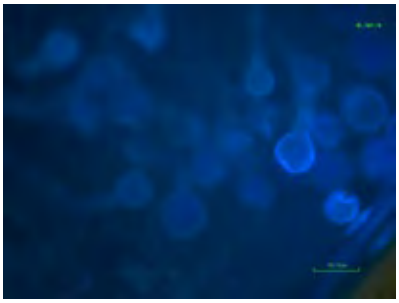

Fig 1-B. Germinating Calcutta 4 pollen on a Mbwazirume stigma treated with PGM

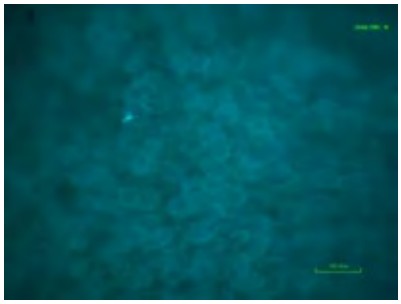

Fig 1-C. None germinating *Heliconia collinsiana* pollen on untreated Calcutta 4 stigma

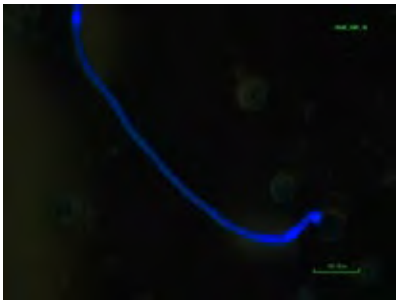

Fig 1-D. Growing *Heliconia collinsiana* pollen tube on Calcutta 4 stigma after pollination with PGM

[1]Bar = 100µm
[2]All images captured at 1 hour after pollination

**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 manipulating flowers with different pollination techniques to come up with the best *in vivo* pollination technique. Since 3% glucose showed that it could germinate pollen fast, it was used on stigmas in an attempt to germinate pollen fast before it desiccates (Fig. 2). Preliminary results show that pollination media on stigmas can increase seed set in bananas (Table 3). But irrespective of the pollination technique used, there is a general tendency of high seed set in bunches pollinated in the dry seasons (Table 4). Early pollination (about a day before flower opening) and pollination in the evening has not had any increase in seed set. Results show that pollen germination media enables germination of pollen that leads to fertilization of ovules in both seed fertile and seed sterile EAHBs but ovules abort after 2 weeks (Figure 3A). In seed fertile edible bananas, seed set seems to be biased at the tip of the fruit (Fig. 3B) and there is also a bias of seed set in the distal hands of the bunch (Table 5). Literature is being reviewed to come up with an explanation of these observations.

I am currently using germination media in combination with growth regulators especially those that are directly or indirectly involved in seed and/or fruit development. Growth regulators used so far include auxins, salicylic acid, abscisic acid (ABA), cytokinins (6 BAP), gibberellin inhibitor (B-nine), Thiourea to inhibit parthenocarpy and auxin inhibitor TIBA. All Plant Growth Regulators tried so far have not shown any sign of overcoming sterility or increasing seed set.

***(A)*** *Flower bract forced open and petals removed to expose stigmas for pollination*

*(**B**) Glucose solution applied with hand sprayer*

***(C)*** *Brushing male flowers on stigmas to expel pollen*

*(**D**) Flower bract returned in position, inflorescence re-bagged and labeled for next pollination*

**Figure 2**: **a photographic description of early pollination of bananas using a 3% glucose solution**

**Table 3.** Mean seed set per 100 fruits in (EAHB) Enzirabahima (3X), Mshale (2X) and Nshonowa (2X) pollinated with Calcutta 4 between January 16 and April 2018 using customary pollination technique and pollination with 3% pollen germination media

| Culivar | Pollination Technique | No of bunches | Av seed set / 100 fruits | % seed increase |
|---|---|---|---|---|
| Enzirabahima | Customary | 77 | 2.15 | |
| | With Glucose | 77 | 3.05 | 41.5 |
| Mshale | Customary | 28 | 34.70 | |
| | With Glucose | 18 | 54.38 | 56.7 |
| Nshonowa | Customary | 31 | 4.95 | |
| | With Glucose | 37 | 11.68 | 135.9 |

**Table 4:** Seed set by month results for one matooke (3X) and two Mchare (2X) female fertile bananas pollinated between January 2016 and May 2018 at NARL

| Pollination month | Enzirabahima (3X) | | | | Mshale (2X) | | | | Nshonowa (2X) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total bunches | With seed | Success Rate (%) | Av Seed / 100fruits | Total bunches | With seed | Success Rate (%) | Av Seed / 100fruits | Total bunches | With seed | Success Rate (%) | Av Seed / 100fruits |
| **Jan** | 22 | 12 | 54.5 | 6.36 | 8 | 8 | 100.0 | 22.50 | 27 | 16 | 59.3 | 7.77 |
| **Feb** | 32 | 19 | 59.4 | 7.06 | 13 | 13 | 100.0 | 81.67 | 18 | 10 | 55.6 | 31.37 |
| **Mar** | 17 | 5 | 29.4 | 12.84 | 8 | 6 | 75.0 | 22.58 | 19 | 14 | 73.7 | 13.67 |
| **Apr** | 21 | 4 | 19.0 | 2.27 | 5 | 3 | 60.0 | 16.21 | 13 | 3 | 23.1 | 14.89 |
| **May** | 22 | 4 | 18.2 | 5.23 | 1 | 0 | 0.0 | 0.00 | 5 | 1 | 20.0 | 7.84 |
| **Jun** | 19 | 9 | 47.4 | 3.89 | 1 | 1 | 100.0 | 6.98 | 7 | 1 | 14.3 | 3.57 |
| **Jul** | 29 | 6 | 20.7 | 3.04 | 3 | 3 | 100.0 | 91.66 | 4 | 0 | 0.0 | 0.00 |
| **Aug** | 34 | 5 | 14.7 | 2.9 | 3 | 2 | 66.7 | 35.14 | | | | |
| **Sep** | 21 | 2 | 9.5 | 2.01 | 1 | 1 | 100.0 | 118.84 | | | | |
| **Oct** | 16 | 2 | 12.5 | 1.69 | 4 | 4 | 100.0 | 68.71 | | | | |
| **Nov** | 15 | 3 | 20.0 | 1.76 | 4 | 3 | 75.0 | 27.52 | | | | |
| **Dec** | 17 | 16 | 94.1 | 8.80 | 5 | 4 | 80.0 | 29.18 | 16 | 10 | 62.5 | 4.83 |

Av Seed/100fruits is the average seed set among bunches with seed

**Table 5.** Percentage seed set by hand position and by number of hands per bunch among female fertile East African Highland Bananas (EAHBs) Enzirabahima (3X), Mshale (2X) and Nshonowa (2X) pollinated with Calcutta 4 between January 16 and April 2018

| Cultivar | Hands per bunch | Bunch no. | Total seed | Seed per bunch | Percentage seed set by hand | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Enzirabahima (3X) | 4 | 3 | 6 | 2.0 | 0.0 | 0.0 | 66.7 | 33.3 | | | | | | |
| | 5 | 10 | 24 | 2.4 | 0.0 | 4.2 | 29.2 | 37.5 | 29.2 | | | | | |
| | 6 | 23 | 150 | 6.5 | 1.3 | 12.7 | 15.3 | 28.0 | 29.3 | 13.3 | | | | |
| | 7 | 21 | 96 | 4.6 | 0.0 | 1.0 | 17.7 | 19.8 | 16.7 | 35.4 | 9.4 | | | |
| | 8 | 10 | 26 | 2.6 | 0.0 | 0.0 | 23.1 | 19.2 | 15.4 | 15.4 | 19.2 | 7.7 | | |
| Mshale (2X) | 5 | 4 | 58 | 14.5 | 5.2 | 20.7 | 29.3 | 32.8 | 12.1 | | | | | |
| | 6 | 13 | 535 | 41.2 | 6.0 | 8.2 | 24.5 | 23.2 | 22.6 | 15.5 | | | | |
| | 7 | 20 | 873 | 43.7 | 11.5 | 18.4 | 15.0 | 16.7 | 14.9 | 13.7 | 9.7 | | | |
| | 8 | 2 | 44 | 22.0 | 0.0 | 2.3 | 9.1 | 13.6 | 22.7 | 40.9 | 11.4 | 6.8 | | |
| Nshonowa (2X) | 5 | 1 | 4 | 4.0 | 0.0 | 75.0 | 25.0 | 0.0 | 0.0 | | | | | |
| | 6 | 3 | 59 | 19.7 | 0.0 | 28.8 | 33.9 | 30.5 | 5.1 | 1.7 | | | | |
| | 7 | 12 | 199 | 16.6 | 17.1 | 17.1 | 11.1 | 14.1 | 15.1 | 8.5 | 17.1 | | | |
| | 8 | 10 | 142 | 14.2 | 2.1 | 2.8 | 6.3 | 9.2 | 11.3 | 24.6 | 39.4 | 4.2 | | |
| | 9 | 3 | 36 | 12.0 | 0.0 | 27.8 | 0.0 | 0.0 | 2.8 | 25.0 | 8.3 | 2.8 | 33.3 | |
| | 10 | 1 | 3 | 3.0 | 0.0 | 0.0 | 0.0 | 66.7 | 0.0 | 0.0 | 0.0 | 0.0 | 33.3 | 0.0 |


Fig. 3-A. Smaller ovules from a Matooke (Mpologoma) bunch that was not pollinated compared to a few bigger aborted ovules from a pollinated bunch


Fig. 3-B. Seed set position in Matooke (Enzirabahima) biased toward the fruit tip.

**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.

**Conclusion / next steps**

All bananas seem to have a potential for producing seed and the secret lies in finding the right procedures to overcome pre- and post-fertilization barriers. Hope is in the use of plant hormones and their inhibitors especially those 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. But all attempts to use plant growth regulator have so far been futile. There is a plan to develop an ovule culture procedure in bananas using wild types (Calcutta 4) and wild relatives (*Heliconia* and *Strelitzia*). Preliminary results are showing poor response of ovules on culture media although this has been done on *Alstroemeria, Cyclamen, Lycopersicon, Nicotiana* and *Vitis.* Attempts to find suitable culture media for banana ovules will continue as well as measures of finding the plant hormones that can overcome sterility. There is a planned doctoral committee meeting on October 11, 2018, the aim is to discuss results achieved so far, papers being drafted and tentative write up of the thesis.

On 22nd April 2018, we held a Skype meeting with two Scientific Advisory Group (SAG) members and we discussed the role of environment on seed fertility in bananas and plantains. The teams involved in the discussion were the SAG members, a team from IITA Nigeria, IITA Arusha, NARO Uganda and partners from Czech Republic. As a way forward, we discussed a list of factors that could be involved in seed set and the decision was to come up with a priority list in a chronological order with the most important factors first. The discussion is still on-going about the manipulative experiments that will overcome sterility in bananas. The discussants also agreed that specific roles will be assigned to stations involved in banana breeding to avoid duplication. These on-going discussions are meant for developing a future work plan.

## WP1 - MSc Research Progress Report (2017-2018)

| | |
|---|---|
| **Research title:** | Enhancing seed set in East African diploid cooking banana (Mchare cultivars) |
| **Name of Student:** | **VERONICA MASSAWE** |
| **Field of study:** | MSc in Life Science (Sustainable Agriculture) |
| **University:** | Nelson Mandela African Institution of Science & Technology (NM-AIST) |
| **Timeline of study:** | January 2018-December 2019 |
| **Supervisors**: | Dr. Pavithravani Venkantaramana (NM-AIST), Prof. Patrick Ndakidemi (NM-AIST) and Dr. Allan Brown (IITA) |

### Research Objectives

i. To characterize floral structures of Mchare cultivars, wild diploids and F1 plants
ii. To quantify pollen grains produced by Mchare cultivars, wild diploids and F1 plants at different bunch development stages
iii. To assess pollen viability and germination in Mchare cultivars, wild diploids and F1 plants at different bunch development stages
iv. To assess the influence of season during pollination on the seed set in Mchare cultivars

### Achievements

1. Successfully completed one semester (course work) at NM-AIST and now undertaking second semester. I undertook courses such as Biostatistics, Plant Molecular breeding, Seed systems and Seed technology, Sustainable Crop Protection and Soil health management, Foundations of Law in Science, Engineering and Technology, Philosophy, Ethics and Social Imperative and graduate seminars.
2. Established a field for data collection

### Introduction

Banana is one of the most important crops, and is among the 10 most important staples in the world (Ortiz & Swennen, 2014). They are part of a well-balanced human diet, a major food staple for more than 400 million people in the tropics (Hölscher et al., 2014). Average annual banana harvest is about 145 million tons worldwide, and 85% comes from smallholder farmers in developing countries (Ortiz & Swennen, 2014). East African diploid cooking bananas (EADB or Mchare) are a staple crop for millions of consumers in Tanzania and Kenya (Ssebuliba et al., 2008; Ssali et al., 2012). These diploids are parthenocarpic bananas, whereby unpollinated ovaries will automatically develop into fruits (Dodds & Simmonds, 1948). Poor seed production in banana especially East African cooking diploids (Mchare) is one of the principle limiting factors in banana improvement at IITA.

Currently, banana and plantain production worldwide is threatened by fungal pathogens such as *Fusarium oxysporum* f. sp. *cubense* (Foc) (Ssali et al., 2012), and *Mycosphaerella fijiensis* and pests such as nematodes and weevils as well as drought spells. This pest and disease complex endangers both the staple cooking bananas of East Africa and the worldwide export market of dessert bananas (Ssebuliba et al., 2006). Adequate levels of resistance have been

identified in wild diploid banana varieties such as “Calcutta 4” (Ssebuliba et al., 2006; 2008) and have been used in improving susceptible banana cultivars including Mchare cultivars (Ssali et al., 2012).

The rate of genetic improvement in cultivated *Musa* depends on the reproductive fertility (Dumpe & Ortiz, 1996). Poor seed production in banana is one of the principle limiting factors in plant improvement, especially in banana. Ortiz et al. (1998) suggested that seed production relies on the fertility of both parents which may be influenced by the environment. In their study on seasonal variation of male fertility, they concluded that pollen stainability (used as a measure of male fertility) varied with the season. Apart from environmental factors, physiological as well as genetic factors affect seed set in banana.

Most cooking bananas are parthenocarpic, and it takes extra efforts to produce seeds from them through artificial (hand) pollination. The seed set is very low regardless of thousands of crosses made throughout the year. At NM-AIST, only 5% of crosses will produce seed. The average number of seeds produced from one successful cross is 2-10 seeds per bunch. This leads to a very slow progress in improving these diploids.

Understanding the environmental, physiological and genetic factors that reduce seed production will allow banana breeders to design strategies to enhance seed set and as a consequence to facilitate the development of more improved varieties for local farmers.

This work will characterize diploid bananas that differ in seed production to understand the process based on morphological structures. In addition, the work will involve plant populations that have been created from crosses made between Mchare cultivars and wild diploids to understand how these factors are transmitted from one generation to the next.

Table 1. Plant materials for evaluation

| | **Mchare cultivars** | | **Hybrids** | |
|---|---|---|---|---|
| 1 | Huti white | | Cross no. | Pedigree |
| 2 | Huti green | 1 | T.2273-2 | Huti white x Calcutta 4 |
| 3 | Mchare Laini | 2 | T.2274-7 | Huti white x Calcutta 4 |
| 4 | Mchare mlelembo | 3 | T.2274-12 | Huti white x Calcutta 4 |
| 5 | Makhyugu I | 4 | T.2274-4 | Huti white x Calcutta 4 |
| 6 | Makkhyugu II | 5 | T.2274-3 | Huti white x Calcutta 4 |
| 7 | Ijihu inkundu | 6 | T.2274-6 | Huti white x Calcutta 4 |
| 8 | Akondro mainty | 7 | T.2317-1 | Huti white x Borneo |
| 9 | Kahuti | 8 | T.2327-1 | Huti white x Borneo |
| 10 | Nshonoa | 9 | T.2269-1 | Huti white x Calcutta 4 |
| | | 10 | T.2203-1 | Nshonowa x Calcutta 4 |
| | **Wild diploids** | 11 | T.2070-1 | Huti white x Borneo |
| | 1 Calcutta 4 | 12 | T.2269-2 | Huti white x Calcutta 4 |
| | 2 Cv rose | 13 | T.2115-1 | Nshonowa x Calcutta 4 |
| | 3 Borneo | 14 | T.1687-1 | Huti white x Calcutta 4 |
| | | 15 | T.1768-1 | Huti white x Borneo |

**Specific objective i:** Characterization of floral structures
Floral structures will be characterized with the aid of banana descriptors developed by IPGRI Descriptors to be assessed include;

- Ovary shape, pigmentation, basic color, size (diameter) arrangement of ovules, stigma color, and style length
- Anther exsertion in relation to the base of the lobes on the compound tepal, anther color and pollen sac color

**Specific objective ii**: Quantification of pollen grains at different bunch development stages
Image analysis software ImageJ will be used to count number of pollen grains per anther. Every day one flower bract opens until full bunch maturity (approximately 4-5 months). Pollen quantification will be conducted at 2 weeks interval for 4 months to assess if pollen production changes as banana bunch mature.

- Data will be collected from 28 genotypes x 3 replications x 2 x 4 months

**Specific objective iii:** assessing pollen viability and germination
Staining procedures use 1% Tri-phenyl tetrazolium chloride solution (TTC) for pollen viability tests . For pollen germination tests, pollen grains will be inoculated in culture medium containing 15% sucrose, 0.01% H3BO3, 0.01% KNO3, 0.03% Ca(NO3)2.4H2O, 0.02% MgSO4.7H2O, solidified with 0.8% agar and pH adjusted to 5.8

- Data will be collected from 28 genotypes x 3 replications x 2 x 4 months

**Specific objective iv*:*** To assess influence of season during pollination on seed set in Mchare cultivars
Pollinations will be done throughout the rainy and dry seasons. Seeds will be extracted from all successful crosses. Number of seeds produced from pollinations made during the rainy season will be compared with those made during dry season to see the seasonal effect of pollination on seed set.

**Way forward**

- Complete semester two (course work) by November 2018
- Collect all the necessary data by September 2019
- Thesis write up and submission by November 2019

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## 7.2 WP2 - PhD Research Progress Report (2017-2018)

**Title:** Evaluation of African bananas for resistance to *Fusarium oxysporum* f. sp. *cubense*
**Name of Student:** **PRIVAT NDAYIHANZAMASO**
**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. *cubense*.
2. Develop a rapid screening method of bananas for resistance to *Fusarium oxysporum* f. sp. *cubense* (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 effect of plant age on disease development has been determined.
   - The use of phenolic acids and qPCR of Foc DNA as indicators of disease resistance was investigated.
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 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. *cubense* (Foc) race 1, a soil-borne fungus responsible for Fusarium wilt of banana, but Pisang Awak, Sukari Ndizi, 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 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 -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-1α). They were tested for specificity on 84 *F. oxysporum* isolates from different parts of the world and included 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).

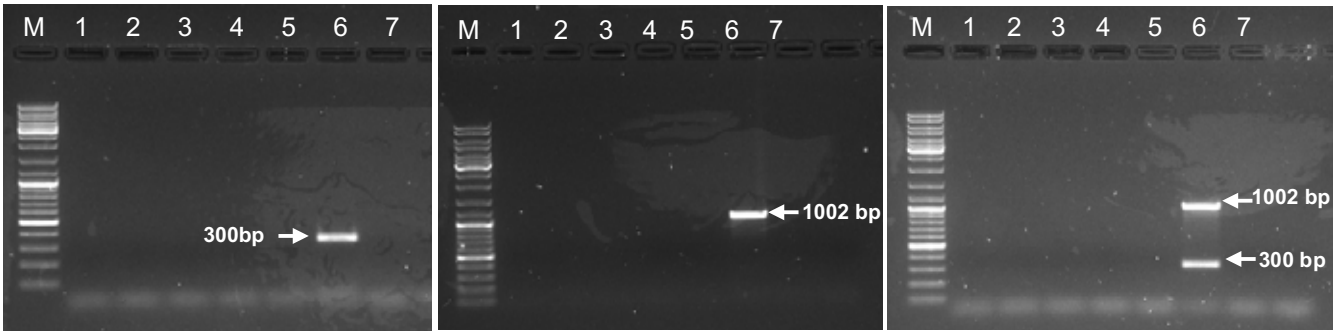
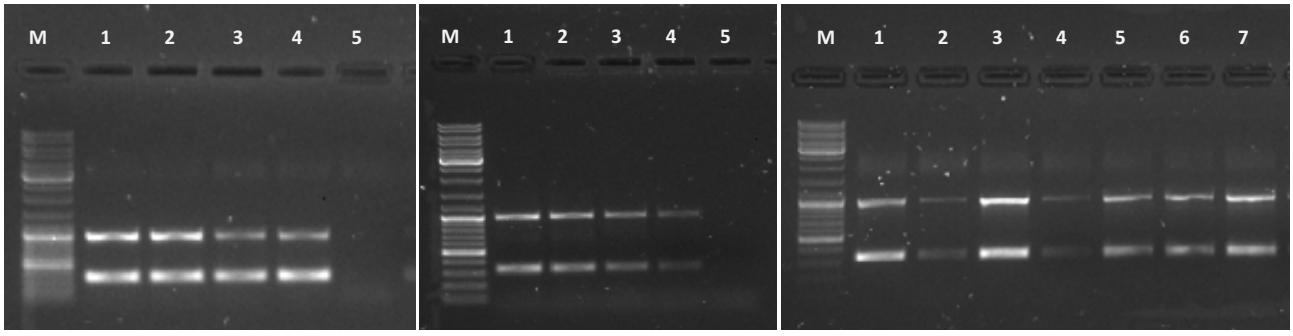



***Figure 1. Specificity testing of two primer sets in individual and multiplex PCR assays for Lineage VI of* Fusarium oxysporum *f. sp.* cubense*. Left: A 300-bp fragment amplified by the FocLin6b-F/R primers, Middle: A 1002-bp fragment amplified by the FocL in VI-F/R primers, Right: Both 300-bp and 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.***




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

***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.***

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% Foc isolates collected at 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. 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 seeds per kg sands (T8,T9 and T10). Three replicates of eight plant each were randomized in a complete block design. A rhizome discolouration index (RDI) with a rating scale ranging from 1 (healthy) to 6 (dead) 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.

Effect of plant age on disease development

The effect of plantlet age on the development of banana Fusarium wilt was investigated in the glasshouse. In this experiment, four banana genotypes differing in resistance to Foc were hardened-off for 1, 2 and 3 months, and planted in soil infested with Foc subtropical race 4 (STR4) on millet seeds at a concentration of 5 g of millet seeds per 1 kg of potting soil. The genotypes included FHIA 17 (tolerant to Foc STR4), FHIA 01 (resistant to Foc STR4), Gros Michel (susceptible to both Foc STR4) and Williams (resistant to Foc STR4). Disease severity was evaluated 6 weeks after inoculation using a rhizome discoloration index (RDI) with a rating scale ranging from 1 to 6. ANOVA (XLSTAT, edition 2017) was used to compare the difference between treatments.

Phenolic compounds as early indicator of disease resistance

Phenolic extraction: Root samples of control and treated banana plants were collected at 0, 10, 20, 30 and 40 hrs after inoculation, and kept at -80 ºC. Samples were freeze-dried and ground in liquid nitrogen to a fine powder. Total soluble phenolic compounds were extracted from 250 mg of freeze-dried roots in 10 ml of 80 % aqueous methanol, while shaking at 150 rpm for 1 hr at room temperature. Samples were then centrifuged for 10 min at 4 000 rpm and 8 ml of the supernatant collected in a new tube while the pellet was dried in a fume hood at room temperature for cell wall-bound phenolic extraction. The supernatant was concentrated to 4 ml under gas nitrogen stream at 40 °C, and the volume increased to 10 ml with water and the pH adjusted to 2-3 using HCl before extraction with 10 ml of ethyl acetate for 5 min at room temperature. The ethyl acetate extract was centrifuged for 5 min at 4 000 rpm, and 8 ml of the upper phase was transferred into a new tube. The 8 ml were reduced to dryness under gas nitrogen at 40°C and stored at -20 °C prior to reconstitution and assays. Cell wall-bound phenolic compounds were extracted from 100 mg of the pellet after alkaline hydrolysis with 4 ml of NaOH (2 N), and shaken for 2 hrs in nitrogen atmosphere (gas nitrogen blown in the tube prior to the shaking). The pH was then adjusted to 2-3 with HCl (12 N). Cell wall-bound phenolic compounds were extracted with ethyl acetate, as previously described for total soluble phenolic compounds and reduced to dryness. Phenolic compounds were reconstituted with 250 µl of 50% aqueous methanol.

Quantification of phenolics in a 96-well microplate reader: The Folin-Ciocalteu assay was used to measure total soluble and cell wall-bound phenolic compounds (Zang *et al*., 2006). The procedure involves the loading 20 µl of each sample and serial standards on a 96-well microplate, followed by the addition of 100 µl of Folin-Ciocalteu reagent (Sigma-Aldrich, South

Africa). Samples were mixed by shaking the microplate and left to stand for 5 min at room temperature. Eighty µl of 7.5 % sodium carbonate solution was then added, and the mixture covered and allowed to stand for 2 hrs in the dark. The absorbance was measured at 725 nm with a spectrophotometric microplate reader. Gallic acid (GA) was used to prepare serial standard solutions of 0, 10, 25, 50, 100, 250 and 500 µg/ml (parts per millions), and distilled water was used as blank. Phenolic contents were quantified using a GA calibration standard curve ranging from 0; 0.1; 0.5; 1; 2; 5 and 10 µg, and results were expressed as µg GA equivalents per g dry roots weight (µg GAE/g).

HPLC analysis of phenolic compounds: Individual phenolic acids were analysed with liquid chromatography-mass spectrometry (LC-MS). An external standard mixture consisted of eight phenolic acids: p-coumaric acid, protocatehuic acid, sinapic acid, GA, 4-hydroxybenzoic acid, ferulic acid, chlorogenic acid and caffeic acid.

The use of qPCR in root and rhizome tissues to identify susceptible and resistant genotypes

Measuring pathogen DNA *in planta* with qPCR can distinguish between susceptible and resistant plants. For instance, Jiménez-Fernández *et al*. (2011) have determined resistance in chickpea cultivars against *F. oxysporum* f. sp. *ciceris* by quantifying fungal DNA in roots 35 days after sowing in infested soil. Pathogen DNA in chickpea roots was positively correlated with the resistance level of chickpea cultivars planted in the field.

A study to determine Foc DNA in the roots and rhizomes of resistant and susceptible banana cultivars by qPCR was performed. Foc VCG 0124 was used as inoculum. Four genotypes Gros Michel (susceptible, used as a positive control), Williams (resistant, used as a negative control), FHIA 01 (resistant) and FHIA 17 (tolerant) were inoculated by dipping their roots in a $10^4$ spores/ml suspension for 2 min, followed by replanting in Foc VCG 0124 (5 g Foc-inoculated millet seeds per 1 kg of potting soil). Root and rhizome samples were collected from three plants per cultivar 2, 3, 4 and 5 weeks after inoculation.

For DNA extraction, root and rhizome samples were surface-disinfected in 2 % NaOCl for 2 min, rinsed twice with tap water, and dried with tissue paper prior to storage at -80 °C. Genomic DNA was then extracted from 100 mg of homogenized, dried and crushed root or rhizome tissues according to the NucleoSpin® Plant II protocol (MACHEREY-NAGEL, GmbH & Co., Germany). Foc DNA in the root and rhizome samples was estimated using a standard curve established with serial dilutions of Foc DNA in a background of banana DNA. The qPCR assay was repeated twice for each sample.

**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 (P<0.001) |
|---|---|---|---|
| **Dipping method + millet seeds (M3)** | $10^2$spores/ml (T8) | 96 | 4.41 ± 0.24[a] |
| | $10^4$spores/ml (T9) | 100 | 4.20 ± 0.24[a] |
| | $10^6$ spores/ml (T10) | 92 | 4.50 ± 0.24[a] |
| **Millet seeds method (M2)** | 1g/kg of soil (T4) | 17 | 1.29 ± 0.24[c] |
| | 2g/kg of soil (T5) | 67 | 2.41 ± 0.24[b] |
| | 5g/kg of soil (T6) | 67 | 2.91 ± 0.24[b] |
| | 10g/kg of soil (T7) | 71 | 2.58 ± 0.24[b] |
| **Drenching method (M1)** | 5 x $10^2$spores/ml (T1) | 8 | 1.13 ± 0.24[c] |
| | 5 x $10^4$spores/ml (T2) | 4 | 1.04 ± 0.24[c] |
| | 5 x$10^6$ spores/ml (T3) | 58 | 2.33 ± 0.24[b] |
| **Controls** | Control for M1 (C1) | 0 | 1.00 ± 0.24[c] |
| | Control for M2 (C2) | 0 | 1.00 ± 0.25[c] |
| | Control for M3 (C3) | 0 | 1.00 ± 0.24[c] |

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 $10^2$ and $10^4$ 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 $10^6$ 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.

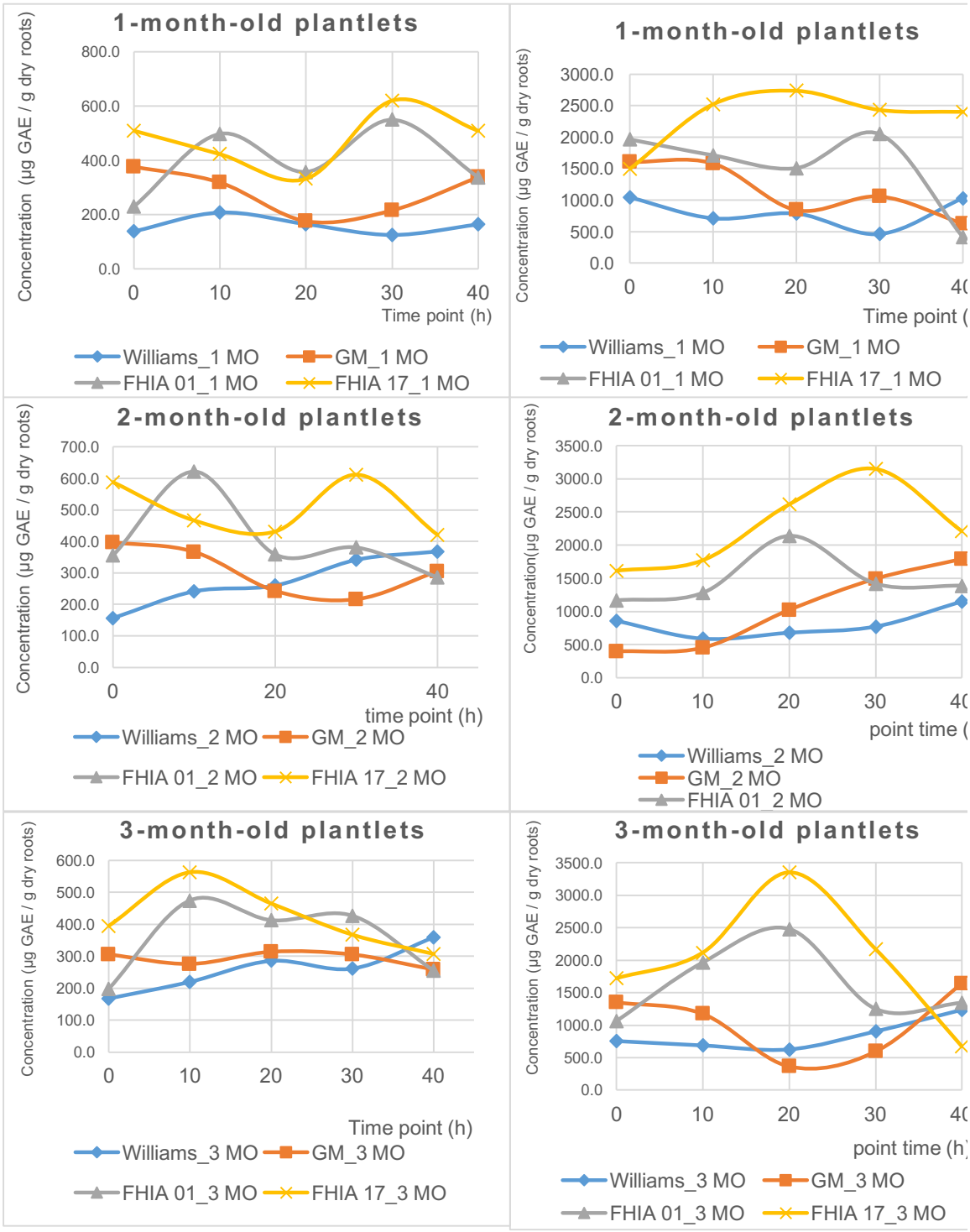
Although the combination of root dipping and infested soil with millet seeds consistently caused the highest ratings to all cultivars, it cannot be used to rank cultivars. The intermediate cultivars were as severely infected as susceptible cultivars. The millet seed method seems to be the most appropriate,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

Phenolic compounds as early indicators of resistance to Foc

Phenolic compounds produced in bananas roots inoculated and non-inoculated with Foc: Following Foc inoculation, the total soluble and cell wall-bound phenolic compounds in 1-, 2- and 3-month-old plantlets of FHIA 01 and FHIA 17 increased up to 2.5 fold (Fig. 3). Cell wall-bound phenolic compounds were 2 to 5 times more abundant than the total soluble phenolic compounds (Fig. 3). The two types of phenolic compounds were, however, positively correlated, irrespective of plantlet age ($r = 0.956$ for 1-mo-old plantlets; $r = 0.994$ for 2- and 3-month-old plantlets, respectively). The increase started less than 10 hours after inoculation and reached a maximum between 10-20 hrs for total soluble phenolic compounds, and 20-30 hrs for cell wall-bound phenolic compounds. Plantlets of Williams and Gros Michel, regardless the age of plants, slightly increased the phenolic production after 20 hrs (Fig. 3).

The amount of total soluble and cell wall-bound phenolic compounds that accumulated in tolerant cultivars (FHIA 01, FHIA 17) in the Foc inoculated plants was higher than in susceptible plants (Williams and Gros Michel), regardless the plantlet age (Table 3 and Table 4). The difference was, however, not significant. Plant age did not have a significant effect on total soluble and cell wall-bound phenolic content (Table 2 and Table 3).

Figure 3: Phenolic compounds in banana roots following infection with *Fusarium oxysporum* f. sp. *cubense* subtropical race 4. Graphs on the left show the total soluble phenolic compounds, and graphs on the right show cell wall bound phenolic compounds. Phenolic compounds were quantified with Folin Ciocalteu reagent as micrograms of gallic acid equivalents per gram dry roots (µg GAE/g dry roots).




Phenolic profiles in roots of banana varieties resistant and susceptible to Foc: Twenty-five phenolic compounds were found in banana roots. Of these, ferulic acid, p-coumaric acid, 4-hydrobenzoic acid (isomer of salicylic acid), benzoic acid, protocatechuic acid, salicylic acid, vanillic acid and quinic acid were the major phenolic acids present constitutively, and their concentration increased after inoculation of plants with Foc. Caffeic acid was resent in FHIA 01 and FHIA 17 before inoculation but it was induced in the Gros Michel and Williams after infection only. Gallic acid was absent in Gros Michel, Williams, but was induced in 1-month-old FHIA 01 and FHIA 17 plantlets. Catechin and epicatechin were induced in FHIA 17 and FHIA 01, but not in Gros Michel and Williams. Salicylic acid, a phenolic compound more commonly known as a phytohormone, was detected in cell wall-bound phenolic compounds.

Of the major phenolic acids, ferulic acid, p-coumaric acid, salicylic acid and vanillic acid seem to distinguish the disease resistance of cultivars inoculated with Foc.

Differentiation of banana varieties by phenolic compound profiles: The total soluble phenolic compounds, cell wall-bound phenolic compounds and individual phenolic acids such as ferulic acid, salicylic acid, p-coumaric acid and vanillic acid acid were able to differentiate the tolerant FHIA 17 and FHIA 01 from the susceptible Gros Michel and Williams cultivars. Difference between these cultivars, however, were not always significant (Table 4). Plant age did not seem to affect the amount of total soluble and cell wall-bound phenolic compounds, and phenolic compounds such ferulic acid, p-coumaric-acid salicylic acid, significantly (Table 4). The accumulation of total soluble and cell wall-bound in the non-inoculated did not differ between the tolerant FHIA 17 and FHIA 01 and the susceptible Gros Michel and Williams cultivars (Table 2 and Table 3). It was not possible to differentiate tolerant from susceptible genotypes in non-inoculated plants (Table 2 and Table 3).

Three-month-old Williams plantlets developed more Fusarium wilt symptoms than 2-month-old plants. Williams is susceptible to Foc STR 4 only when subjected to stressful conditions such cold temperature. The high disease severity observed in 3-month-old plants of Williams may have resulted from limiting potting conditions such small size of the pot and nutritional deficiencies. This indicates that source of stresses, environmental or nutritional, should be addressed prior to inoculation as they influence the screening results by predisposing plants to more disease development.

The disease severity was affected by the plant age but the accumulation of phenolic compounds did not. This means that phenolic compounds are not the only factor that defines resistance in bananas to Fusarium wilt. One-month-old plantlets, regardless of their susceptibility or resistance, developed more disease than older plantlets during small plant screening. Such young plantlets, therefore, may not be appropriate to use during greenhouse screening of genotypes for disease resistance. The use of 2- or 3-month-old plants discriminated well between susceptible and resistant bananas, and are recommended for small plant screening for Foc.

Phenolic acids accumulated in banana roots of infected plants with Foc were able differentiate the disease resistance of banana genotypes even though the difference among the four cultivars was always significant. Some phenolic acids, such caffeic acid and gallic acid, were either absent or induced in susceptible cultivars, but their ability to differentiate between resistant and susceptible banana cultivars need to be further investigated.

**Table 2:** Total soluble phenolic compounds in banana roots of four banana genotypes inoculated and non-inoculated with *Fusarium oxysporum* f. sp. *cubense* subtropical race 4.

| Cultivar | Total soluble phenolic compounds (µg GAE / g dry roots) | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated plants | | | Non-inoculated plants (control) | | |
| | 1 month | 2 months | 3 months | 1 month | 2 months | 3 months |
| FHIA 17 | 479.1 a | 504.1 a | 419.4 ab | 514.1 abc | 554.9 ab | 587.9 a |
| FHIA 01 | 394.6 abc | 400.9 abc | 353.3 bcd | 440.5 abcd | 325.9 de | 391.9 bcde |
| Gros Michel | 285.2 cd | 305.3 bcd | 291.4 cd | 386.3 bcde | 307.2 de | 357.3 cde |
| Williams | 160.0 e | 273.6 de | 259.2 de | 359.8 cde | 271.2 de | 235.9 e |
| Pr > F(Model) | < 0.0001 | | | 0.012 | | |
| Significant | Yes | | | Yes | | |

**Table 3**: Cell wall-bound phenolic compounds in banana roots of four banana genotypes inoculated and non-inoculated with *Fusarium oxysporum* f. sp. *cubense* subtropical race 4.

| Cultivar | Cell wall-bound phenolic compounds (µg GAE / g dry roots) | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated plants | | | Non-inoculated plants (control) | | |
| | 1 month | 2 months | 3 months | 1 month | 2 months | 3 months |
| FHIA 17 | 2318.1 a | 2273.9 ab | 2008.1 abc | 1758.7 b | 1715.0 b | 2130.0 a |
| FHIA 01 | 1527.7 cde | 1476.7 cdef | 1622.3 bcd | 1540.6 bc | 1580.1 bc | 1717.2 b |
| Gros Michel | 1142.9 def | 1031.0 def | 1028.4 def | 1351.8 cd | 1491.1 bc | 1712.9 b |
| Williams | 803.6 f | 809.1 f | 841.9 ef | 1307.6 cd | 1187.7 d | 1543.2 bc |
| Pr > F(Model) | 0.0002 | | | < 0.0001 | | |
| Significant | Yes | | | Yes | | |

Table 4: Segregation of *Fusarium oxysporum* f. sp. *cubense* subtropical race 4-infected banana cultivars based on the disease severity and the accumulation of phenolic compounds in roots

| Cultivar * age | Rhizome discolouration index (RDI) | Cell wall-bound phenolic compounds (µg GAE / g dry roots) | Total soluble phenolic compounds (µg GAE / g dry roots) | Ferulic acid (µg/ g dry roots) | Salicylic acid (µg/ g dry roots) | p-coumaric acid (µg/ g dry roots) | Vanillic acid (µg/ g dry roots) | Caffeic acid (µg/ g dry roots) | Free 4-HBA (µg/ g dry roots) | Bound 4-HBA (µg/ g dry roots) | Quinic acid (µg/ g dry roots) | Benzoic acid (µg/ g dry roots) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FHIA 17*1 mo | 2.8 a | 2315.8 a | 479.1 a | 444.2 abc | 113.8 abc | 131.3 ab | 23.7 abc | 5.7 abc | 197.0 ab | 533.8 a | 29.0 ab | 1.5 a |
| FHIA 01*1 mo | 2.4 ab | 1527.0 cde | 394.6 abc | 311.7 bcde | 163.4 a | 143.2 a | 27.4 ab | 7.3 abc | 140.9 bcde | 337.4 ab | 28.3 ab | 0.4 d |
| Gros Michel*1 mo | 2.4 ab | 1140.0 def | 285.2 cd | 158.0 e | 109.4 abc | 61.8 bc | 14.6 bcd | 3.1 c | 167.3 bc | 258.6 b | 13.9 bcd | 1.2 abc |
| Williams*1 mo | 2.5 ab | 804.7 f | 160.0 e | 145.7 e | 168.4 a | 65.5 bc | 18.5 abcd | 5.2 abc | 117.3 cdef | 314.0 ab | 6.5 d | 0.4 d |
| FHIA 17*2 mo | 1.3 d | 2271.6 ab | 504.1 a | 534.4 a | 172.5 a | 150.7 a | 27.5 ab | 9.0 a | 152.3 bcd | 423.9 ab | 26.3 abc | 1.2 ab |
| FHIA 01*2 mo | 1.4 d | 1471.4 cdef | 400.9 abc | 303.9 bcde | 162.8 a | 118.1 abc | 29.3 a | 3.7 bc | 142.5 bcde | 393.8 ab | 14.2 bcd | 0.3 d |
| Gros Michel*2 mo | 2.3 ab | 1029.0 def | 305.3 bcd | 163.6 e | 152.7 ab | 65.1 bc | 8.5 d | 7.2 abc | 206.6 ab | 452.6 ab | 23.9 abc | 0.9 abcd |
| Williams*2 mo | 1.7 cd | 822.2 f | 273.6 de | 275.6 cde | 50.9 c | 93.3 abc | 15.5 abcd | 3.3 c | 246.7 a | 362.3 ab | 12.8 cd | 0.5 cd |
| FHIA 17*3 mo | 1.4 d | 2007.7 abc | 419.4 ab | 473.4 ab | 122.4 abc | 148.0 a | 23.3 abc | 6.5 abc | 86.5 def | 412.6 ab | 29.8 a | 0.8 abcd |
| FHIA 01*3 mo | 1.4 d | 1619.1 bcd | 353.3 bcd | 383.8 abcd | 1584 a | 159.0 a | 24.4 abc | 8.1 ab | 60.9 f | 352.7 ab | 6.8 d | 1.0 abcd |
| Gros Michel*3 mo | 2.4 ab | 1022.1 def | 291.4 cd | 201.1 de | 106.1 abc | 91.3 abc | 23.0 abc | 6.6 abc | 77.7 ef | 371.6 ab | 33.7 a | 0.4 d |
| Williams*3 mo | 2.1 bc | 853.2 ef | 259.2 de | 208.8 de | 64.4 bc | 55.9 c | 11.2 cd | 3.8 bc | 184.8 abc | 470.5 ab | 28.4 ab | 0.7 bcd |
| Pr > F(Model) | < 0.0001 | < 0.0001 | < 0.0001 | 0.001 | 0.141 | 0.019 | 0.061 | 0.206 | < 0.0001 | 0.678 | 0.002 | 0.016 |
| Significant | Yes | Yes | Yes | Yes | No | Yes | No | No | Yes | No | Yes | Yes |

mo: month-old; HBA: hydrobenzoic acid

The use of q-PCR to separate Foc-susceptible from -resistant banana varieties

Foc DNA was low in the roots of Williams, FHIA 01 and FHIA 17 2 weeks after inoculation, and was found in their rhizomes only 4 weeks after inoculation (Fig. 4). Significant differences in DNA levels in the rhizomes of susceptible and resistant varieties were observed only 3, 4 and 5 weeks after inoculation. Foc DNA was higher in rhizome samples than in the root samples of the susceptible Gros Michel (Fig. 4). Conversely, Foc DNA was low in both the roots and rhizomes of cultivars Williams, FHIA 01 and FHIA 17.

Earlier studies have demonstrated that Foc races 1 and 4 reach rhizome tissues of Cavendish bananas 2 weeks after inoculation, but that Foc race 1 does not cause disease (Dita *et al*., 2010; Guo *et al*., 2015). The detection of Foc race 1 in roots and rhizomes of Williams, a Cavendish cultivar, is not surprising. However, the very low concentrations of Foc DNA in Williams compared to the susceptible Gros Michel shows the ability of Cavendish bananas to resist Foc race 1, even though they are infected. The DNA concentration of Foc race 1 in FHIA 01 and FHIA 17 did not differ significantly from that in Williams (the resistant control), but did differ significantly from that in Gros Michel (the susceptible control). To validate the usefulness of qPCR in screening banana plantlets, a range of varieties with different reactions to Foc will be included in future experiments, and their internal symptoms scored.

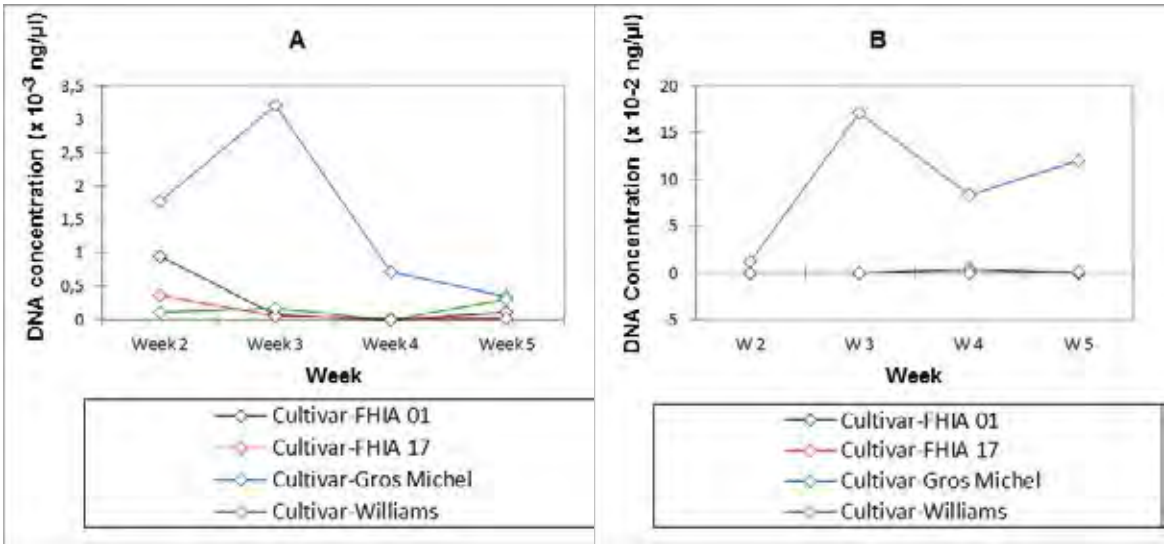



**Figure 4:** Amount of *Fusarium oxysporum* f. sp. *cubense* (Foc) DNA in roots and rhizomes of Gros Michel (susceptible), Williams (resistant), FHIA 01 (resistant) and FHIA 17 (tolerant) bananas using a Foc Lineage VI-specific quantitative (q) PCR. A: Foc DNA quantified in the root samples taken on weeks 2, 3, 4 and 5 after inoculation with Foc. B: Foc DNA quantified in the rhizome samples taken on the same period.

**Objective 3. Evaluate Mchare diploids and NARITA hybrids for resistance to Foc Lineage VI**

Mchare 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 5). 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 and was later confirmed to be an EAHB cultivar. EAHB cultivars are resistant to 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 Ndizi, which was the susceptible control. Muraru, Kahuti, Kamunyila, Hutishamba and Njuru did not develop Fusarium wilt symptoms (Table 5).

*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 5). 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.

**Table 5**: 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[b] | Intermediate | 1 | Nshonowa | MMC 423 | 33 | 1.70 + 0.09[a] | Susceptible |
| 2 | Huti green bell | ITC1559 | 12.5 | 1.29 ± 0.14 [b] | Intermediate | 2 | Mshare | MMC 501 | 23 | 1.37 + 0.09[b] | Intermediate |
| 3 | Mshare | | 17 | 1.21 ± 0.14 [b] | Intermediate | 3 | Mshare Mlelembo | MMC 453 | 10 | 1.10 + 0.09[c] | Intermediate |
| 4 | Ijihu Inkundu | ITC1460 | 12.5 | 1.17 ± 0.14 [b] | Intermediate | 4 | Muraru | MMC 421 | 0 | 1.00 + 0.09[c] | Resistant |
| 5 | Makyughu I | ITC1454 | 12.5 | 1.14 ± 0.14 [b] | Intermediate | 5 | Kahuti | MMC 483 | 0 | 1.00 + 0.09[c] | Resistant |
| 6 | Mshare Mlelembo | ITC1455 | 8 | 1.13 ± 0.14 [b] | Intermediate | 6 | Kamunyila | MMC 479 | 0 | 1,00 + 0,09[c] | Resistant |
| 7 | Makyughu II | ITC1446 | 4 | 1.09 ± 0.14 [b] | Intermediate | 7 | Hutishamba | MMC 486 | 0 | 1.00 + 0.09[c] | Resistant |
| 8 | Akondro Mainty | ITC0281 | 8 | 1.08 ± 0.14 [b] | Intermediate | 8 | Njuru | MMC 418 | 0 | 1.00 + 0.09[c] | Resistant |
| 9 | Nshonowa | | 0 | 1.00 ± 0.14 [b] | Resistant | 9 | Sukari Ndiizi** | | 57 | 1.73 + 0.09[a] | Susceptible |
| 10 | Kahuti | ITC1468 | - | - | Not tested | 10 | Mbwazirume*** | | 0 | 1.00 + 0.09[c] | Resistant |
| 11 | Gros Michel** | | 33 | 1.83 ± 0.14[a] | Susceptible | | | | | | |
| 12 | Grande Naine*** | | 0 | 1.00 ± 0.14 [b] | Resistant | | | | | | |

* RDI: rhizome discolouration index

** Susceptible control

*** Resistant control

**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

- Validate the effect of plant age in the rapid screening methods and the use phenolic compounds and qPCR of Foc DNA as early screening indicators of resistance to Foc. Experiments will end with 2018.

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

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

## WP2 - PhD Research Progress Report (2017-2018)

**TITLE:** Genetic diversity of *Pseudocercospora* spp. associated with banana Sigatoka in East Africa
**Name of Student:** **JANET N. KIMUNYE**
**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**

- A manuscript **"Distribution of *Pseudocercospora* species causing Sigatoka leaf diseases of banana in Uganda and Tanzania"** has been submitted to Plant Disease journal.
- Presented part of my work as a poster during the annual Breeding Better Bananas project meeting in Arusha "**Occurrence and distribution of *Pseudocercospora fijiensis* mating types in Uganda and Tanzania**". The poster was awarded 2nd place prize.
- A total of 318 isolates were recovered from NARITA testing sites and characterised using mating type markers. Both mating types were recovered in Luwero, Mbarara, and Bukoba, while only mating type 1 was recovered in Mbeya.
- DNA has been isolated from 422 isolates and genetic characterization using SSR markers will be conducted between October – December 2018 at Stellenbosch University.
- Trial for protocol validation was established and evaluation for response to Sigatoka has been done four times. Data analysis is ongoing.
- Evaluation of Mchare diploids for response to Sigatoka revealed that all eight Mchare varieties were equally susceptible with disease severity index (DSI) of 39.4%.
- Evaluation of the 1st and 2nd cycles of NARITA hybrids established in 2 sites in Uganda and 3 sites in Tanzania was completed and the data is being uploaded into MusaBase. 3rd cycle evaluations are ongoing. Results indicate that the sites

differ in disease severity with Kawanda having more disease pressure (AUDP 281.3). The sites (environment) had the highest contribution (59.6%) to cultivar response.

- Development of protocols for rapid evaluation of banana germplasm for sigatoka resistance is in progress. Cell-free filtrates were able to differentiate between susceptible and resistant banana cultivars. Effector proteins have been received and utility validation is ongoing.

**Background/introduction**

Banana yields in the Great Lakes region of Africa is <20 t ha $^{-1}$year$^{-1}$ (FAO, 2009) compared to the yield potential of >70 t ha year (van Asten *et al.*, 2005). The difference between the actual and potential yield has been attributed to pests and diseases (Gold *et al.*, 1999) and abiotic constraints (Wairegi *et al.*, 2010; Wairegi & van Asten, 2011). Sigatoka is one of the most important banana diseases and is known to reduce banana yields by as much as 50% while affecting fruit quality (Akele *et al.*, 2000). *Pseudocercospora musae* and *P. fijiensis* have been reported in most banana growing regions both in Uganda and Tanzania while *P. eumusae* has not been reported in the region. The projected climate change 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 (Carlier *et al.*, 2000), resulting in higher genetic diversity and emergence of new pathotypes. 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 levels of genetic diversity have been reported in *Pseudocercospora* populations. 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 reported that the *Pseudocercospora* population in Africa was more or less homogeneous (Carlier *et al.*, 2002; Fahleson *et al.*, 2009). It is however important to monitor the status of the pathogen population structure 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 commonly used method, but it is expensive, lengthy and its reliability is affected by availability of natural inoculum and unpredictable weather patterns. As a result, development of rapid and cost-effective screening techniques 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) reported the potential for using effector proteins to identify resistance sources in banana. If validated, use of effector proteins present a rapid cheap, and reliable assay for identifying sigatoka resistance bananas. 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 for this study are:

1. *Pseudocercospora* pathogens causing sigatoka in the Great Lakes region of Africa are homogeneous (i.e. show 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 a reliable tool for discriminating resistant and susceptible banana varieties
4. The response of NARITA hybrids to Sigatoka is similar across sites

This study will determine *Pseudocercospora* species distribution in Uganda and Tanzania, assess genetic and pathogenic variability within *Pseudocercospora* species, evaluate response of NARITA hybrids to *Pseudocercospora* species under different environments and develop rapid screening methods to support the breeding pipeline to rapidly develop sigatoka resistant banana varieties.

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

To determine Sigatoka leaf spot distribution and severity, field surveys were conducted in Kilimanjaro, Mbeya, Bukoba (Tanzania), Luwero 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 and disease severity was determined using a 0-6 scale (Gauhl et al., 1997). A disease severity index was computed as DSI = [Σnb/ (N-1) T] *100. Diseased leaf samples were collected, and the pathogen inciting the disease was identified using species-specific molecular markers. Most of the sites visited in Mbeya, Bukoba and Luwero were in the low and mid altitude range, while sites in Arusha and Mbarara were in the mid and high-altitude range. Disease severity was significantly higher in Uganda with mean DSI 39.3% compared to Tanzania (DSI 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).

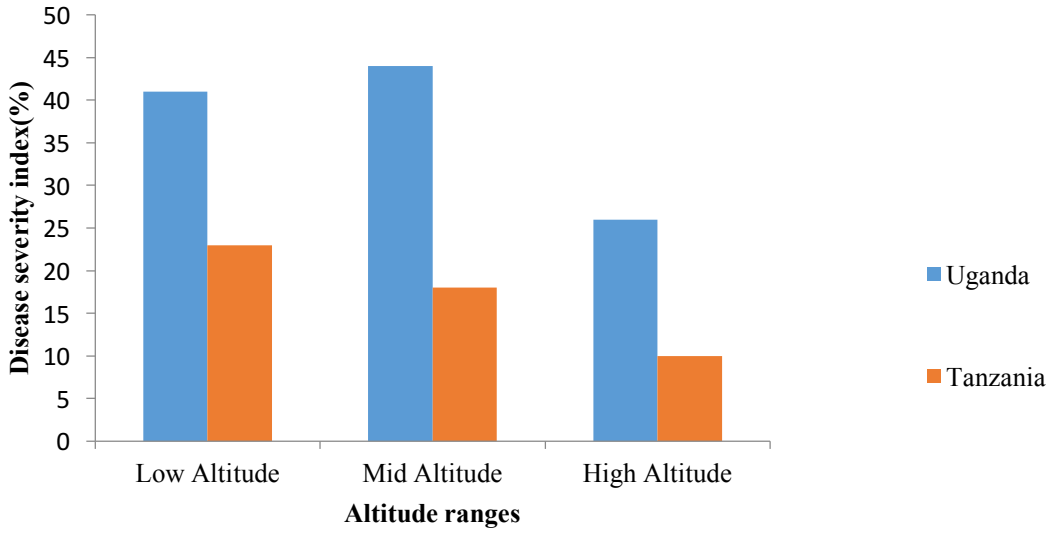



**Figure 1**: Sigatoka Leaf disease severity indices at low altitude <1200 m a.s.l, mid altitude 1201-1500 and High altitude >1500 m a.s.l ranges in Uganda and Tanzania

*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 masl tested positive for *P. fijiensis* (Table 1). These results point to a shift in environmental suitability for survival of *P. fijiensis.*

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

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

Historical weather data 1980-2016 and 1980-2010 in Uganda and Tanzania respectively revealed a significant increase p>0.05 in both minimum and maximum temperatures at the rate of 0.06°C/year in Mbarara and 0.03°C/year in Mbeya (Figure 2) while annual rainfall has declined over the same period at the rate of -0.008mm/yr. This gradual increase in minimum temperatures means that the high-altitude areas are gradually getting warmer surpassing the 15°C threshold earlier set for proliferation of black Sigatoka pathogen. This could explain the occurrence of *P. fijiensis* at high altitudes where the pathogen was previously considered not important. For example, in Uganda, *P. fijiensis* was absent at altitudes above 1350 masl and minimum temperatures below 15°C (Tushemereirwe et al., 1994; Johanson et al., 1996) but in this study the pathogen was detected at altitudes above 1800 m. The closely related *P. musae* (Yellow Sigatoka pathogen) that was considered a high-altitude pathogen was not detected in this study.

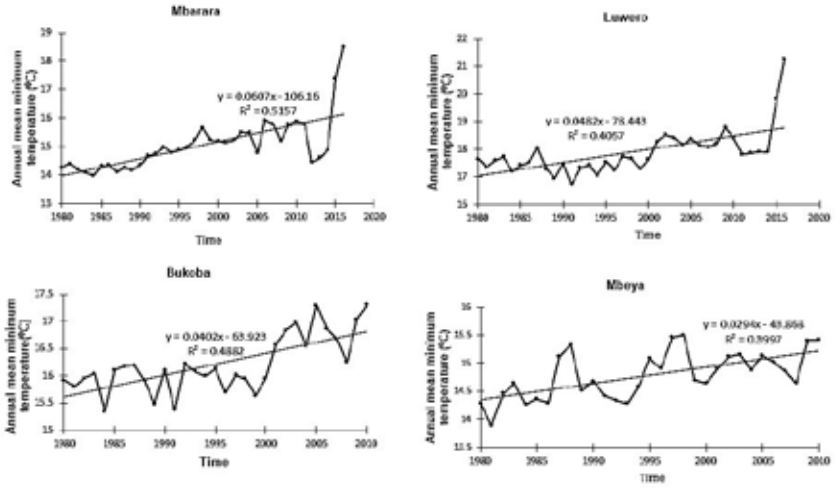



**Figure 2: Trends in minimum temperature from 1980-2016 in Uganda and 1980-2010 in Tanzania:** Isolation, detection and mating type analysis

Recovery of pathogen on station and from survey areas was done using the ascospore ejection method to generate single spore isolates. Pathogen detection was done by PCR using species specific primers for *P. fijiensis* (primers MF137/R635), *P. musae* (MM137/R635). Mating type idiomorphs 1 and 2 were detected using MAT 1 and MAT 2 gene-primers (Conde – Ferraez et al., 2010). A total of 318 single spore isolates of different morpho-types were 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 3).

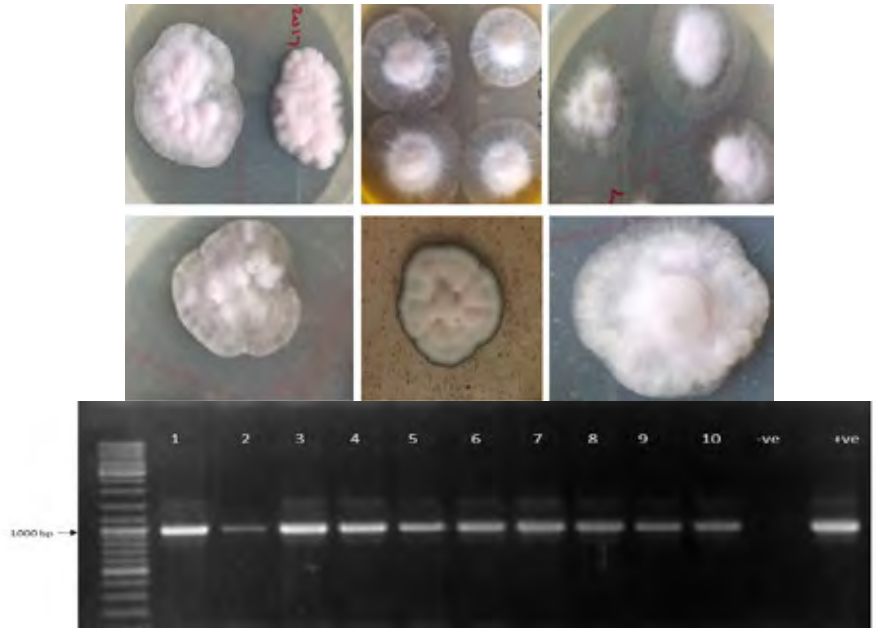



**Figure 3: A) Different morphology of isolates recovered on station and from screening sites; B) Gel showing the *P. fijiensis* specific fragment amplified using primers MF137/R635. The 1000bp amplicon indicates presence of *P. fijiensis* DNA.**

Amplification with mating type specific primers confirmed that both mating types were present in the pathogen population at almost equal frequencies except in Mbeya where all isolates were Mating type 1 (Table 2). This suggests that sexual reproduction frequently occurs within the pathogen populations which may lead to high genetic variability. This may in turn impact on pathogenic variability which has an implication on durability of introduced resistance. Further characterisation to determine extent of genetic and pathogenic variability is in progress.

**Table 2: Distribution of mating types in Uganda and Tanzania**

| Region | No of farms sampled | Mating type 1 | Mating type 2 | Chi-square value | P Value |
|---|---|---|---|---|---|
| Kawanda (NARO Research farm) | 1 | 34 | 29 | 0.396 | 0.53 |
| Sendusu (IITA Research farm) | 1 | 35 | 16 | 7.078 | 0.008 |
| Luwero | 14 | 34 | 43 | 1.052 | 0.3 |
| Mbarara | 11 | 30 | 36 | 0.54 | 0.46 |
| Mbeya | 8 | 32 | 0 | 32 | $1.54x10^{-3}$ |
| Bukoba | 3 | 5 | 6 | 0.09 | 0.76 |
| Kilimanjaro | 4 | 14 | 4 | 5.55 | 0.02 |

An interesting observation was the size polymorphism within the mating type 1 region. This was observed in the isolates from Mbeya and Bukoba where the MAT1 specific primers amplified a shorter fragment (approximately 480 bp instead of the expected 702 bp fragment (Figure 4). This shows that the mating type region may have undergone evolutionary changes leading to loss of part of the fragment.

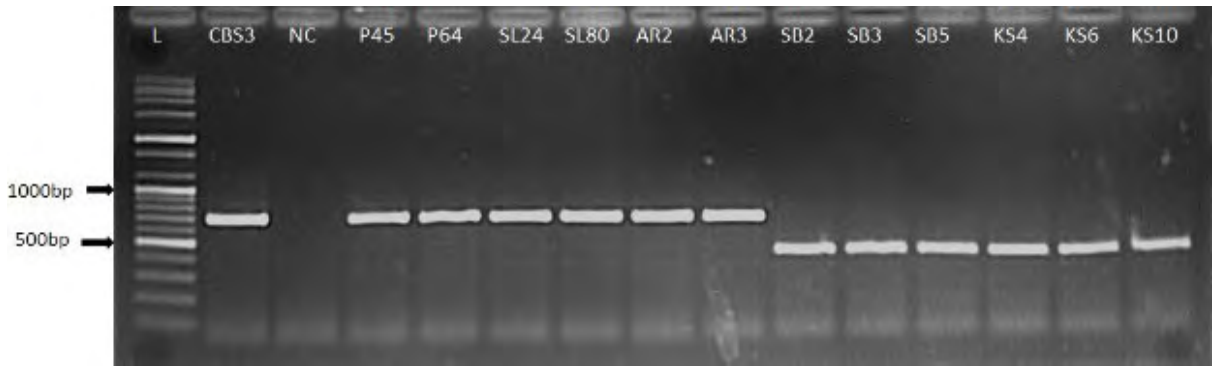



Figure 4: Amplification profile of selected isolates using *P. fijiensis* Mat1-1 specific primer (Mat1-1F/Mat1-1R). L is DNA Ladder, Lane 2 is a Mat1 isolate from CBS; Lane 3 is a no template control; lanes 4-8 are isolates from Kawanda and Luwero; lanes 9-14 are isolates from Mbeya and Bukoba

Sequencing of this region reveal loss of ~75bp and 166bp at the start and end of the sequence respectively on Mbeya isolate as compared to the CBS isolate. Molecular phylogeny analysis of this region indicates the relatedness of these isolates. The Mbeya MAT1 isolate was more like the MAT2 CBS isolate (Figure 5a&b). These are preliminary results and more samples have been submitted for sequencing to shed more light on the Mbeya and Bukoba *P. fijiensis* populations.

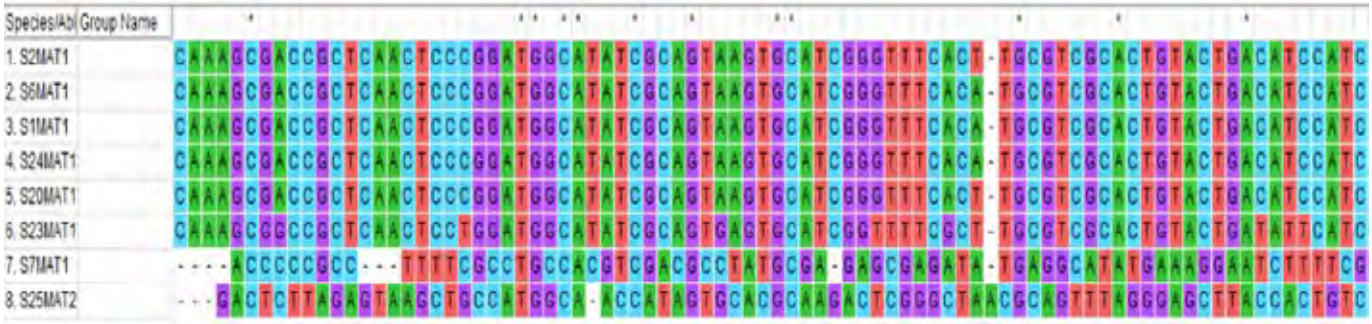



**Figure 5a: Part of the mating type sequence aligned for selected isolates Sequence alignment of selected isolates using ClustalW in MEGA 7 program.**

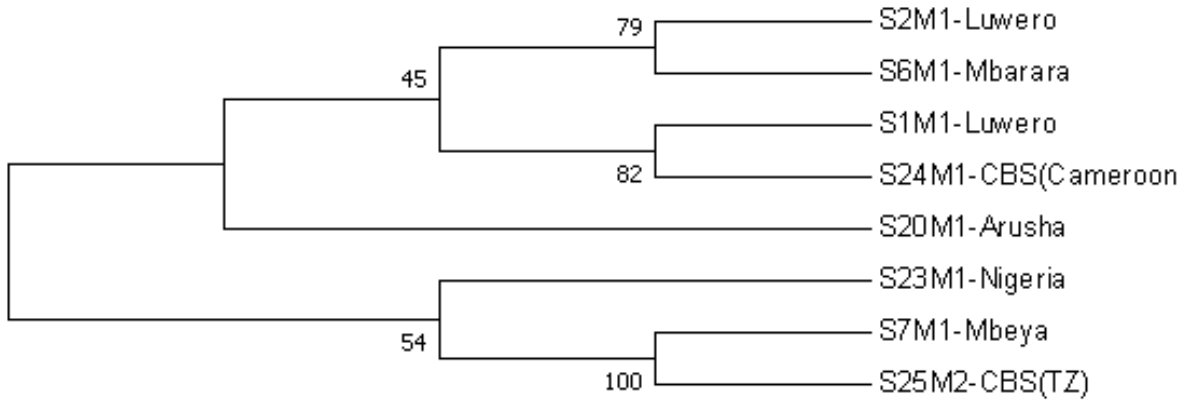



Figure 5b. Molecular Phylogenetic analysis by Maximum Likelihood method on selected *P. fijiensis* isolates from Uganda and Tanzania. M1 is mating type 1 and M2 is mating type 2

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

Different inoculants i.e. mycelial fragments, conidial suspensions, culture filtrates and effector protein at different rates are being evaluated for suitability in screening for Sigatoka resistance. Detached leaf assays have been used previously for rapid screening for Sigatoka resistance (Twizeyimana et al., 2007). Different types of inoculum types at different levels were tested.

**Mycelial fragments:** Detached leaves from Williams, Pisang lilin 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 lilin and Williams, and Calcutta 4, respectively. Symptoms appeared as light brown streaks that later darkened and enlarged, only in Pisang lilin and Williams but not in Calcutta 4.

More disease streaks were observed on leaf discs inoculated with lower concentrations of mycelial fragments (Fig. 6). 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, had more streaks compared to Pisang lilin and Calcutta 4. This observation is suspected to be a result of self-inhibition like 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.

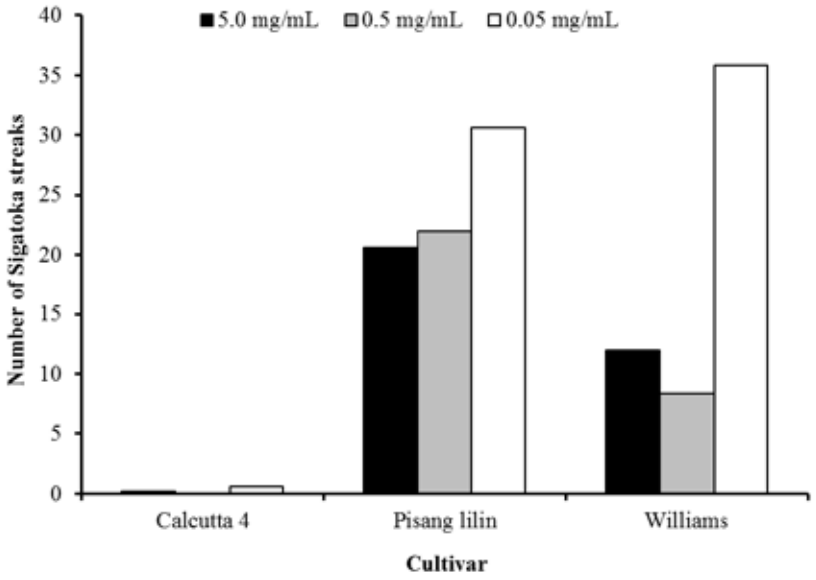



Figure 6**:** Effect of different concentrations of inoculum of *Pseudocercospora fijiensis* on disease severity on three *Musa* cultivars with different levels of susceptibility to black Sigatoka. Disease severity was assessed *in vitro* on leaf discs at 49 days after inoculation.

Optimisation of inoculum type and level experiments are ongoing. Young Williams plants (4-month-old) derived from macro-propagation were used to test different mycelia suspension concentrations. The concentrations used were 50mg/ml, 25mg/ml, 5mg/ml and 0.5mg/ml. Severity on inoculated leaves was recorded on a 0-6 scale (Gauhl *et al*., 1997) on a weekly interval. The severity values were converted to AUDPC to compare the different

concentrations. The different concentrations varied significantly (F=16.53; P<0.001) with 5mg/ml having the highest AUDPC and 50mg/ml the lowest (Figure 7).

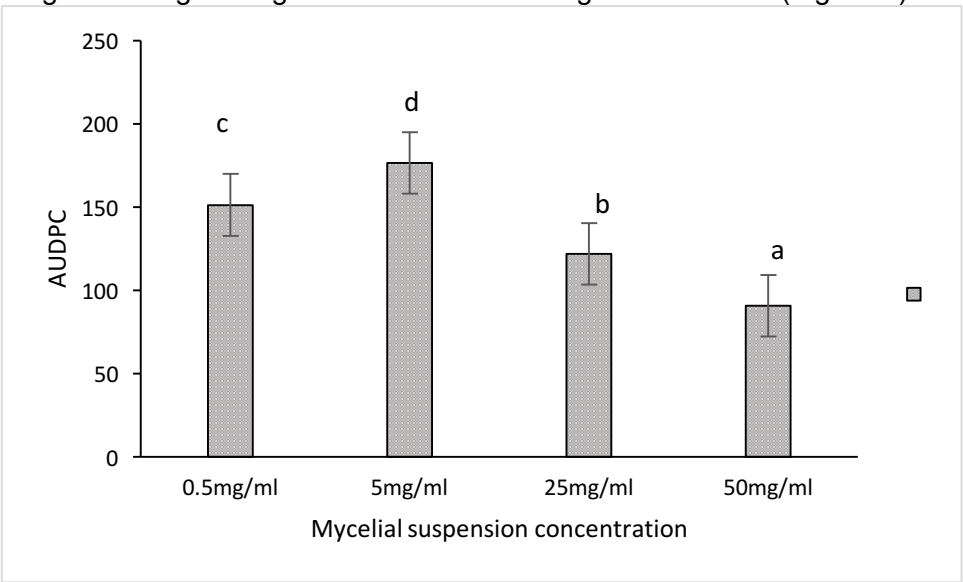



**Figure 7: Average AUDPC values on plants inoculated with different concentrations of *P. fijiensis* mycelia suspension**

The mean incubation period (number of days from inoculation to date 1st symptoms are observed) was 24.1 days. However, this varied across the concentrations with 5mg/ml having the shortest 19.8 days and 50 mg/ml having the longest incubation period at 34.4 days. 25, 5 and 0.5mg/ml incubation periods did not differ significantly.

Experiments to determine the level of inoculum on small plants that give disease response similar to field response were established in a glasshouse in Kawanda. Mycelial suspension was obtained from actively growing cultures on 20%V8 media plates. The plates were grown under 12:12 photoperiod at room temperature for two weeks. The mycelia were harvested, macerated into a suspension using sterile distilled water in a mortar. The suspension was filtered through double layer of cheese cloth, quantified on a haemocytometer and diluted to different concentrations. Three cultivars, Williams (susceptible), Pisang lilin (partially resistant) and Calcutta 4 (resistant) were used. Data collection is in progress. Initial results show that symptom development on Williams is like field infection, however Calcutta 4 does not develop typical Sigatoka symptoms but instead develops what could be described as HR as early as 3 days post inoculation (DPI) especially at high inoculum concentrations (Figure 8). This reaction is however not as strong as what is induced by culture filtrate.

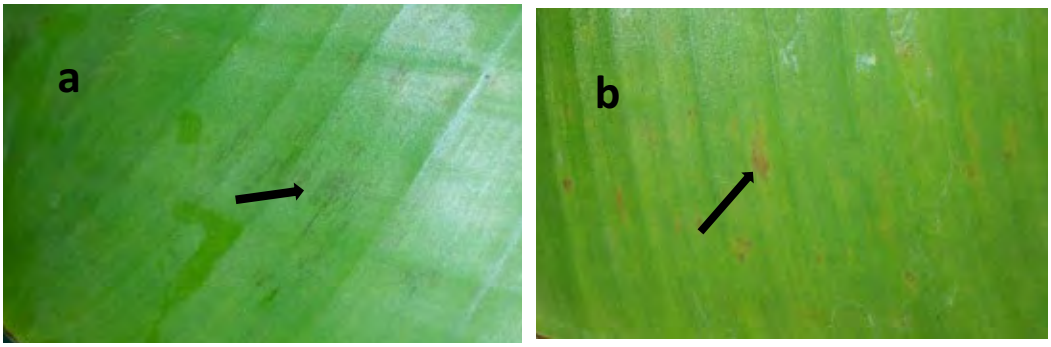



**Figure 8: Early response of Calcutta 4 in response to inoculation with *P. fijiensis* mycelia suspension; a) is 3 days post inoculation (DPI), b) 10 DPI**

**Cell-free culture filtrates:** *P. fijiensis* cell free culture filtrate was produced by inoculating V8 broth media with mycelia fragments, incubated on a shaker at 100 rpm at room temperature for 1 month. The filtrate was sieved through 4 layers of cheese cloth, centrifuged at 10,000 rpm for 5 minutes then filtered through 0.2 µm filters. The cell free culture filtrate was infiltrated on to a resistant (Calcutta), partially resistant (Pisang lilin) and susceptible (Williams) cultivars using a needle-less syringe. A clear hypersensitive reaction was observed on Calcutta 4 starting from day 4 post infiltration (Figure 9). The filtrate formed a water-soaked lesion at the point of administration on Pisang lilin while no response was observed on Williams 10 days after infiltration (Figure 9). This test will be extended to other cultivars varying in resistance to *P. fijiensis* and probably identify the factor responsible for inducing HR on Calcutta 4.

Williams- Susceptible | Pisang Lilin-Partially resistant | Calcutta 4-Resistant

**Figure 9: Response of Musa sp. to infiltration with *P. fijiensis* cell free culture filtrates after 10 days**

**Conidia production:** 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.

**Effector proteins:** Sequences of Avr4 (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 were used to amplify the gene sequences for cloning and heterologous expression of the proteins in *Pichia pastoris*. The effector protein was ordered from Bon opus biosciences (USA) and was delivered on 29th August 2018. Testing and optimising use of the effector protein as a rapid screening tool is ongoing.

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

Evaluation of NARITA trials are done on a quarterly basis across the five testing sites. Three plants per genotype per replication are selected for evaluation. Disease severity is recorded using the modified Gauhl's 0-6 scale (Gauhl et al., 2000). Disease severity index was computed as

DSI = [Σnb/ (N-1) T] *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

Four evaluations have been done for all sites capturing disease on cycle 1&2 plants. Evaluation of cycle 3 plants is ongoing. To assess disease intensity over time, disease severity index (DSI) per genotype in each region was converted to Area under disease progression curve (AUDPC) as per the equation (Madden et al., 2007).
AUDPC =∑ni=1[(Xi+1 + Xi)/2][ti+1 - ti]

Where, Xi= DSI at ith day, ti = the time in days after appearance of the disease at ith day, and n = the total number of observations.

Out of the possible 26 NARITAS, only 15 were common across the testing sites plus four controls i.e. Cachaco, Gros michel, Mbwazirume and Williams. The rest that were missing in some sites as well as the local checks that were unique to each site were eliminated from this analysis. There was significant difference in genotype AUDPC, Environments (regions) and Genotype x Environment interaction (P<0.001) (Table 3).

**Table 3: Two-way analysis of variance using the mean AUDPC from 19 genotypes grown in five different environments**

| Source | DF | Sum of squares | Mean of squares | F Value | P Value | % Genotype +Environment +GXE |
|---|---|---|---|---|---|---|
| **Genotype** | 18 | 839902 | 46661 | 21.18 | <0.001 | 26.3 |
| **Region (Environment)** | 4 | 1900221 | 475055 | 215.6 | <0.001 | 59.6 |
| **Genotype x Environment** | 72 | 450341 | 6255 | 2.84 | <0.001 | 14.1 |

The highest mean AUDPC was recorded in Kawanda (281.3), Bukoba (213.7), Mbeya (188.3), Mbarara (141.7) and the lowest in Kilimanjaro (60.3). Two-way ANOVA of AUDPC from 19 genotypes grown over 5 environments indicated that AUDPC in different genotypes was significantly different from each other ($F=21.18$, $P<0.001$), and that AUDPC of genotypes grown in different environments was also significantly different ($F=215.6$, $P<0.001$); the interaction between the genotype and environment was highly significant ($F=2.84$, $P<0.001$; Table 3). The environment contributed 59.6% to the total variance and the interaction between the genotype and environment contributed 14.1% of the total variance, while the genotype only contributed 26.3% to the total variance. Different environment variables including rainfall, temperature, humidity, organic matter content and silicon levels all influence genotype response to Sigatoka. These variables need to be interrogated to provide an insight into possible contribution variations observed.

The two diseases parameters DSI and AUDPC were highly correlated ($R^2=0.97$) and ranked the genotypes in a similar manner. Genotype stability across the environment was investigated based on the average standard deviation of each genotype across the five environments. The most stable and best performing NARITAs in response to Sigatoka infection were NARITA4, NARITA 14, NARITA 2 while the most unstable NARITAs were NARITA 10 and NARITA 18 (Table 4).

**Table 4: Ranking of NARITA's based on the mean DSI, mean AUDPC and average Standard Deviation (SD) across the five testing sites in Uganda and Tanzania**

| Cultivar | Mean DSI | Mean AUDPC | Average SD |
|---|---|---|---|
| **NARITA 4** | 10.14 | 103.1 | 6.674 |
| **NARITA 14** | 11.6 | 116.7 | 8.176 |
| **NARITA 2** | 12.2 | 117.8 | 8.916 |
| **NARITA 21** | 13.86 | 129.8 | 8.756 |
| **NARITA 8** | 14.32 | 132.5 | 9.472 |
| **NARITA 22** | 13.74 | 133.5 | 8.674 |
| **NARITA 7** | 15.63 | 155.1 | 9.58 |
| **NARITA 9** | 16.74 | 156.6 | 11.734 |
| **NARITA 11** | 15.46 | 158.9 | 11.142 |
| **NARITA 23** | 15.35 | 166.8 | 10.484 |
| **NARITA 6** | 20.22 | 195.2 | 14.658 |
| **NARITA 10** | 23.46 | 202.6 | 16.036 |
| **Gros michel** | 22.02 | 203.3 | 13.382 |
| **Cachaco** | 21.71 | 204 | 12.958 |
| **NARITA 12** | 19.44 | 212 | 12.538 |
| **NARITA 13** | 19.87 | 215 | 13.19 |
| **NARITA 18** | 24.53 | 240.5 | 15.124 |
| **Mbwazirume** | 25.43 | 246.4 | 14.85 |
| **Williams** | 30.33 | 278.2 | 16.452 |

**Mchare:** Mchare genotypes were planted at Kawanda and were evaluated as described for NARITAs. 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 5). 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 5: 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**

- Finalise genetic characterisation of *P. fijiensis* populations and write manuscript
- Conduct virulence assays
- Finalise optimisation of inoculum level
- Test the use of effector protein for screening
- Write manuscript on evaluations of NARITAS

## WP2 - MSc Research Progress Report (2017-2018)

**Research title:** Evaluation of selected diploid banana genotypes for resistance to weevils (*Cosmopolites sordidus*) in Uganda.

**Name of Student:** **KEMIGISA JULIET**

**Field of study**: MSc of Science in Botany (Microbiology and Plant pathology)

**University:** Makerere University- Uganda

**Timeline of study:** 1st August 2016 to 31st July 2019

**Supervisors:** Dr. Robooni Tumuhimbise (NARO), Dr. Jerome Kubiriba (NARO), Assoc. Prof Arthur K. Tugume (Makerere University)

**Research Objectives**

1. To assess the response of selected diploid banana genotypes to weevil infestation
2. To determine the effect of weevils on agronomic traits of selected diploid banana genotypes

**Achievements**

**2016 to 2017**

3. Successfully completed year one (course work) at Makerere university. I under took 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.
4. Established a field screening trial of the selected diploid banana genotypes for weevils resistance screening.
5. Established a pot screening bioassay for screening selected diploids for resistance to banana weevils.

**2017 to 2018**

1. Screened, collected and analyzed data for the pot screening bioassay for resistance to weevils (Objective one achieved 90%).
2. Collected 65% data from the field screening trial of the selected diploid banana genotypes for weevil resistance screening.
3. The research proposal was successfully accepted by the higher academic committee at Makerere University (July 2018) and was submitted.
4. Submitted the progress reports to the University.

**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 estimated yield damages of 14 to 60% and have led to the disappearance of some popular local East African Highland cultivars. Attempt to control weevils by cultural, biological and chemical methods are feasible but not sustainable due to limited resources to 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 lack of research into resistant sources of banana weevils. This MSc study focuses on identifying sources of resistance to weevils from selected diploid banana genotypes and to determine their agronomic value in support of breeding. This will consequently benefit small-scale banana farmers through growing improved weevil resistant varieties, reducing labour costs and chemical use. This will also result into increased banana production and sustainable productivity.

## Experimental site

The study is being carried out at the National Agricultural Research Laboratories (NARL) – Kawanda, located 13 km North of Kampala and at 1195 m above sea level (0°25'N, 32°32'E).

## Materials used in the study

Nine banana genotypes (Table 1) were sourced from the International Transit Centre (ITC)-Bioversity International and multiplied in the tissue culture lab at NARL. The nine diploids belong to 'The Pisang Jari Buaya family'. Four controls were obtained from the NARL plantations in Kawanda. One genotype (Calcutta 4) obtained from Kawanda is used as a male parent and is resistant to major pests and diseases (Kiggundu et al., 2003). Three others are triploids, the EAHBs (Kibuzi and Nakitembe) are highly susceptible to banana weevils while Kayinja is resistant.

**Objective 1. To assess the response of selected diploid banana genotypes to weevils infestation**.

The currently available methods for screening banana genotypes against weevils are field trials or hardened tissue culture plantlets in screenhouses. Field screening is a long term process that takes 3 to 5 years in collecting valuable data from the 1$^{st}$ to the 4$^{th}$ cycle since weevil attacks are rather observed in older plantations than in younger ones. A greenhouse screening method predicts plant resistance to weevils in 8 months. Though it is an accepted method, it has not been validated in comparison to the field screening trial.

**Table 1. Banana genotypes screened for weevil resistance**

| Ploidy | Study material | Special traits | Response against weevils invasion |
|---|---|---|---|
| **AA** | Morongo Datu | Resistant to *R. similis* | Unknown |
| **AA** | Pisang Gigi buaya | Partially resistant to *R. similis* | Unknown |
| **AA** | Pisang Tunjuk | Resistant to *R. similis* | Unknown |
| **AA** | SH-3142 | Resistant to *R. similis* | Unknown |
| **AA** | Pisang Rotan | Resistant to *R. similis* | Unknown |
| **AA** | Huwundu vita | Resistant to *R. similis* | Unknown |
| **AA** | Gabah Gabah | Resistant to *R. similis* | Unknown |
| **AA** | Morong Princessa | Resistant to *R. similis* | Unknown |
| **AA** | Saing Hil | Resistant to *R. similis* | Unknown |
| **AA** | Calcutta 4 | Early flowering, resistant to pests and diseases | Resistant (Kiggundu, Gold, Labuschagne, Vuylsteke, & Louw, 2003) |
| **ABB** | Kayinja | Big bunch | Resistant (Kiggundu et al., 2003) |
| **AAA-EA** | Kibuzi | Big bunch | Susceptible (Kiggund et al., 2003) |
| **AAA-EA** | Nakitembe | Susceptible to nematodes | Susceptible (Kiggundu, et al., 2003) |

## Pot screening

Hardened tissue culture plantlets of ten genotypes were used in this experiment (Sadik, Nyine, & Pillay, 2010). The plantlets were potted in plastic buckets (5 L) filled with sterilized soil, sand and decomposed farmyard manure at a ratio of 3:1:1. Eight of the genotypes are diploids obtained from ITC, two are controls (Calcutta 4 and Nakitembe) obtained from NARL plantations. The experiment was arranged in a Randomized Complete Block Design (RCBD) with five replicates and 15 copies of each genotype. Plantlets were allowed to establish in pots for 60 days under screenhouse conditions. Then four adult weevils were released in each bucket and sealed off with nylon nets to stop weevils from escaping. After 60 days of weevil feeding, data collection on parameters such as corm damage assessment due to weevils, number of larvae and adult weevils recovered in pots was recorded. The weevil damage assessment was carried out by visual observation of the weevil galleries around the peripheral of the corm. The corm was then cut cross-sectional wise at 3 cm (lower position) and at collar region (upper position 6cm). Data were collected from the outer (OX) and inner (IX) corm. The total cross-sectional (XT) damage was obtained by averaging OX and IX. Data were analysed using Gensatat software and ANOVA tables generated indicating differences in weevil damage among the genotypes.

The results show significant differences in the resistances among the different genotypes for both peripheral and cross sectional weevil damages. Cultivars Calcutta 4, Pisang Tunjuk,

Saing Hil, Rotan and Morongo Datu showed promising resistance. The results of this experiment will be compared with data from the field screening trial to select diploid resistance to weevils.

**Table 2. Mean cross sectional and peripheral damage after 60 days**

| Genotype | XT | peripheral |
|---|---|---|
| **Calcutta 4** | 12.3a | 15.2a |
| **Pisang Tunjuk** | 13.1a | 16.5a |
| **Saing Hil** | 23.1a | 45.9b |
| **Pisang Rotan** | 30.9a | 38.4a |
| **Morongo Datu** | 36.0a | 34.0a |
| **Pisang Gigi Buaya** | 38.1b | 40.9b |
| **Huwundu Vita** | 40.0b | 43.5b |
| **Gabah Gabah** | 44.1b | 50.8b |
| **SH-3142** | 50.3b | 58.6b |
| **Nakitembe** | 80.4c | 83.2c |
| **Mean** | **36.8** | **42.7** |
| **LSD 0.05** | **23.7** | **25.4** |
| **P-value** | **<0.001** | **<0.001** |

Means with the same letters are not significantly different (p>0.005)

XT- total cross-sectional damage of the corm

**Table 3. Mean correlations of the weevil damages at the peripheral and cross sectional after 60 days.**

| | Peripheral | XO_3cm | XI_3cm | XO_6cm | XI_6cm | XT |
|---|---|---|---|---|---|---|
| **Peripheral** | - | | | | | |
| **XO_3cm** | 0.8998 | - | | | | |
| **XI_3cm** | 0.8109 | 0.8237 | - | | | |
| **XO_6cm** | 0.7945 | 0.8186 | 0.662 | - | | |
| **XI_6cm** | 0.7768 | 0.7901 | 0.801 | 0.7441 | - | |
| **XT** | 0.9003 | 0.9414 | 0.911 | 0.8733 | 0.9172 | - |

XO-Outer cross-sectional damage
XI- Inner cross-sectional damage
XT-total cross-sectional damage

### Field screening

The nine diploids and three controls (Calcutta 4, Kayinja and Kibuzi) were planted in a RCBD. At 9 months after planting, banana mats were infested with 10 adult weevils (five males and five females) per plant in the ratio of 1:1 female to male (Kiggundu et al., 2003). Weevil damage assessment on corms is conducted at most one week after harvest. Data collection is under way for the first (50 %) and second cycle (15 %). Since the genotypes used in the study were different, their dates to flowering and maturity differ and hence delay data completion.

**Objective 2. To determine the agronomic traits of selected diploid banana genotypes against weevil infestation**

**Experimental design**

Hardened tissue culture plantlets of twelve genotypes (Table 1) were planted at a spacing of 3m x 3m per plant in a RCBD. At 9 months after planting, banana mats were infested with 10 adult weevils per plant in the ratio of 1:1 female to male. Agronomic data (Table 4) collection is underway for the first and second cycle.

**Table 4. Data collected at flowering and harvest**

**Agronomic traits**

1. Days (period) to flowering
2. Plant girth (cm) at 100 cm and height (cm) of the pseudo-stem at flowering
3. Number of functional leaves at flowering and harvest
4. Height (cm) of the tallest sucker at flowering and at harvest
5. Period (days) to maturity of the flowered banana
6. Pollen quantity (on a scale of 1-4)
7. Bunch weight (kg) at harvest
8. Number of banana clusters and fingers on the 2$^{nd}$ cluster (top and bottom)
9. Banana fruit size (length and circumference)

**Weevil assessment**

1. Corm diameter(cm) at 5cm (lower) and at 10cm (upper) regions
2. Percentage peripheral and cross sectional weevil damage

**Challenges faced**

1. Delayed flowering and maturing of some banana cultivars hence causing the need for extension of the study time
2. Delayed approval of the study proposal at Makerere University

**Conclusion**

- Collect all the necessary data for the field trial by December 2018
- Thesis write up and submission by March 2019

**References**

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Sadik, K., Nyine, M., & Pillay, M. (2010). A screening method for banana weevil ( Cosmopolites sordidus Germar ) resistance using reference genotypes, *9*(30), 4725–4730.

## WP2 - MSc Research Progress Report (2017-2018)

**Research title:** Improving embryo germination of banana seeds derived from tetraploid and diploid crosses
**Name of Student:** **LUYIGA JANE**
**Field of study:** MSc in Crop Science (Biotechnology)
**University:** Makerere University
**Timeline of study:** 1st August 2017 to 31st July 2019
**Supervisors**: Prof. Swennen Rony (IITA), Dr. Uwimana Brigitte (IITA), Master Amah Delphine and Dr. Wasswa Peter (Makerere University)

**Research Objectives**

1. To assess the effect of different imbibition times on seed germination rate
2. To establish the optimal BAP and GA concentration for embryo germination

**Achievements**

1. Successfully completed year one (course work) at Makerere University. I undertook courses such as Applied Statistics and Biometrics, Plant Cell and Tissue Culture, Graduate Seminars, Advanced Molecular Biology and Genetics, Advanced Plant Microbiology, Gene transcription: mechanisms and control, Molecular Plant-Microbe Interactions, Principles of Population & Evolutionary Biology, Bioinformatics, Research Methodology, Bio-policy, Bio-safety and Bioethics, Advances in molecular genetics & Functional genomics and Agricultural Marketing Management.
2. Pollination of the female bananas is underway

**Introduction**

Bananas and plantains (*Musa* species) are important food and cash crops worldwide (FAOSTAT, 2015). The East and Central African (ECA) region, comprising of Burundi, Democratic Republic of Congo (DR Congo), Kenya, Rwanda, Tanzania and Uganda accounts for about 53% of the banana in sub-Saharan Africa and approximately one third of the global *Musa* production (FAOSTAT, 2015). In Uganda, farmers grow a number of banana varieties and these include the plantains (roasting banana) commonly known as gonja, the sweet banana (dessert) commonly known as sukaliNdizi, and bogoya, the cooking types belonging to the East African highland banana (EAHB) subgroup locally called matooke and the beer type (Mbidde) used to produce alcoholic and non-alcoholic beverages (Karamura, 1999).

Most cultivated banana varieties are parthenocarpic triploids characterized by low fertility or complete sterility (Vuylsteke et al., 1993). They were derived from two seedy species *Musa acuminata* and *Musa balbsiana*, which contributed the A and B genomes, respectively (Ortiz, 2013). Bananas have diverse genomic constitutions and ploidy levels such as diploids (AA, AB, BB), triploids (AAA, AAB, ABB) and tetraploids (AAAA, AAAB, AABB, ABBB). Reduction in banana yields has been attributed to increasing pest and disease pressure accelerated by climate change (Ghini et al., 2008). Yet chemical control measures are not sustainable due to environmental pollution and economic burden to smallholder farmers. Conventional cross breeding is used to improve resistance of cultivated bananas against production constraints (Jenny et al., 2002). However, the success of a breeding programme depends on the compatibility of parents crossed, the number of good seeds produced and the ability of embryos to regenerate into plantlets. Improvement of triploid banana species have been

achieved through crossing triploid landraces with wild diploids to produce tetraploids, the selected tetraploids are then crossed with improved diploids to produce sterile secondary triploids (Pillay et al., 2002). The improved diploids have useful traits introduced from wild sources and they are being used for the improvement of many different types of bananas (Escalant and Jain, 2004). The improved diploids are used as male parents in crosses with the desired triploids or tetraploids as female parent.

The low number of seeds available in banana breeding programs and problems of seed germination led to the use of embryo culture to increase the number of seedlings. Embryo culture is the sterile isolation and *in vitro* growth of mature embryos on artificial medium with the goal of acquiring a viable plant (Pierik, 1987). At the IITA banana breeding programme, the maximum germination rate of embryos from tetraploid by diploid crosses is 4.7%. The failure of most of the seed embryos to germinate is not well documented although embryo dormancy could be one of the reasons. Therefore, there is a need to understand the optimal culture conditions that could significantly increase the rate of embryo germination because the cost of obtaining banana seeds is very high in terms of money, time and labour thus, if the embryos do not germinate, then all efforts are nullified.

Improving embryo germination will complement the study on improving seed set in banana by improving germination efficiency of banana embryos, hence improve the output of crosses in banana breeding experiments.
This research seeks to improve embryo germination by focusing on the optimal concentration of hormones (cytokinins and gibberellic acid) and to find out whether imbibition can affect germination of embryos.

**The crosses between parents from which seeds will be obtained**

| **Tetraploids** | **Diploids** | **No. of pollinated plants** |
|---|---|---|
| 1438K-1 | Malaccensis | 19 |
| 917K-2 | Calcutta 4 | 6 |
| 1201K-1 | 7197-2 | 19 |
| 917K-2 | Cv rose | 9 |
| 660K-1 | Calcutta 4 | 11 |

**Specific objective 1:**To assess the effect of different imbibition times on seed germination rate

The extracted seeds will be divided into two equal groups. The first group will be cracked on the day of extraction and the second group will be cracked and cultured after five days of soaking.

**Specific objective 2: To establish the optimal hormone 6-Benzylaminopurine (BAP) and Gibberellic acid (GA)concentration for embryo germination**

The extracted seeds will be cracked and inoculated on culture media containing different concentrations of BAP at 0 mg/L, 0.5 mg/L and 1 mg/L and GA at 0 mg/L, 0.5 mg/L and 1 mg/L. There will be a combination of the two hormones having different concentrations

**Way forward**

- Collect all the necessary data by May, 2019
- Thesis write up and submission by July, 2019

**References**

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## 7.3 WP3 - PhD Research Progress Report (2016-2018)

**TITLE:** Genetic Analysis of Resistance Against *Fusarium oxysporum* f.sp. *cubense* (Foc) in Selected Banana Populations

**Name of Student:** **ARINAITWE IVAN KABIITA**

**Supervisors:** Rofina Yasmin Othman
Jennifer Ann Harikrishna
Chee How Teo

Fatimah Kayat
Robooni Tumuhimbise
Uwimana Brigitte

**Timeline of study:** 2016-2020

**University:** University of Malaya

**Research Objectives**
List the individual topics of study – objectives or study areas

1. To identify contrasting diploid parents for use in *Foc* race 1 and race 4 genetic studies,
2. To develop and phenotype 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. To identify polymorphic and heritable molecular markers for *Foc* race 1 and race 4, and
5. To perform a QTL analysis for *Foc* race 1 and race 4.

**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 have been rescreened at UM.
2. Two unrelated diploid mapping populations for *Foc* race 1 resistances developed. One population of Kokopo x Monyet is phenotyped (90%) and another of Mshale x Calcutta 4 planted in the field where the suckers will be picked and multiplied in tissue. The multiplied genotypes will be screened for *Foc* race 1.

3. Different molecular markers IRAP, SSR and ISSR have been evaluated on the parents contrasting for *Foc* race 1. 2 IRAP, 14 SSR and 10 ISSR markers showing polymorphism within parents of Monyet and Kokopo and 2 IRAP, 15 SSR and 10 ISSR markers showing polymorphism within parents of Mshale and Calcutta 4 have been identified and used to screen the subsequent $F_1$ hybrids. Heritable markers for both populations have been identified.

4. DNA for both populations sent for SNP typing.

**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. *Fusarium* wilt causes banana yield loss of about 30-40 % with a yield loss of 2-90% estimated in South India alone (Mustaffa & Thangavelu, 2011; Kumar, 2006).

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 (van Bueren *et al*., 2010).

**Objective / Study 1. To identify contrasting diploid parents for use in *Foc* race 1 and race 4 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.

**Table 3. Banana diploids screened for resistance to Fusarium wilt**

| | Diploid parents (Race 1) | F2 diploid banana plants (Race 1) | OP-*malaccensis* (Race 4) |
|---|---|---|---|
| | 1 TMB2X614-1 | 123 F2 diploid banana plants | 45 plants from an open pollinated bunch of malaccensis |
| | 2 Pahang | | |
| | 3 Kokopo | | |
| | 4 Long tavoy | | |
| | 5 Calcutta 4 | | |
| | 6 Zebrina | | |
| | 7 Kasaska | | |
| | 8 Borneo | | |
| | 9 Pisang Lilin | | |
| | 10 Monyet | | |
| | 11 Mwitu Pemba | | |
| | 12 Huti shamba | | |
| | 13 Kahuti | | |
| | 14 Mlilembo | | |
| | 15 Muraru | | |
| | 16 Nshonowa | | |
| | 17 Njuru | | |
| 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 at 14 days interval from the date of inoculation to see if there is any leaf showing symptoms and data was used 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 4. Scale for scoring different parameters for Fusarium disease resistance (Viljoen *et al.*, 2017).**

| Disease rating scale | Leaf symptom index | Stem splitting | Rhizome discoloration index |
|---|---|---|---|
| 1 | No yellowing | No cracking | No internal symptoms |
| 2 | Yellowing of < 1/3 of the leaves | Slight cracking | Few internal spots |
| 3 | Yellowing of 1/3 to 2/3 of leaves | Advanced | <1/3 discolored |
| 4 | Yellowing of > 2/3 of leaves | | 1/3-2/3 Discoloured |
| 5 | Plant dead | | >1/3 Discoloured |
| 6 | | | Entire inner rhizome |

**Table 5. Interpretation of LSI and RDI, DSI (Sutanto *et al*, 2011).**

| 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 6. Analysis of variance for LSI and RDI for screened banana diploids**

| | Diploid parents | | | F2 diploid banana plants | | | OP-*malaccensis* | | |
|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | RDI | LSI | d.f | RDI | LSI | d.f | RDI | LSI |
| Total | 131 | 3.17 | 0.56 | 530 | 4.2 | 0.4 | 77 | 2.6 | 0.3 |
| Rep | 5 | 2.2 | 0.17 | 4 | 3.4 | 1.1 | 1 | 19.8 | 0.3 |
| Genotype | 21 | 11.90*** | 1.61*** | 124 | 9.3*** | 0.9*** | 44 | 3.8*** | $0.3^{ns}$ |
| Residual | 105 | 1.48 | 0.37 | 402 | 3.1 | 0.3 | 32 | 1.3 | 0.2 |

*** P>0.001. ns= non-significant

**Table 7. Categorisation of the genotypes within germplasm using DSI (RDI)**

| | Diploid parents | | | | F2 diploid banana plants | | | | OP-*malaccensis* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | PR | S | HS | R | PR | S | HS | R | PR | S | HS |
| | Long tavoy | TMB2X614-1, Mwitu Pemba, Monyet, Pisang Lilin, Borneo, Kasaska, Zebrina, Pahang | Kokopo (3.5)<br><br>Hutishima Kahuti Mililembo Muraru Nsonowa Njuru | | 55, 62, 80, 82, 120, 109, 234 | 2, 3, 4, 7, 8, 11, 13, 14, 16, 17, 19, 25, 26 , 30, 35, 37, 39, 41, 42, 49, 51, 52, 54, 59, 61, 63, 64, 65, 67, 69, 74, 77, 79, 81, 83, 84, 85, 87, 91, 94, 96, 110, 113, 117, 120, 128, 131, 132, 135,137, 138, 141, 142, 143, 144, 146, 151, 153, 159, 160, 161, 165, 171, 174, 178, 184, 196, 204, 205, 215, 216, 217, 218, 219, 221, 222, 227, 229 | 1, 5,10, 15,18, 20, 33, 38, 43, 51, 56, 66, 90, 102, 112, 114, 121, 125, 134 135, 139, 143,169 179, 205, 211, 223, 230 | 68, 162, 164 | LJ56 | LJ37 LJ41 LJ26 LJ49 LJ52 LJ1 LJ38 LJ61 | LJ2 LJ32 LJ16 LJ28 LJ29 LJ13 LJ33 LJ31 LJ17 | LJ50 LJ39 LJ75 LJ40 LJ47 LJ74 LJ34 LJ51 LJ62 LJ48 LJ3 LJ5 LJ14 LJ9 LJ15 LJ19 LJ45 LJ20 LJ25 LJ42 |
| **TOTAL** | 1 | 9 | 7 | | 7 | 81 | 32 | 3 | | | | |
| **Resistant** | **TMB2X8075 (DSI=1)** | | | | **Mpologoma (DSI=1.2)** | | | | | | | |
| **Susceptible** | **Mshale (DSI=4.2)** | | | | **Kayinja (DSI=5.4)** | | | | | | | |

**Objective / Study 2. To develop and phenotype unrelated diploid mapping populations for *Foc* race 1 and race 4 resistances**

Developing two populations for Foc race 1.

1. A resistant Monyet was crossed with a susceptible Kokopo banana plant to generate an $F_1$ population.

The 142 $F_1$ genotypes were screened with Foc race 1 in a pot experiment as described in objective one.

**Results**

**Table 8. DSI screening results for Kokopo x Monyet $F_1$ population**

| | Resistant | Partially resistant | Susceptible | Highly susceptible |
|---|---|---|---|---|
| **Rhizome Discoloration** | 8 | 100 | 33 | 1 |
| **Leaf severity index** | 0 | 73 | 64 | 5 |
| **Stem Splitting** | 139 | 3 | 0 | |

Analysis of variance for the $F_1$ diploid population revealed a significant difference among the genotypes at P<0.001 for RDI, LSI and PS, Table 7. This is evidence that he population is segregating to Foc race 1 resistance. Most of the $F_1$'s were triploids about 87.1%, 8.2% were tetraploids and 4.7% were diploids.

**Table 9. Mean square of an F1 population derived from a cross between Monyet and Kokopo screened for Foc race 1**

| Source of variation | d.f | RDI | LSI | PS |
|---|---|---|---|---|
| Total | 851 | 2.37 | 0.48 | 0.21 |
| Rep | 5 | 2.36 | 0.42 | 0.32 |
| Genotype | 141 | 5.66*** | 0.81*** | 0.34*** |
| Residual | 705 | 1.71 | 0.41 | 0.17 |

*** P<0.001

1. A resistant Calcutta 4 was crossed with a susceptible Mshale banana plant to generate an $F_1$ population of 105 genotypes.
2. The Mshale x Calcutta $F_1$ population of 105 genotypes was planted in the field in January 2018.

**Conclusion / next steps**

Summarises the plan for screening remaining Kokopo X Monyet $F_1$ hybrids, Calcutta 4 x Mshale $F_1$ hybrids and screening open pollinated malaccensis plants to identify parents varying for Foc race 4. Table 8

**Table 10. Timelines for screening the remaining genotypes and populations**

| Kokopo x Monyet | Mshale x Calcutta | OP-malacensis |
|---|---|---|
| 1. 50 Copies are TC<br>2. They could be screened January 2019 | 1. 105 genotypes planted in field in January 2018<br>2. To be screened in 2019 | 1. Parents at UM screened with Foc 4<br>2. Selected parents to be multiplied and planted for crossing |

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

i. Nature of inheritance

Nature of inheritance was determined using frequency histograms.

ii. Genetic ratios

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

iii. Broad sense heritability (H) was computed with formula below

$H = VG/VP$

**Results (Foc race 1 in Monyet x Kokopo population)**

Frequency histograms revealed continuous variation with skewness to the right for RDI and LSI (Figure 1 and 2). For pseudostem splitting, the histogram revealed two categories that is discrete variation, Figure 3.

**i. Nature of inheritance**

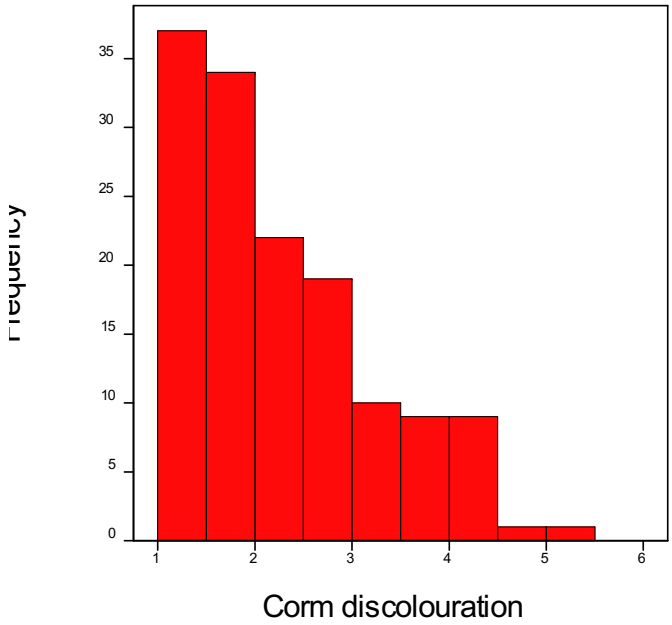


**Figure 1. Cumulative histogram showing nature of inheritance for corm discoloration: Continuous variation (Quantitative/polygenic trait(s)**

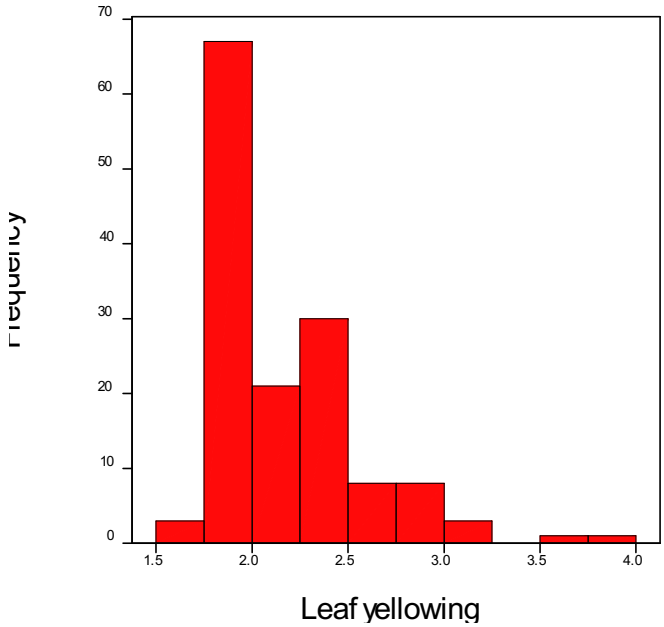


**Figure 2. Cumulative histogram showing nature of inheritance for leaf yellowing: continuous variation (Quantitative/polygenic trait(s)**

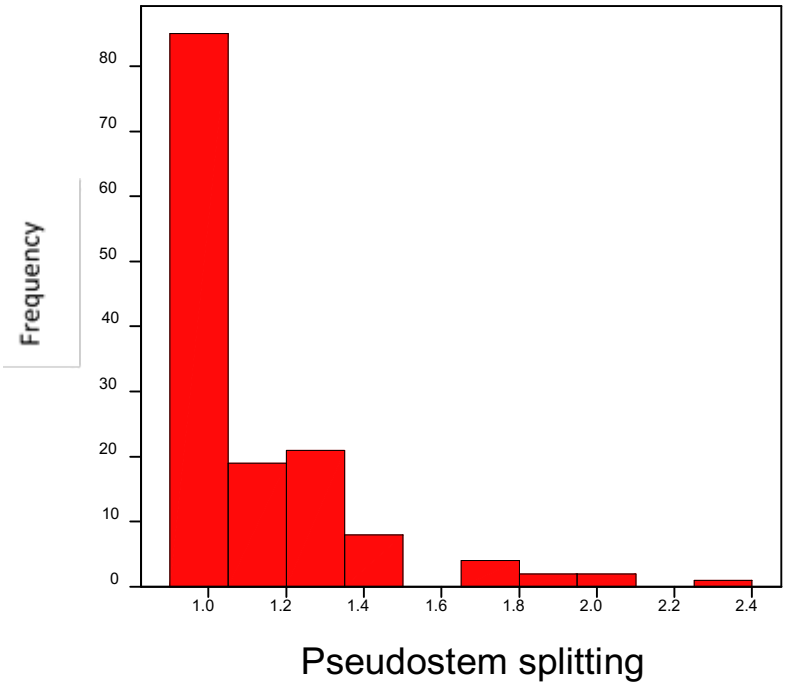


**Figure 3. Cumulative histogram showing nature of inheritance for stem splitting: Qualitative (monogenic) traits**

### ii. Genetic ratios

Three ratios tested non-significant to Chi square (3:1, 11:5 and 13:3) indicating that the genes controlling resistance to Foc race 1 fall within those ratios, Table 9.

**Table 11. Chi-square test for corm discolouration showing non-significant ratios**

| Ratio | Group (G) | Observed (O) | Expected (E) | O-E | (O-E) | (O-E)2/E | X2 | DF | Probability |
|---|---|---|---|---|---|---|---|---|---|
| 03:01 | G1 | 108 | 106.5 | 1.5 | 2.25 | 0.021 | 0.085 | 1 | **0.77** |
| | G2 | 34 | 35.5 | -1.5 | 2.25 | 0.064 | | | |
| 11:05 | G1 | 108 | 97.625 | 10.38 | 107.64 | 1.103 | 3.528 | 1 | **0.06** |
| | G2 | 34 | 44.375 | - 10.38 | 107.64 | 2.426 | | | |
| 13:03 | G1 | 108 | 115.38 | -7.375 | 54.39 | 0.471 | 2.514 | 1 | **0.11** |
| | G2 | 34 | 26.63 | 7.375 | 54.39 | 2.042 | | | |

### iii. Broad sense heritability

Computing the broad sense heritability variance components indicated that parameters for resistance to Foc race 1 had a relatively low heritability. Corm discoloration which is the main parameter for estimating fusarium wilt resistance in banana had a heritability of 27.8%. Leaf yellowing and pseudostem splitting had broad sense heritabilities of 13.9% and 14.7% respectively, Table 10.

**Table 12. Broad sense heritability for fusarium resistance parameters**

| Source of variation | d.f. | Corm discoloration | Leaf yellowing | Pseudostem spliting |
|---|---|---|---|---|
| Rep | 5 | 2.4 | 0.4 | 0.3 |
| Gen | 141 | 5.7 | 0.8 | 0.4 |
| Residual | 705 | 1.7 | 0.4 | 0.2 |
| VE | | 1.7 | 0.4 | 0.2 |
| VG | | 0.7 | 0.1 | 0.03 |
| VP | | 2.4 | 0.5 | 0.2 |
| Heritability (%) | | 27.8 | 13.9 | 14.7 |

**Objective / Study 4. To identify polymorphic and heritable molecular markers 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 **4 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**

**A. Identifying markers showing polymorphism within parents contrasting for Foc race 1 parents.**

### 1. Monyet and Kokopo

Some of the markers screened with the Kokopo and Monyet parents showed polymorphism among the two parents. 2 IRAPS, 10 ISSRs and 15 SSRs showed polymorphism between Kokopo and Monyet and were subsequently used to screen their $F_1$ hybrids to identify markers that are heritable.

**Table 13. IRAP and ISSR markers that showed polymorphism between the contrasting parents of Monyet and Kokopo**

| Category | | Primer Name | Annealing Temps. | Sequence |
|---|---|---|---|---|
| **IRAP** | | | 62°C | |
| | 1 | GyLTRev | | 5'CTTAGGCAAAACCAGCTAAGTCCG 3' |
| | | | | |
| | 2 | Sukkula | | 5' GATAGGGTCGCATCTTGGGCGTGAC 3' |
| **ISSR** | | | 50°C | |
| | 1 | CTC6T | | 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 14. 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'AGTAATCTTTTGTCCTTCAGCT3' | |
| | 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'TACAAAGAGAAAGTGCAGGGGAATA3' | 55 |
| | | AGMI142 | Reverse | 5'CNGCTATAAAGACCACCAGCTTCAT3' | |
| | 11 | AGMI137 | Forward | 5'CTTCCTTTCTGTCTTTTTGATTGTA3' | 56 |
| | | AGMI138 | Reverse | 5'GCAAGTCCTTCTGAATCTTAT3' | |
| | 12 | AGMI159 | Forward | 5'GTTTGGTTGATCCTCCCTTTA3' | 56 |
| | | AGMI160 | Reverse | 5'GAAAACAAGAGAGAGAGAGAGAG3' | |
| | 13 | AGMI203 | Forward | 5'TGCTGCCTTCATCGCTACTA3' | 56 |
| | | AGMI204 | Reverse | 5'GGAACATCGCCCCCGCCAC3' | |
| | 14 | AGMI147 | Forward | 5'CTGCAGCAACCCAAATTTATTTC3' | 56 |
| | | AGMI148 | Reverse | 5'AAATAAGCTCATATGGGTACAGTCA3' | |
| | 15 | AGMI143 | Forward | 5'TCAAGAGCAATGAAGACCTCAAA3' | 56 |
| | | AGMI144 | Reverse | 5'TTTTACATGTACAAGGTCAAGCAAT3' | |

### 2. Mshale and Calcutta 4

Some of the markers screened with the Mshale and Clcutta 4 parents showed polymorphism among the two parents. 2 IRAPS, 10 ISSRs and 15 SSRs showed polymorphism between Mshale and Calcutta 4 and were subsequently used to screen their $F_1$ hybrids to identify markers that are heritable.

**Table 15. 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'CTTAGGCAAAACCAGCTAAGTCCG 3' |
| | 2 | Sukkula | | 5' GATAGGGTCGCATCTTGGGCGTGAC 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 16. 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'GTGGTTTGGCAGTGGAATGGAA3' | 47 |
| | | AGMI134 | Reverse | 5'GTATGGCTCAGCTGTATCCATC3' | |
| | 3 | AGMI155 | Forward | 5'CGAAACCTGCTGGACGAGT3' | 50 |
| | | AGMI156 | Reverse | 5'CGGGACCCAAGGAGGAGG3' | |
| | 4 | AGMI187 | Forward | 5'GCAACTTTGGCAGCATTTT3' | 52 |
| | | AGMI188 | Reverse | 5'TGAGATATAGAGGAAAATAATGTTA3' | |
| | 5 | AGMI131 | Forward | 5'ATCTTTTCTTATCCTTCTAACG3' | 52 |
| | | AGMI132 | Reverse | 5'CGCTTTAGATTCTGTTTAAG3' | |
| | 6 | AGMI201 | Forward | TGGTTGAGTAGATCTTCTTGTGTTC | 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'CTGCAGCAACCCAAATTTATTTC3' | 55.2 |
| | | AGMI148 | Reverse | 5'AAATAAGCTCATATGGGTACAGTCA3' | |
| | 10 | AGMI139 | Forward | 5'GGGGAACAGCACGGTCACAT3' | 56 |
| | | AGMI140 | Reverse | 5'ACGATGACAACCATTACTAC3' | |
| | 11 | AGMI137 | Forward | 5'CTTCCTTTCTGTCTTTTTGATTGTA3' | 56 |
| | | AGMI138 | Reverse | 5'GCAAGTCCTTCTGAATCTTAT3' | |
| | 12 | MusaBAG1_SSR1_F | Forward | 5'GACTCTGGAGCATCTTGTCCAT3' | 56 |
| | | MusaBAG1_SSR1_R | Reverse | 5'CTTTATCTTCGCCAACCCTAACGG3' | |
| | 13 | AGMI203 | Forward | 5'TGCTGCCTTCATCGCTACTA3' | 58 |
| | | AGMI204 | Reverse | 5'GGAACATCGCCCCCGCCAC3' | |
| | 14 | AGMI143 | Forward | 5'TCAAGAGCAATGAAGACCTCAAA3' | 58 |
| | | AGMI144 | Reverse | 5'TTTTACATGTACAAGGTCAAGCAAT3' | |
| | 15 | MusaBAG1_SSR3_F | Forward | 5'GGATGGAATTCTCCTCCATCTC3' | 58 |
| | | MusaBAG1_SSR3_R | Reverse | 5'GGAAGGAGAAGGATGCATGAAACAGG3' | |

## B. Polymorhic and heritable markers within Kokopo x Monyet F1 hybrid population

### 1. Kokopo x Monyet

Screening the markers that revealed polymorphism with Kokopo and Monyet parents with their $F_1$ hybrids revealed important polymorphism within the population. 2 IRAP, 5 ISSR and 10 SSR markers showed heritable bands from the parents to the $F_1$ offspring. The inherited bands will be used to determine the recombination frequency and subsequently determine if they are linked to Fusarium wilt.

**Table 17. Polymorphic and heritable markers within Kokopo x Monyet F1 hybrid population**

| | IRAP | % Polymorphism |
|---|---|---|
| 1 | GyLTRev | 35.7 |
| 2 | Sukkula | |
| | ISSR | |
| 1 | GTG6A | 20 |
| 2 | AC10T | 11.8 |
| 3 | CTC6T | 9.1 |
| 4 | CA10G | 8.3 |
| 5 | GTG6T | 5.6 |
| | | |
| | SSR | |
| 1 | AGMI 131-132 | |
| 2 | AGMI 137-138 | |
| 3 | AGMI 139-140 | |
| 4 | AGMI 145-146 | |
| 5 | AGMI 147-148 | |
| 6 | AGMI 187-188 | |
| 7 | AGMI 141-142 | |
| 8 | AGMI 189-190 | |
| 9 | AGMI 197-198 | |
| 10 | AGMI 199-200 | |

**2. Mshale x Calcutta 4**

Screening the markers that revealed polymorphism with Mshale x Calcutta 4 parents with their $F_1$ hybrids revealed important polymorphism within the population. 2 IRAP, 5 ISSR and 13 SSR markers showed heritable bands from the parents to the $F_1$ offspring. The inherited bands will be used to determine the recombination frequency and subsequently determine if they are linked to Fusarium wilt.

**Table 18. Polymorphic and heritable markers within Mshale x Calcutta 4 F1 hybrid population**

| | IRAP | % Polymorphism |
|---|---|---|
| 1 | GyLTRev | 50 |
| 2 | Sukkula | |
| | ISSR | |
| 1 | GTG6A | 35.7 |
| 2 | AC10T | 14.3 |
| 3 | AC10G | 26.7 |
| 4 | CAC6T | 23.5 |
| 5 | ACC6G | 11.1 |
| | | |
| | SSR | |
| 1 | AGMI 131-132 | |
| 2 | AGMI 137-138 | |
| 3 | AGMI 139-140 | |
| 4 | AGMI 145-146 | |
| 5 | AGMI 147-148 | |
| 6 | AGMI 187-188 | |
| 7 | AGMI 133-134 | |
| 8 | AGMI 155-156 | |
| 9 | AGMI 143-144 | |
| 10 | AGMI 201-202 | |
| 11 | AGMI 203-204 | |
| 12 | MusaBAG1_SSR1 | |
| 13 | MusaBAG1_SSR3 | |

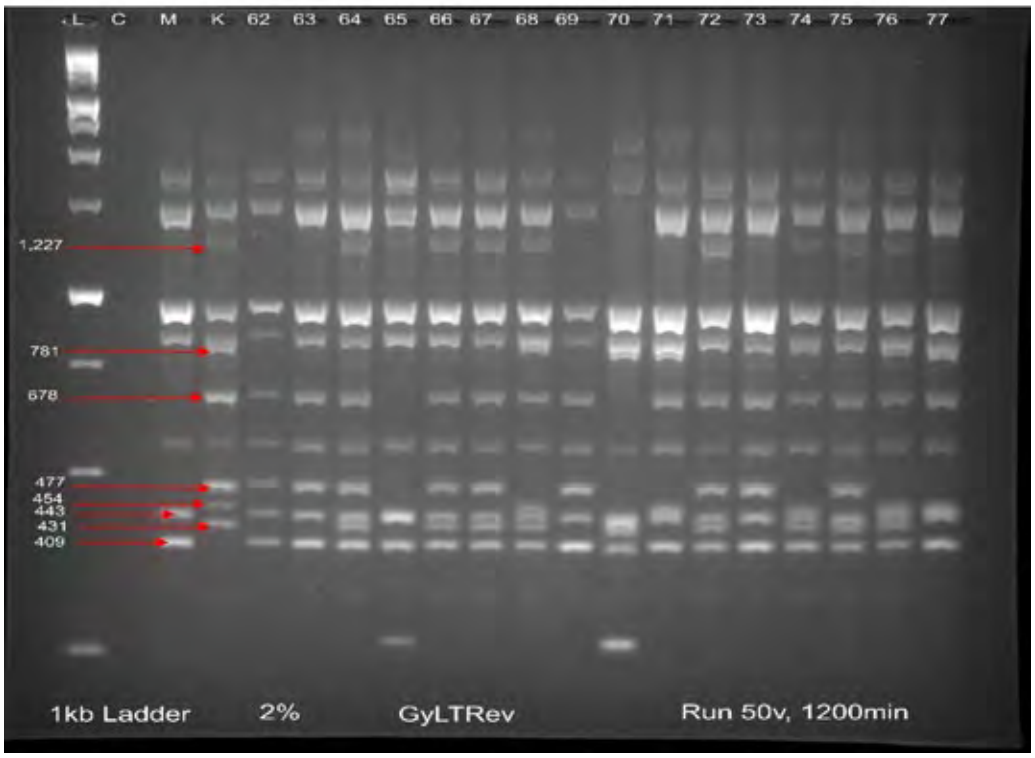


**Figure 4. Gel picture showing polymorphic and heritable bands of Monyet x Kokopo using IRAP- GyLTRev**

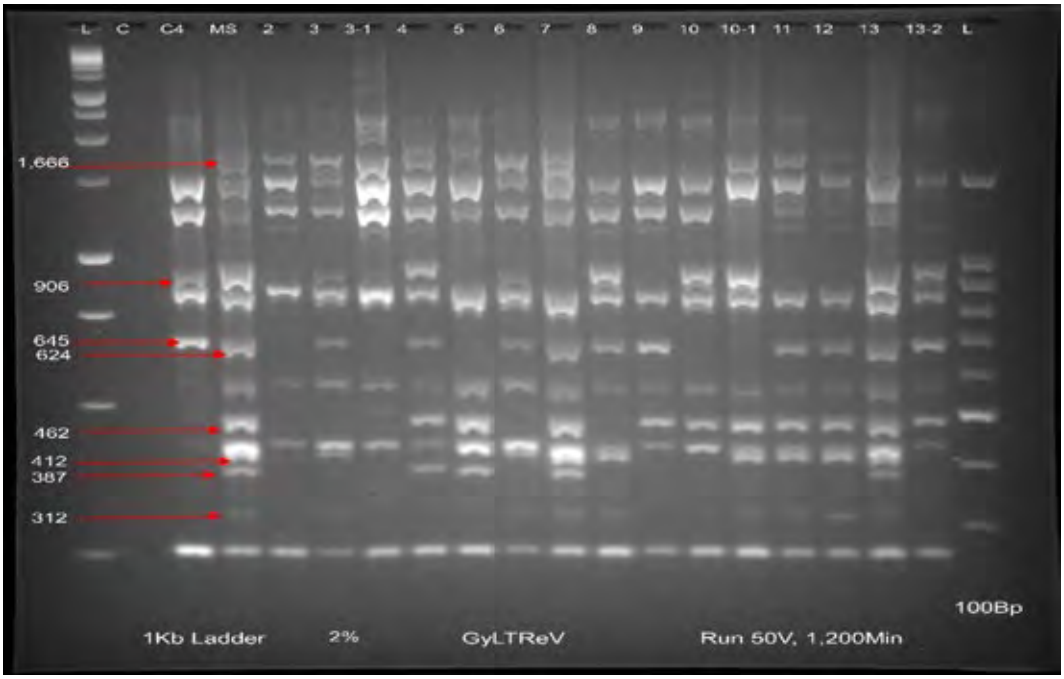


**Figure 5. Gel picture showing polymorphic and heritable bands of Mshale x Calcutta 4 IRAP-GyLT Mshale x Calcutta 4**

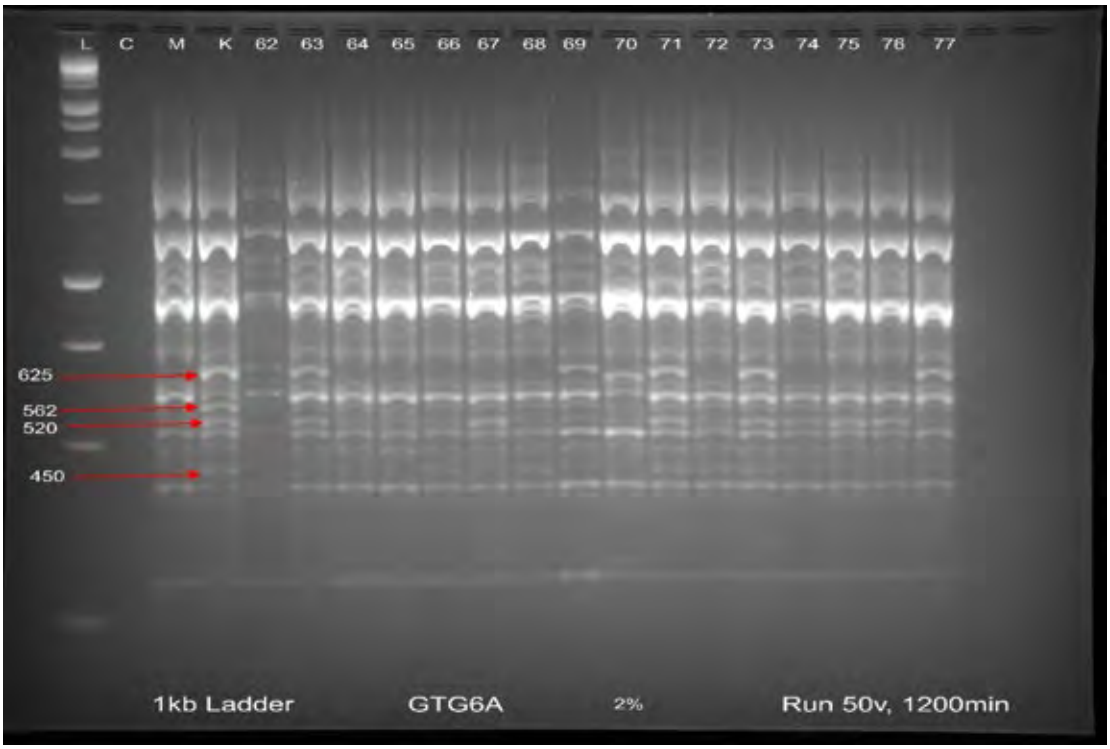


**Figure 6. Gel picture showing polymorphic and heritable bands of Monyet x Kokopo ISSR-GTG6A**

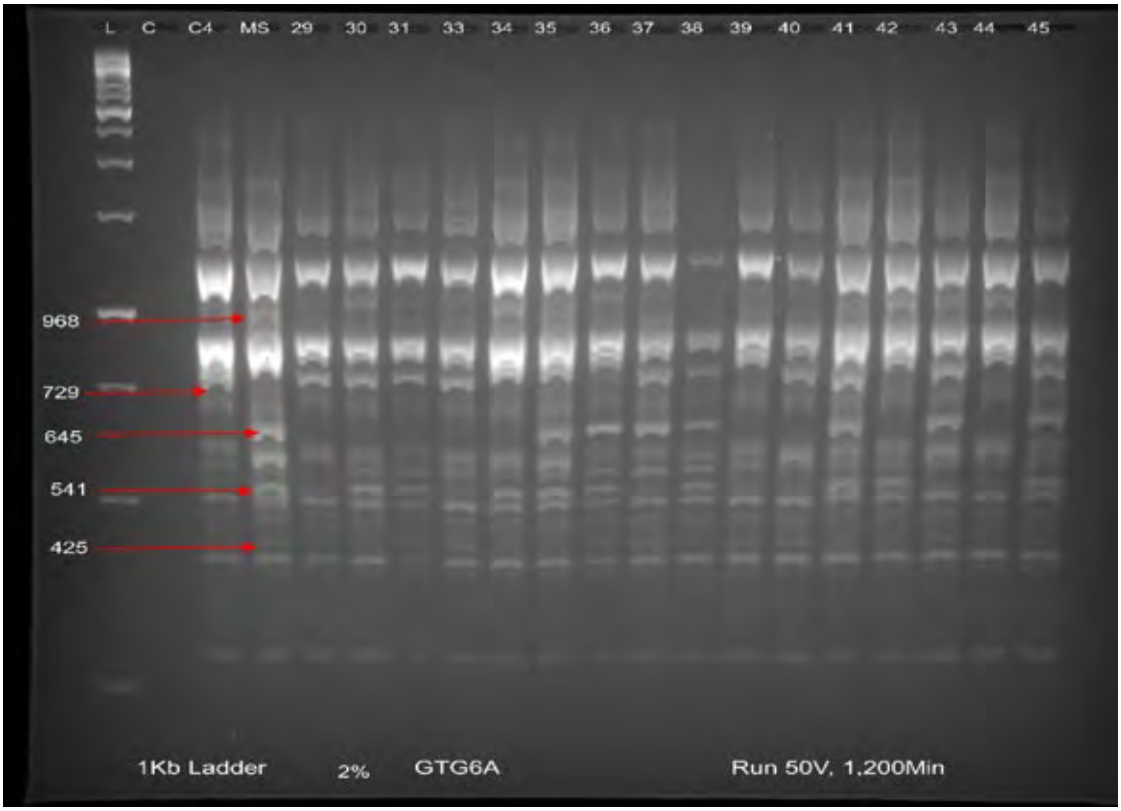


**Figure 7 Gel picture showing polymorphic and heritable bands of Mshale x Calcutta4 ISSR-GTG6A**

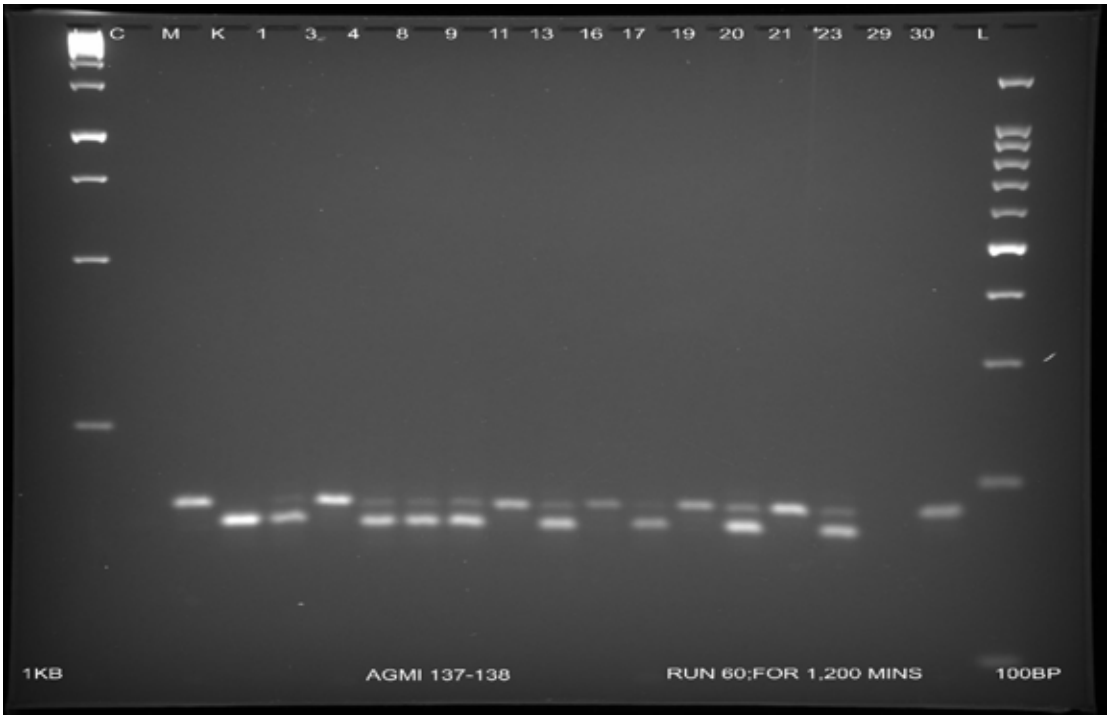


**Figure 8 Gel picture showing polymorphic and heritable bands of Monyet x Kokopo SSR-AGMI 137-138**

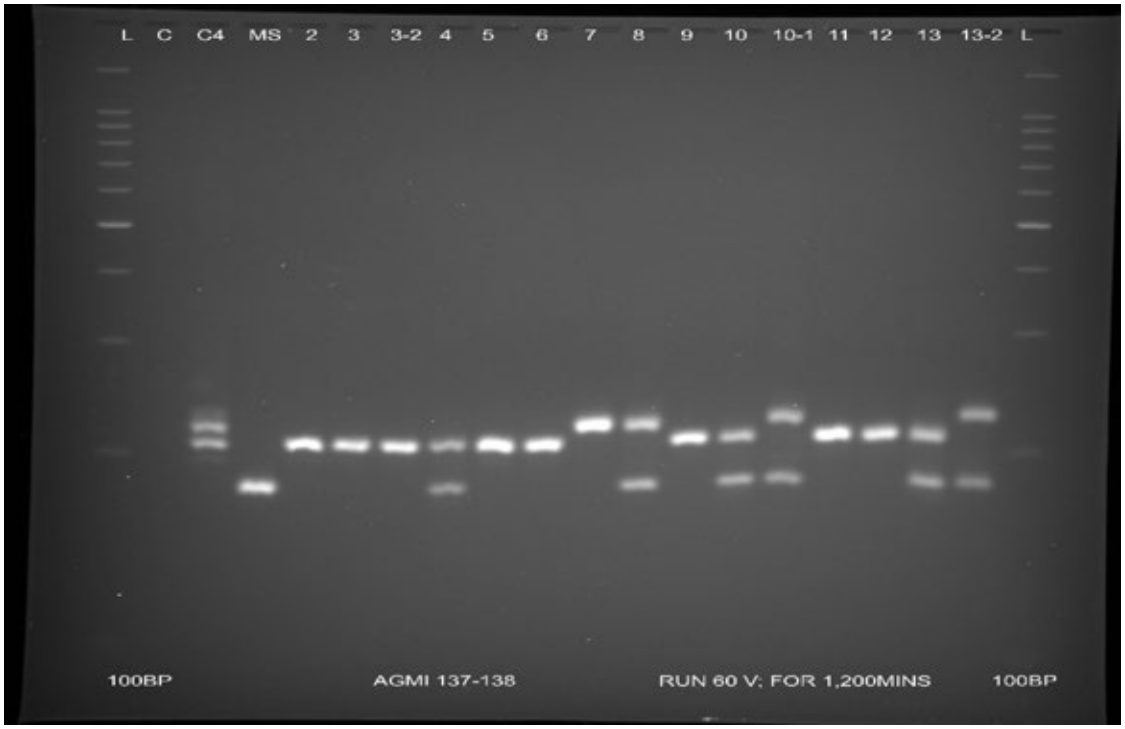


Figure 9. Gel picture showing polymorphic and heritable bands of Mshale x Calcutta4 SSR- AGMI 137-138

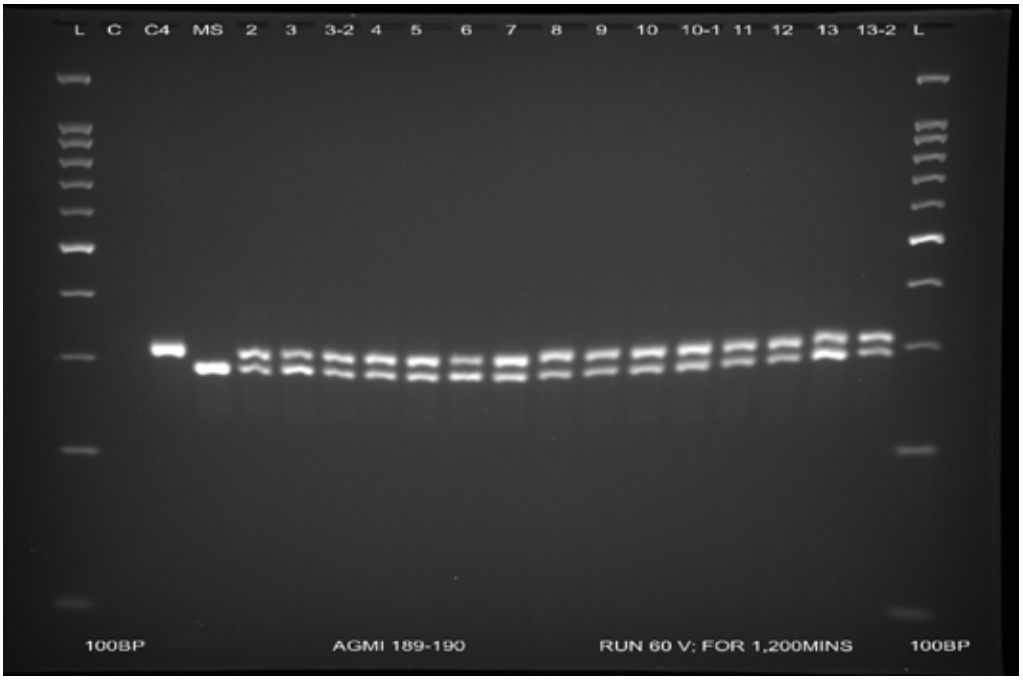


Figure 10. Gel picture showing polymorphic and heritable bands of Mshale x Calcutta4 SSR- AGMI 189-190

**Objective / Study 5. To perform a QTL analysis for *Foc* race 1 and race 4**

1. For IRAP, ISSR and SSR, QTL analysis will be performed using: GACD (Zhang *et al*., 2016). GACD: Integrated Software for Genetic Analysis in Clonal F1 and Double Cross Populations.)

**NB. 197 genotypes have been genotyped with IRAP, ISSR and SSR. Analysis of 140 genotypes that have been phenotyped is ongoing at UM.**

2. For SNP markers,

- DNA is already sent to Biosciences eastern and central Africa (BecA) for genotyping and awaiting results to perform QTL analysis for the already phenotyped genotypes.

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## WP3 - PhD Research Progress Report (2017-2018)

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

**Name of Student**: **MOHAMED HUSSEIN MPINA**

**Supervisors**: Main supervisors: Prof. Altus Viljoen (Stellenbosch University)
Co-supervisor: Dr. Allan Brown (IITA), Dr. George Mahuku (IITA)

**Timeline of study**: 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

**Objectives or study areas**

- Segregation test for Paliama x Borneo mapping population for resistance to Foc race 1
- Phenotyping of Paliama x Borneo mapping population for resistance to Foc race 1
- Phenotyping Malaccensis x Malaccensis mapping population for resistance to Foc race 1
- Genotyping (SNP calling and linkage mapping)
- Marker-trait association (QTL mapping) for resistance to Foc race 1

**Achievements**: Highlight significant achievements

Paliama X Borneo 190 $F_1$ genotypes

1. Paliama (susceptible) and Borneo (moderate resistant) differs in reaction to Foc race 1
2. $F_1$ genotypes showed continuous frequency distribution in reaction to Foc race 1
3. The phenotypic data for Foc race 1 consists of 145 (76%) Paliama x Borneo $F_1$ genotypes
4. The phenotypic data for Foc race 1 of 30 other Paliama x Borneo $F_1$ genotypes will be available by September 2018
5. DNA are extracted for 190 genotypes of Paliama x Borneo population
6. DNA for 190 genotypes are sent for genotyping

Malaccensis X Malaccensis 188 genotypes

7. The genotypes showed continuous frequency distribution in reaction to Foc race 1
8. The phenotypic data for Foc race 1 of other 35 Malaccensis x Malaccensis genotypes will be available by September 2018

**Background/introduction**

Brief background

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is a plant disease affecting many banana cultivars grown by smallholder farmers in the Africa Great Lakes Region. Long persistence of *Foc* in the soil and challenging facing fungicide applications complicates control measures. Therefore, resistance among banana cultivars remain the most effective and sustainable management option. With the exception of the East Africa highland bananas (EAHB), most other banana cultivated in the East Africa region including ''Sukari Ndizi'' (AAB) and ''Mchare'' (AA) are susceptible to *Foc* race 1. *Foc* race 1 is thus considered a major banana production constraint in the Africa Great Lakes region.

Banana resistance to *Foc* race 1 has been reported in several wild diploid bananas as *M. acuminata* ssp. *malaccensis* and *burmannica*. However, introgression of resistance into edible cultivars is slow due to sterility and the long banana life cycle. Genetic markers for the early selection of resistance traits will speed up banana breeding for *Foc* resistance and other important traits. The development of genetic markers is currently hindered by the presence of relatively few banana-mapping populations and a lack of dense genetic linkage maps.

The aim of this study is to elucidate the genetics of resistance in banana and identify genetic markers associated with resistance to *Foc* race 1, with 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
- 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 the African Great Lakes region. This study will be conducted at IITA Arusha station and at the 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 (susceptible) and Borneo (moderate resistant) differs in reaction to Foc race 1
  - $F_1$ genotypes showed continuous frequency distribution in reaction to Foc race 1
  - The phenotypic data for Foc race 1 of 145 Paliama x Borneo $F_1$ genotypes (76%) are available
  - The phenotypic data for Foc race 1 of other 30 Paliama x Borneo $F_1$ genotypes will be available by September
  - The genotypes showed continuous frequency distribution in reaction to Foc race 1
  - The phenotypic data for Foc race 1 of 65 genotypes of Malaccensis x Malaccensis are available
  - The phenotypic data for Foc race 1 of other 35 Malaccensis x Malaccensis genotypes will be available by September 2018

When all required genotypes are evaluated, data will be subjected to analysis of variance (ANOVA). Joint Segregation analysis and heritability analysis will be performed.

**Objective / Study 2**

**To construct high-density genetic linkage map with a diploid population**

- Methods: SNP calling and linkage analysis with Join map version 4.1
- Results: Linkage maps will be constructed.
  - DNA were extracted for 190 genotypes of Paliama x Borneo population
  - DNA for 190 genotypes were sent for genotyping

**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**

- Phenotyping of the Paliama x Borneo mapping population for Foc race 1 will continue
- TC work for Malaccensis x Malaccensis genotypes is continuing
- Phenotyping of Malaccensis x Malaccensis is continuing

## WP3 - MSc Research Progress Report (2017-2018)

**TITLE:** Development of transcriptome-based Simple Sequence Repeat Markers and their application in genotyping Ugandan banana weevils (*Cosmopolites sordidus*)

**Name of Student:** **ALI MILTON**

**Supervisor**: Dr. Robooni Tumuhimbise, Dr. Muhanguzi Denis

**Timeline of study:** 8th August 2016 to 22rd April 2019

**University:** Makerere University

### Research Objectives

List the individual topics of study – objectives or study areas

i. To identify and develop SSR markers from the banana weevil transcriptome
ii. To assess the level of genetic diversity among and within banana weevil populations using the newly developed SSR markers
iii. To assess the weevil damage among the new banana hybrids using different weevil populations.

Highlight significant achievements – e.g. in bullets

- The SSRs were identified from the weevil transcript and the primer markers designed were delivered and optimization will start this month
- From this study objective, it was discovered that the SSR markers generated were di, tri- tetra-, penta and hexa nucleotide SSRs. SSR markers for primer design were selected based on a minimum length of 18 to 20 bp. These parameters were chosen because the polymorphism level decreases with SSRs shorter than 18 bases (Cho *et al.*, 2000). The total SSR identified were 2,098 SSRs out of total of 48,864 reads. The frequency of finding a SSR motif in the reads was 4.3 %. In banana weevil transcriptome the dinucleotide motifs (dimers) are the most abundant (72 %), followed trinucleotide (24.5 %), tetra-nucleotide (3.1 %), hexa-nucleotide (0.1 %) and penta-nucleotide (0.1 %) respectively. Most of the banana weevil transcriptome were rich in AT/TA dimers. Among the trimers, GCG/CGC was the most abundant, followed by CTC/GAG
- The weevil populations from all the selected agro-ecological zones have been sampled and DNA extracted from them.
- The selected hybrids for screening to be used are the NARITAs and their parents. This has weaned and will be screened later after acquiring large corms.
- Research proposal submitted and defense at university completed.

### Background/introduction

The banana corm weevil, *Cosmopolites sordidus* (Germar), is an important economic pest in Uganda that can cause severe yield loss of 40-100 % depending upon the stage at which infestation occurs. In spite of its economic importance, little is known about the population structure of this pest in Uganda. Understanding the genetic diversity of weevil biotypes can provide important information on the population structure of this pest, which will aid in designing suitable strategies for its control and management, especially with respect to insecticide resistance. The study of genetic diversity has been done using Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), however these markers are not

reproducible, are costly and dominant. The most suitable technique could be the use of simple sequence repeats (SSR), but their development relies on genomes and transcriptome sequences. The objective of this study is to identify and develop SSR markers from the banana weevil transcriptome**,** to assess the level of genetic diversity among and within banana weevil populations using the newly developed SSR markers, and to assess the weevil damage among the new banana hybrids using different weevil populations. The SSR markers will be identified using the simple sequence identification tool and markers picked on basis of primer parameters. These primers will be evaluated for amplification and polymorphism using GenAlEx for analysis of molecular variance (AMOVA) to determine the genetic structure and genetic diversity present within and among populations. Pairwise comparison will be conducted to test genetic divergence among populations ($F_{ST}$). The banana hybrids will be then be assessed for weevil damage using the weevil populations and analyzed by GenStat 14$^{th}$ edition to determine the total corm damage.

**Objective / Study 1**. **Developing SSR markers from banana weevil transcriptome**

The SSR markers generated were di, tri- tetra-, penta and hexa nucleotide SSRs. SSR markers for primer design were selected based on a minimum length of 18 to 20 bp. These parameters were chosen because the polymorphism level decreases with SSRs shorter than 18 bases (Cho *et al.*, 2000). The total SSR identified were 2,098 SSRs out of total of 48,864 reads. The frequency of finding a SSR motif in the reads was 4.3 %. In banana weevil transcriptome the dinucleotide motifs (dimers) are the most abundant (72 %), followed trinucleotide (24.5 %), tetra-nucleotide (3.1 %), hexa-nucleotide (0.1 %) and penta-nucleotide (0.1 %) respectively. Most of the banana weevil transcriptome were rich in AT/TA dimers. Among the trimers, GCG/CGC was the most abundant, followed by CTC/GAG as in figure 1 below

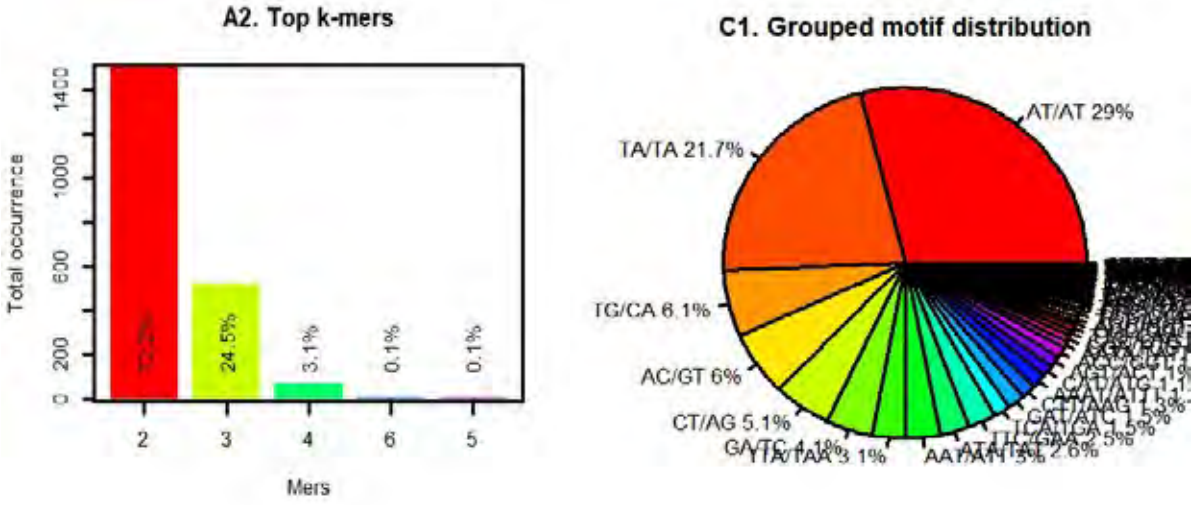



**Figure 1 Selected statistical graphics by GMATA for the banana weevil transcriptome SSRs analyzed in GMATA using the default settings. (A2) Shows the mer or length distribution of the repeated motif, (C1) shows the distribution of the repeated motif nucleotides**

The primer pairs were picked according to the following parameters: 20-28 nucleotide primer length, TM of 57°C-63°C, GC content of 40-60 %. The sequence size used for designing primers ranged also from 536 base pairs to 8,336 base pairs with the polymerase chain

reaction product size ranging from 400-800 base pairs. The following are the primer pairs selected and send for synthesis in Eurofin as attached on the table 1.

Table 1. SSR primer pairs to be screened for amplification and polymorphism

| Sequence Id | Primer Sequence ( 5'-3' ) | Motif | PCR Product |
|---|---|---|---|
| BWF1 | CATGTCAACACCAATGAAGA | ATAT | 572 |
| BWR1 | TGAGTGATCCGGTAAATAGG | | |
| BWF2 | GATGGCTTGTATGCTGATTT | TGG | 486 |
| BWR2 | ACTCTGATCCCCAATTTTCT | | |
| BWF3 | CGAGCAGAGAAGTAAACAGA | TAA | 507 |
| BWR3 | CTGCCTCAAAATGATAAAGG | | |
| BWF4 | ACATGGGATGTCCTTTCTTT | TATA | 381 |
| BWR4 | TTGGTATTTCGACCTGCTT | | |
| BWF5 | GAGCTATGAAGACACAGAAACA | TGA | 499 |
| BWR5 | TAACTCAGAAAGGGGAAACA | | |
| BWF6 | GCAGCAAAGCCAAAAGGT | AAG | 732 |
| BWR6 | CCTGAGTTAAGCCTGTAGTTG | | |
| BWF7 | GTATTGCAAAGCAAACAGG | ATT | 760 |
| BWR7 | GCAGCTTAATCTCACCAAAA | | |
| BWF8 | CAGGTCTGTTGTGATTCTGTT | TAA | 507 |
| BWR8 | CTGTTCAATTGTGTGTGTGG | | |
| BWF9 | ATCAAACATGCAATCTCTCC | TGAT | 490 |
| BWR9 | AATAAGCAAGAGACCCAACA | | |
| BWF10 | TGCGAAATACTATAGGAAGG | TAT | 501 |
| BWR10 | AAGAATTGTGCAAGACCAAG | | |
| BWF11 | TAAAAGTTTGCAGGAACAGG | ACA | 490 |
| BWR11 | AATAACGAAAACGAGTGTGG | | |
| BWF12 | TTAGCAGCTTGACCTATTCC | TGT | 472 |
| BWR12 | ATCAGTCAGCATCAACAACA | | |
| BWF13 | GTTCTTCGAGGTCCTTTTCT | TTCT | 495 |
| BWR13 | CAGATTTGACGGAGTATGTTG | | |
| BWF14 | GACGTATTTTACGCCATCTT | ACC | 497 |
| BWR14 | GGTATGGGAATTTCTTGTTG | | |
| BWF15 | GACGTATTTTACGCCATCTT | CCT | 497 |
| BWR15 | GGTATGGGAATTTCTTGTTG | | |
| BWF16 | GTCGGTTCAAACGAAACA | ACG | 515 |
| BWR16 | ATGATGAAACCACAAAGGAG | | |
| BWF17 | GTCGGTTCAAACGAAACA | CGC | 515 |
| BWR17 | ATGATGAAACCACAAAGGAG | | |
| BWF18 | GTCGGTTCAAACGAAACA | ACG | 515 |
| BWR18 | ATGATGAAACCACAAAGGAG | | |
| BWF19 | TTGATCCTGAATTGCACAC | ATT | 445 |
| BWR19 | TTTTGTCTGGACGGATCTT | | |
| BWF20 | TGTCTTCAGTACATCCCTGTT | TTTA | 482 |
| BWR20 | CTGATCAGTTTCGATGTTTG | | |
| BWF21 | GCTAATAGGAAGCAAACGAA | ATTT | 535 |
| BWR21 | AGCGCCATCTATAGGAAAA | | |
| BWF22 | GATAACTTCACCACCTCCAA | TTC | 536 |
| BWR22 | TAGAAGAGGAAGAGGCAACA | | |

**Objective / Study 2 Assessing the genetic diversity among banana weevils with newly developed microsatellite markers.**

The DNA of banana weevils from twelve districts has been extracted and is being analyzed with the primers.

Table 2. Concentration of DNA extracted from different weevil populations

| Sample ID | Sample Type | Nucleic Acid Conc. | Unit | 260/280 | 260/230 | 25ng/µl | H2O | Factor |
|---|---|---|---|---|---|---|---|---|
| 1 | DNA | 6200.2 | ng/µl | 1.72 | 1.39 | 0.2016 | 49.798 | 50 |
| 2 | DNA | 1344.5 | ng/µl | 1.72 | 1.22 | 0.9297 | 49.07 | 50 |
| 3 | DNA | 1041.5 | ng/µl | 2.01 | 1.91 | 1.2002 | 48.8 | 50 |
| 4 | DNA | 600.8 | ng/µl | 1.64 | 1.19 | 2.0806 | 47.919 | 50 |
| 5 | DNA | 601.6 | ng/µl | 1.9 | 1.74 | 2.0778 | 47.922 | 50 |
| 6 | DNA | 891.8 | ng/µl | 2 | 1.71 | 1.4017 | 48.598 | 50 |
| 7 | DNA | 1215.8 | ng/µl | 1.35 | 0.8 | 1.0281 | 48.972 | 50 |
| 8 | DNA | 794.8 | ng/µl | 1.99 | 1.58 | 1.5727 | 48.427 | 50 |
| 9 | DNA | 640.3 | ng/µl | 1.74 | 1.19 | 1.9522 | 48.048 | 50 |
| 10 | DNA | 717 | ng/µl | 1.65 | 1.08 | 1.7434 | 48.257 | 50 |
| 11 | DNA | 1205.4 | ng/µl | 1.76 | 1.42 | 1.037 | 48.963 | 50 |
| 12 | DNA | 729.1 | ng/µl | 1.95 | 1.59 | 1.7144 | 48.286 | 50 |
| 13 | DNA | 637.8 | ng/µl | 2.12 | 3.27 | 1.9599 | 48.04 | 50 |
| 14 | DNA | 917.2 | ng/µl | 1.63 | 1.14 | 1.3628 | 48.637 | 50 |
| 15 | DNA | 1334.7 | ng/µl | 1.74 | 1.31 | 0.9365 | 49.063 | 50 |
| 16 | DNA | 1031.4 | ng/µl | 1.8 | 1.4 | 1.2119 | 48.788 | 50 |
| 17 | DNA | 1284 | ng/µl | 1.27 | 0.77 | 0.9735 | 49.026 | 50 |
| 18 | DNA | 464.4 | ng/µl | 1.84 | 1.53 | 2.6916 | 47.308 | 50 |
| 19 | DNA | 2790.7 | ng/µl | 1.11 | 0.64 | 0.4479 | 49.552 | 50 |
| 20 | DNA | 1732.4 | ng/µl | 1.53 | 1.01 | 0.7215 | 49.278 | 50 |
| 21 | DNA | 871.5 | ng/µl | 1.85 | 1.49 | 1.4343 | 48.566 | 50 |
| 22 | DNA | 499.3 | ng/µl | 1.89 | 1.59 | 2.5035 | 47.496 | 50 |
| 23 | DNA | 1048.2 | ng/µl | 1.78 | 1.47 | 1.1925 | 48.807 | 50 |

**Objective/Study 3 Assessing selected banana hybrids and their parents for their response to banana weevils**

All the NARITA hybrids, their parents and Calcutta 4, KM5 and Mbwazirumwe have been weaned in the nursery with required copies of each ranging from 6 to 12. The following is the table of NARITA and their accessions that have been weaned in the nursery.

Table 3. NARITA hybrids and their parents weaned for assessing weevil damage

| Name | Hybrid code | Female parent | Male parent |
|---|---|---|---|
| NARITA 23 | 21086S-1 | Kazirakwe | 7197-2 |
| NARITA 18 | 14539S-4 | 365K-1 | 660K-1 |
| NARITA 7 (M9) | 12419S-13 | 1201K-1 | SH3217 |
| NARITA 22 | 19798S-2 | 917K-2 | 9128-3 |
| NARITA 8 | 12468S-18 | 917K-2 | SH3217 |
| NARITA 14 | 12949S-2 | 917K-2 | 7197-2 |

| | | | |
|---|---|---|---|
| NARITA 4 | 9187S-8 | 660K-1 | 9128-3 |
| NARIT21 | 17503S-3 | 1201K-1 | 7197-2 |
| NARITA 9 | 12468S-6 | 917K-2 | SH3217 |
| NARITA 12 | 12479S-13 | 1201K-1 | 9128-3 |
| NARITA 11 | 12479S-1 | 1201K-1 | 9128-3 |
| NARITA 26 | HJ | Unknown | Unknown |
| NARITA 15 | 13284S-1 | 660K-1 | 9128-3 |
| NARITA 10 | 12477S-13 | 917K-2 | SH3217 |
| NARITA 1 | 7798S-2 | 917K-2 | 9128-3 |
| NARITA 13 | 12618S-1 | 1201K-1 | SH3362 |
| NARITA 3 | 9494S-10 | 917K-2 | SH3362 |
| NARITA 25 | HX | Unknown | Unknown |
| NARITA 24 | HB | Unknown | Unknown |
| NARITA 2 | 9750S-13 | 401k-1 | 9128-3 |
| NARITA 20 | 16457S-2 | Entukura | 365K-1 |
| NARITA 19 | 16242S-1 | 1201K-1 | 8075-7 |
| NARITA 17 | 13573S-1 | 1438K-1 | 9719-7 |
| NARITA 16 | 135225S-5 | 917K-2 | SH3362 |
| NARITA 5 | 8386S-19 | 917K-2 | SH3217 |
| NARITA 6 | 11274S-3 | 222K-1 | 9128-3 |
| NARITA 27 | 9518S-12 | 222K-1 | SH 3362 |

**Conclusion / next steps**

- Phenotpying of the NARITA hybrids and their parents for weevil damage
- Conclude multiplication of NARITA hybrids and their parents in tissue culture
- Genotyping the banana weevil populations is on-going

## WP3 - MSc Research Progress Report (2017-2018)

**Title of the research:** QTL mapping for resistance to nematodes (*Radopholus similis*) in a diploid segregating banana population

**Name of Student:** **JEAN CLAUDE HABINEZA**

**Supervisors:** Dr. Brigitte Uwimana (IITA), Dr. Coyne Danny (IITA) and Dr. Richard Edema (Makerere University)

**Timeline of study:** From 1st June 2017 up to 31st May 2018 (12 months)

**University:** Makerere University

### General objectives

To contribute to the improvement of the efficiency of banana breeding for nematode resistance

### Specific objectives

- To identify improved diploids ($F_1$ hybrids) with resistance to nematodes that can be used in breeding
- To understand the inheritance patterns of nematode resistance in a banana $F_1$ diploid population
- To identify and map QTLs associated with traits for *R. similis* resistance

### Summary of activities

- Phenotyping
- Genotyping

**Activity I: Phenotyping**

This activity is related to the first and second objective entitled:

1) To identify improved diploids (F1 hybrids) with resistance to nematodes that can be used in breeding

2) To understand the inheritance patterns of nematode resistance in a banana F1 diploid population

This activity included the following:

- Screening of at least 116 genotypes from a cross between Zebrina GF and Calcutta 4 (F1 diploid population). Parents and checks are tested for the effect of the experiment across time
- Analysis

**Experimental design**

- RCBD design with three replications with 33 genotypes including 29 hybrids, 2 parents and 2 checks (KM5 &Valery)

## Achievements

111 genotypes have been screened and all data have been entered. The analysis and writing about this activity is ongoing. The first draft has been sent to supervisors for review. Below is a summary of tentative results:

| Item | Results |
|---|---|
| Screening | Out of 111 screened genotypes, 14 were found resistant, 39 partially resistant and 58 susceptible. Classification was done according to Dunnett classification. |
| Heritability | The estimation of broad sense heritability was 0.58 for total nematode count and 0.41 for necrosis damage. |
| Correlation | The two traits (Total nematodes count and % necrosis) were found to be positively correlated with a correlation coefficient of 0.59. |
| Gene action | The resistance for *R. similis* is likely to be controlled by two genes with additive gene actions as the observed values from screening coincide with the epistatic ratio of 9:6:1. This was done by comparing observed values and expected values of dihybrid segregation using Chi square goodness of fit. |

**Activity II: Genotyping**

This activity is related to the third objective entitled "To identify and map QTLs associated with traits for *R. similis* resistance" and it involves the following:

- DNA extraction
- DNA sequencing
- Analysis

## Achievements

DNA has been extracted for phenotyped genotypes and sent to Kenya for sequencing. For this activity, we are still waiting for results so that we can go ahead with the analysis. The analysis will cover the construction of linkage map and QTL mapping by linkage analysis. Genestat 19$^{th}$ edition software will be used.

**Challenges**

The main challenge is the delay of DNA sequencing results which actually is affecting me because I am out of time as I was supposed to submit the thesis at the end of August, 2018.

# WP3 - MSc RESEARCH PROGRESS REPORT

**TITLE:** Study of QTLs for banana resistance to weevil (*Cosmopolites sordidus* Germar)

**NAME OF STUDENT:** **MWANJE GERALD**

**SUPERVISOR**: Dr. Brigitte Uwimana and Prof. Patrick Rubaihayo

**UNIVERSITY**: Makerere, Kampala, Uganda

**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**

- Phenotypic data have been collected from 107 $F_1$ progenies of Monyet × Kokopo which were established in the first, second and the third series.
- More 32 genotypes have been established in the pot experiment.
- DNA has also been extracted from all the genotypes in the Monyet × Kokopo population and sent for genotyping.

**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 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 $F_1$ 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 Calcutta4 and Yangambi km5 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 107 genotypes have been phenotyped whose preliminary results are shown in table 1.

The phenotypic data from the population has been used to determine the broad sense heritability (H) using the variance component formula:

$H=\frac{\delta G}{\delta P}$ where ($\delta P=\delta e+\delta G$) and $\delta e$ is Residual error mean square

**Table 19. Statistical comparison of percentage total corm damage for genotypes from (Monyet x Kokopo) population with the resistant and susceptible controls using Dunnet's test**

| Statistical comparison with | Statistical comparison with | Host response | Number of genotypes |
|---|---|---|---|
| Significantly different | Not significantly different | Susceptible | 23 |
| Not significantly different | Significantly different | Resistant | 55 |
| Not significantly different | Not significantly different | Inconclusive | 29 |
| Significantly different | Significantly different | Partial resistant | 0 |

**Table 20. Heritability values for the different resistance parameters**

| **Parameter** | **Broad sense heritability (%)** |
|---|---|
| Peripheral damage | 22 |
| Total cross section damage | 51 |
| Total cross sectional outer damage | 49 |
| Total cross sectional inner damage | 49 |

**Objective two: To identify and map QTLs associated with banana resistance traits to weevil**

DNA was extracted according to CTAB (Cetyltrimethylammoniumbromide) procedure (Weising *et al*., 1995),which was modified for *Musa* by Samarasinghe *et al*. (2001). It was later sent for genotyping.

**Pending work**

Mapping of the QTLs which is to be done after receiving the genotyping data.

# WP3 - BSc Research Progress Report (2017-2018)

**Research title:** Characterization of banana at IITA

**Name of Student**: **GERARD NDAYISHIMIYE**
**University:** EARTH University, Costa Rica

**Timeline of study:** 1st August 2017 to 31st July 2019

**Supervisors**: Dr Alan Brown (IITA), Luis Pocosangre (EARTH University)

## Introducción

La pasantía es una oportunidad para el desarrollo personal en área profesional ya que nos abre los ojos y mente para la realidad de los entornos fuera de aula y la vida estudiantil. Estoy realizando mi pasantía en una institución internacional de agricultura tropical (IITA) en su proyecto de banana en Arusha Tanzania. IITA tiene diferentes proyectos que se realiza para apoyar las comunidades. Se trabaja con siete cultivos; banana y plátano, yuca, caupi (Cowpea), maíz, café, soya y ñame. Toda la investigación que se hace es para desarrollo y alimentación de las comunidades. Mi interés de elegir esta institución fue el placer que yo he tenido de trabajar con banana y plátano ya que en futuro me gustaría tener una finca integrada en la cual plátano y banano son mis cultivos principales ya que son los cultivos más consumidos en Ruanda. Este será una de las soluciones de mitigar la pobreza y el hambre en mi comunidad.

IITA en su proyecto de investigación de banana y plátano es mi lugar de aprendizaje por sus actividades de investigación para encontrar variedades tolerantes a la sequía y algunas enfermedades. Por lo tanto este coincide con el clima de mi país, en lo cual época de lluvia es muy corta.

## Objetivos profesionales

- Identificar y caracterizar fenotípicamente algunas especies de banana in IITA
- Conocer y describir las especies de banana con que IITA está trabajando
- Elaborar un manual con fotos de las diferentes variedades de bananas y plátano de IITA
- Investigar un poco sobre fusarium raza 1

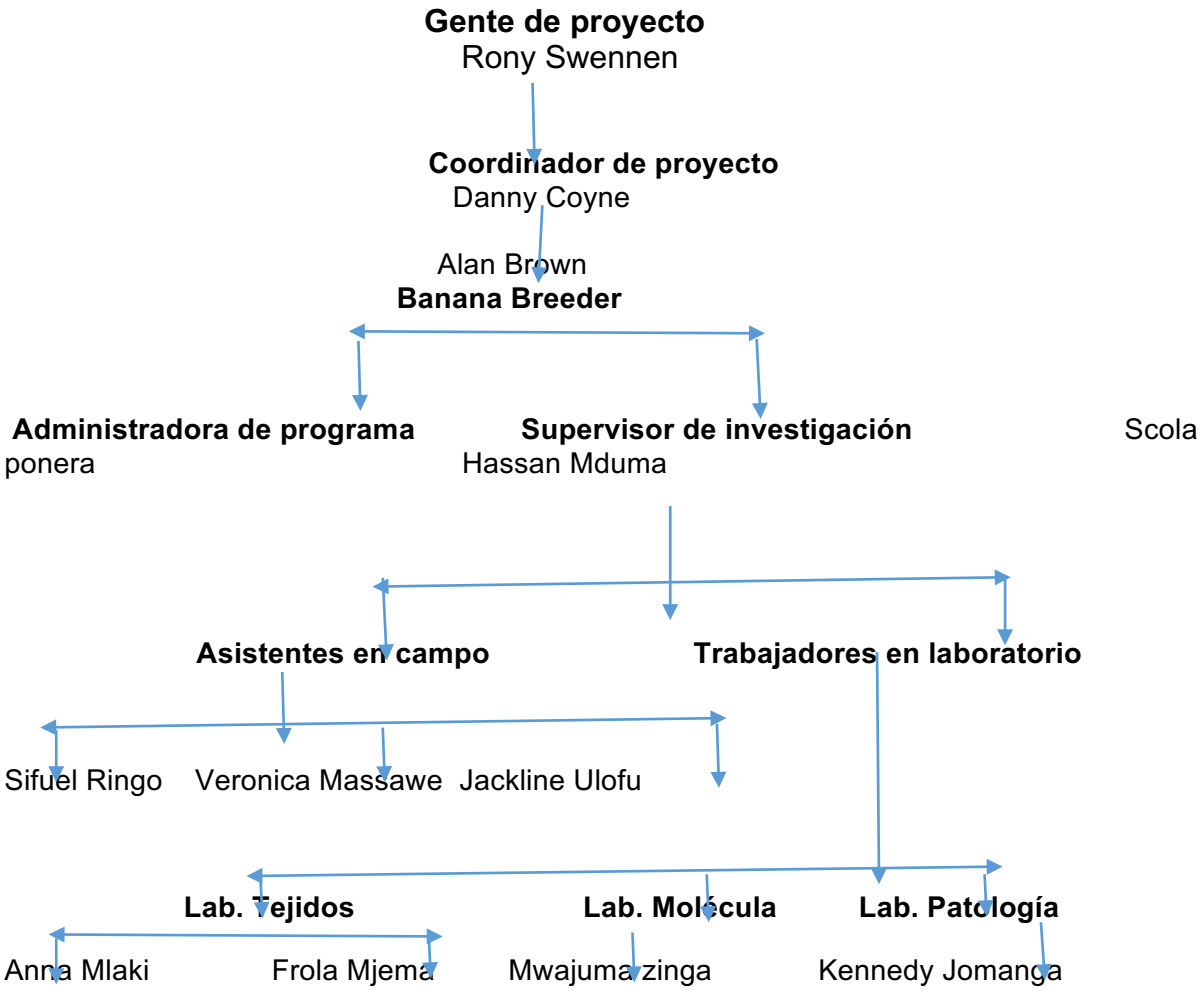
## Objetivos personales

- Desarrollar zona de confianza a través interactuar con la gente con experiencia en su especialización.
- Seguir aprendiendo como adaptar a un nuevo entorno
- Aprender un poco de swahili y algunas culturas para facilitar la comunicación en las regiones locales en Tanzania

**Entorno de la empresa anfitriona**

Nombre y puesto del supervisor: Alan Brown, banana breeder

**Modelo organizacional**




## Principales actividades de la institución

IITA- Arusha está trabajando en el proyecto de investigación de banana y plátano. Las principales actividades de ellos, son la toma de datos en el campo de investigación, eso se hace diariamente, además se requiere conocer las diferentes variedades de plátano y banano lo cual hace mi carga de tomar fotos de diferentes partes de cada variedad en el campo y hacer la colección de las fotos de diferentes variedades en una hoja de Excel, con el documento estandarizado, de esta institución, hago toma de datos en el campo y después correlacionar los con las fotos tomados con el fin de publicarlo para compartir y comparar las mismas variedades de otros proyecto que ellos tienen en otro sitio en Uganda.

También trabaja en mejoramiento de banana y plátano, por lo tanto tiene actividades de laboratorios. Cuenta con tres laboratorios, laboratorio de tejidos, laboratorio de moléculas y

laboratorio de fitopatología. Con la gente encargada de trabajar en laboratorio, estoy realizando mi investigación de fusarium raza 1 para proteger un campo nuevo de investigación que apena está empezando para las especies de banano y plátano tolerantes de sequía.

## Peso de la empresa dentro de su sector

IITA es una institución con gran nombre no solo en Tanzania pero también todo el continente africano por sus actividades y aporte en desarrollo de en áreas de agricultura para mitigar el hambre. Se ha venido trabajando con siete cultivos más importantes ante mencionado por sus alto niveles de demanda para alimentación. Todo estos cultivos, ellos trabaja para aumentar la producción es por eso que mejoramiento genética en su parte de enfoque. Trabaja en cuatro zonas de África oeste, este, sur y África de centro y tiene su sede principal en Nigeria

## Impacto social y ambiental

Esta institución tiene muchas actividades en las comunidades donde se muestra su impacto socio-ambiental, han desarrollado las actividades como el mejoramiento genética de los siete cultivos ante mencionado para mitigar la pobreza y el hambre y malnutrición.
Después de encontrar las nuevas variedades, no se venden pero se donan a las comunidades. Además IITA trabaja con la gente directamente y enseñar les las prácticas para conservación de los recursos reduciendo los riesgos de consumidor y promocionando la calidad y productividad en agricultura.

IITA es una organización sin fines de lucros, tiene colaboración con otras agentes como Africa RISING, Bioversity, CGIAR, todas estas instituciones se dedican en las investigaciones, capacitaciones y mucho más en los países en vía de desarrollo para asegurar la seguridad alimentaria y erradicar la pobreza extrema.

## Informe de la parte técnica de la pasantía

## Actualización de objetivos y actividades y proyectos específicos asignados.

Las actividades planificadas y objetivos en el plan de trabajo, se han mantenido aunque como resultados de la investigación y las recomendaciones de mi investigación de fusarium raza 1, me asignaron a coordinar un proyecto nuevo para implementar medidas y controles fitosanitarios. Se decidió la construcción de la desinfección de pies y lavamanos en las entradas de una nueva plantación de plátano y banana ya que están en el proceso de poner todos los campos de investigación en un solo lugar y hacer cercas para asegurar las medidas fitosanitarias y minimizar los movimientos de las personas y otros portadores de plagas en enfermedades. Este se planificó estar terminado el día 7 de diciembre.

## Análisis y discusión de los resultados/logros.

Se ha podido identificar y caracterizar fenotípicamente cincuenta plantas hasta al momento, las cuales estaban en las condiciones, edad y con los requisitos para ser identificadas. Además se pudo tomar los datos y fotografías para hacer un manual de identificación de las diferentes especies de musa de IITA.

Tanto como la parte de investigación de fusarium, se ha podido identificar los síntomas en el campo, medios de dispersión. Por fin se dio recomendaciones donde nació el proyecto de implementación de las medidas fitosanitario de fusarium y otras plagas y enfermedades, las cuales se dispersen a través del suelo y contacto con personas y otros animales.

## Informe de la parte personal

## Análisis y discusión de crecimiento personal y situaciones conflictivas.

Tal como estaba planificado en el plan de trabajo, se nota el crecimiento personal en la parte de toma de decisión y resolver los problemas.
En el plan de trabajo se planificó que se empezaba el trabajo con toma de fotos de pronto, hubo un problemas que según los criterios de toma de datos y fotos, hay que considerar una planta con fruta bien madura para que tenga todos los requisitos, sin embargo, al inicio no habían plantas suficientes para toma de datos y fotos, pero esto , se solucionó con una propuesta de tomar fotos de algunas partes de una planta y luego cuando se tenga una planta con todos los requisitos de la misma variedad, volver en la plantación y tomar los datos y fotos los cuales hacían faltan.

Tanto la parte personal se cambió la mirada de mi futuro ya que hubo unos ajustes en los planes de maestría. Esta pasantía me ha abierto los ojos. Las zonas rurales en los países con clima seco, se necesitan plantas resistentes a la sequía resistentes a las plagas y enfermedades además unos sistemas de riego más baratos. Aun no se necesitan la tecnología más amplia en sus prácticas de agricultura como lo imaginaba antes.

## Entorno empresarial o laboral

## Prácticas para proteger ambiente y la responsabilidad social.

IITA junto con Bioversity trabajan con agricultores ofreciendo las nuevas variedades y talleres para facilitar entendimiento y el proceso de cultivar esas nuevas variedades para aumentar la productividad y por fin mitigando el hambre y la pobreza.

Además IITA junto con Africa RISING, enseñan los agricultores las prácticas sostenibles de agricultura para ambos la protección de medio ambiente y la productividad.

IITA provee oportunidades de sus empleos para seguir con educación, los trabadores con licenciatura tienen oportunidad de seguir la maestría hasta tener PHD, este aplica a todos según su nivel de educación, se trata de proveer oportunidades a través de financiamiento de los estudios de empleados, lo cual significa mucho para el desarrollo de cada uno de los empleados.

## Prácticas económicas desde la perspectiva de la sostenibilidad.

Es un poco difícil determinar la sostenibilidad económica de IITA ya que utiliza las donaciones para realizar sus investigaciones aunque hace poco empezado un proceso de desarrollar algunos proyectos para tener algunas fuentes de fondos propios ya que desde al inicio se han venido utilizando dinero proviene de diferentes donantes. Sin embargo, se puede comentar que, si, es económicamente sostenible ya que la donación recibida, se utiliza bien tal como se ven en los impactos y resultados donde se invierten estas donaciones.

## 7.4 WP4 - PhD Research Proposal Report (2016 – 2018)

**Title:** Which variety for which farm? Creating locationspecific information for banana variety release decisions and banana variety choice recommendations in Uganda and Tanzania

**Name of Student:** **DAVID BROWN**
**Supervisor:** Inge van den Bergh, Jacob van Etten
**Timeline of study:** 2018-2022
**University:** Wageningen University, Netherlands

# PE&RC PhD PROJECT PROPOSAL

*Please read the appendix with instructions first*

| **1. GENERAL PROJECT INFORMATION** | |
|---|---|
| Main PE&RC affiliated Institute / University | Wageningen University |
| Main PE&RC research group | Laboratory of Geo-information Science and Remote Sensing (GRS) |
| Other PE&RC groups involved | |
| Project Title (English) | Which variety for which farm? Creating location-specific information for banana variety release decisions and banana variety choice recommendations in Uganda and Tanzania. |
| Project duration | FROM 15/03/2018 TO 15/03/2022 |
| Where will the research be conducted (country) | Costa Rica, Uganda and Tanzania. |
| At which University will the thesis be defended? | Wageningen University |
| Funding source(s) for this project (1, 2, or 3?) | 1 ~~(internal)~~ / 2 ~~(NWO)~~ / 3 (external) (strikethrough) Name of funding source: Bioversity International, through an IITA-coordinated grant from the Bill & Melinda Gates Foundation (BMGF) and financial support from the CGIAR Research Program on Roots, Tubers and Bananas (CRP RTB). |

| **2. THE PhD CANDIDATE** | |
|---|---|
| Full name of the PhD candidate | David Brown Fuentes |
| Gender | MALE |
| Nationality | Costa Rica |
| Date of birth | 21-02-1982 |
| Period of appointment | FROM 15/03/2018 TO 15/03/2022 |
| Hours per week | 36 |

**3. SUPERVISION**

| **Project role** | **Name + title** | **Specialisation** | **Organisation** | **Hours/week** |
|---|---|---|---|---|
| Promotor | 1.Arnold Bregt | Geographical information systems; Spatial data infrastructures; Spatial models. | Wageningen University | |
| Daily supervisor | 1.Sytze de Bruin | Geographical information science; spatial analysis; geostatistics; fitness-for-purpose. | Wageningen University | |
| | 2.Inge Van den Bergh | Banana (Musa spp); germplasm screening; varietal selection for end-user-preferred traits; | Bioversity International | |

| | | | | |
|---|---|---|---|---|
| | | information and knowledge sharing. | | |
| Advisor | 1.Jacob van Etten | Participatory methods; crowdsourced citizen science; crop trials; geospatial and environmental analysis. | Bioversity International | |
| Technician | N.A. | | | |
| Other | N.A. | | | |

| **4. COLLABORATION** | | |
|---|---|---|
| **Type of organisation** | **Name of organisation** | **Name + title of collaborator(s)** |
| University | N.A. | |
| Research Institute | 1. Bioversity International<br>2. Bioversity International<br>3. International Institute of Tropical Agriculture (IITA) | Inge Van den Bergh<br>Jacob van Etten |
| Government agency | 1. National Agricultural Research Organization (NARO) Uganda<br>2. Agricultural Research Institute, Department of Research and Development, Ministry of Agriculture & Food, Tanzania | |
| Others (e.g., FAO,WHO) | N.A. | |

| **5. ETHICS** | |
|---|---|
| Will vertebrates be used in animal experiments? | ~~YES~~ / NO |
| Are there other ethical issues to be considered with respect to this project? | ~~YES~~ / NO |
| If YES, please elaborate: | |

2015.01 **PAGE * ALPHABETIC E**

# PE&RC PhD PROJECT PROPOSAL

*In case a peer-reviewed full project proposal is available (e.g., NWO or EU), please send that proposal along together with the reviewers comments and the acceptance letter.*


**6. SUMMARY** (max. 250 words)

Variety choice is very important to farmers. The best variety to be grown on a farm depends on the local environmental conditions, production constraints as well as socioeconomic context. Both in Uganda and Tanzania, banana is crucial as a staple food and source of income. In both countries, varieties are tested before being officially released to farmers, but the current approach lacks detailed location-specific information of variety performance. This type of information is very important for decision makers involved in the variety release processes in Uganda and Tanzania, but specially for farmers. The current project aims to develop methods for providing location-specific information for key decision making in variety release and variety choice recommendations in Uganda and Tanzania. To achieve this goal, the project is structured in four stages. The first stage focuses on the development of a method for location-specific banana variety rankings using legacy data from on-station trials, adding environmental data to existing methods based on Plackett-Luce models. The second stage will enhance the method using new data from on-farm trials and incorporating socio-economic data to provide targeted variety choice recommendations. The third stage aims to identify the current information flows and actors involved in the variety release processes, using social network analysis, to understand how to incorporate the information generated at stage 2. Finally, the fourth stage will elaborate prototypes of information products and tools specifically targeting the actors identified at stage 3. Focus will be on fitness-for-purpose assessment of visualization methods based on users' feedback.


**7. DETAILED DESCRIPTION OF THE RESEARCH PLAN** (max. 2500 words + 1 page literature list)

**Introduction**

Wrong variety choices can lead to unwanted results for farmers, such as low yields and lack of consumer acceptance. In Uganda and Tanzania, where bananas are very important not only as an economic income source but also as staple food (Bagamba et al. 2010; Tushemereirwe et al. 2015), banana varieties are released after passing through an official process of evaluation. This procedure aims to ensure agronomic value along with distinctiveness, uniformity and stability (Ssebuliba 2010; USAID and Fintrac Inc. 2013). Varieties are also evaluated to guarantee their suitability to local target environments but the current approach involving on-station trials lacks detailed location-specific information of variety performance. On-farm trials are providing a more accurate representation of target farm environments, but are still not covering the broad range of target environments by using traditional Participatory Varietal Selection (PVS) methods, such as "mother-baby" trials approach. Nevertheless, such location-specific variety performance information is important for decision making concerning varieties to be released and variety recommendations for farmers (Hyman et al. 2013). Farmer-participatory methods using crowdsourcing (Van Etten et al. 2016) produce considerable larger amounts of localized data, represent a broader range of farmers' and local circumstances and provide many pseudo-replicates for each variety tested. There are still challenges to transform data obtained by such methods into information that supports location-specific variety recommendations. Meta-analyses as proposed by Simko and Pechenick (2010) allow to combine data from heterogeneous trials datasets, but they do not account for location-specific information such as temperature, rainfall and soil properties, affecting variety performance. Along with the generation of location-specific information, an additional challenge is to identify the most effective way to present and incorporate information into variety release processes. The incorporation of new information products highly depends on the organizational culture (de Vos 2007) and the perceptions of individuals within organizations (Omran and van Etten 2007). It is very important to understand the current environment, how the information flows and where, within the variety release processes, the new information products should be incorporated. The Variety release procedures in Uganda and Tanzania involve many organizations and stakeholders. To date, it is largely unknown how these actors interact with each other and how they access information. Social network analysis (Haythornthwaite 1996) may facilitate the development of new information products and tools, considering the needs of users. Furthermore, to effectively deliver the information, it should be done through channels and formats tailored for end users. Information visualization can facilitate data understanding and help users to gain insights (Chen 2010) . Within the context of agricultural research for development, development of a new product requires special attention to user feedback (Sumberg et al. 2013). The development of information products as proposed in the current PhD project will require the identification of end users and to get feedback from them on how information is used, and which representations are deemed useful.

Therefore, the overall **goal** of my research is:

> *To develop methods producing location-specific information on crop varieties performance, that help decision makers involved in the crop variety recommendations and variety release processes.*

The research will specifically address East African Highland Bananas in Uganda and Tanzania, but developed methods are aimed to be of broader applicability.

To further focus my research, I aim to answer the following **research questions**:

1. What are the geospatial factors that influence on-station variety performance and how can these be accounted for in meta-analyses of existing crop trial data?

2. What are the environmental and socio-economic factors that influence on-farm variety performance and how can these be considered in the analysis of large on-farm trials?

3. What are current actors and information flows between them in decision-making procedures used in variety release procedures and related extension programs and what are perceived information problems?

4. Which information products derived from variety trial data can improve key decision making in variety release and generation of location-specific variety recommendations?

**Research methodology**

The proposed approach is organized in 4 stages, each addressing a specific research question. Figure 1 shows how stages relate to each other. At stage 1, the meta-analysis methodology of Simko and Pechenick (2010) will be extended to incorporate geospatial indicators related to environmental conditions affecting crop performance in on-station trials. The aim of this first stage is two-fold. First, to extract information from legacy heterogeneous trials databases, considered incompatibles. Second, to incorporate partitioning based on environmental characteristics in the variety ranking process. At stage 2, on-farm trials and socioeconomic data will be incorporated into an enhanced version of the location-specific model. At stage 3, actors involved into the variety release processes and their perceptions about current and proposed information products will be identified. Stage 4 will build on insights developed in all previous stages to design prototype information products for decision making concerning variety recommendation and variety release processes.

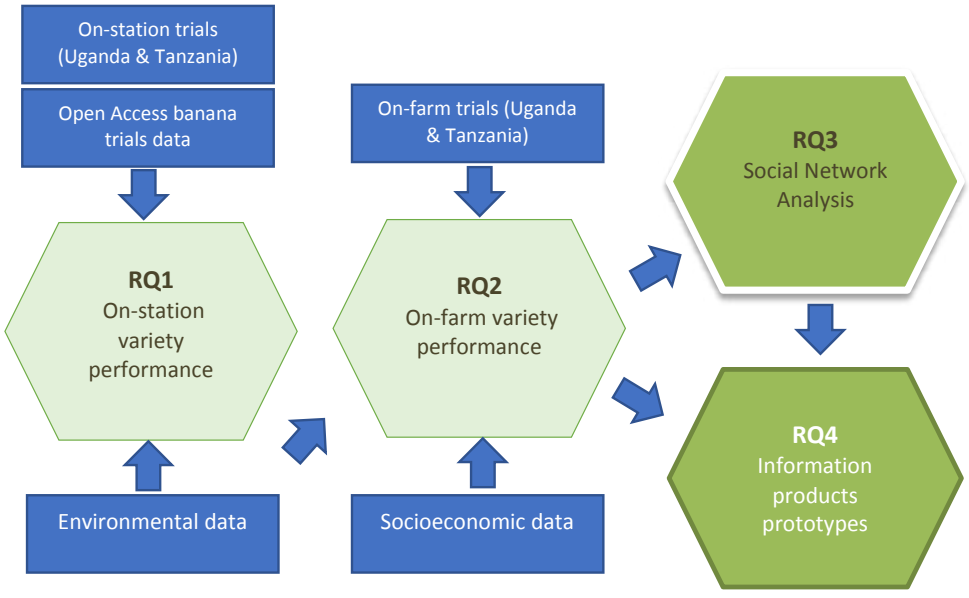



Figure 1. Conceptual model of research methodology

**Methods to address RQ1**

The challenge is that trials datasets are heterogeneous in format while the varieties tested in different trials overlap only partially. Fortunately, rank aggregating methods (Simko and Pechenick 2010) can deal with incomplete rankings. However, different environmental conditions affecting crop performance in the considered agricultural trials are not yet accounted for. Here, I propose to use model-based recursive partitioning (Strobl et al. 2011) on environmental covariates in combination with the Plackett-Luce model (Plackett 1975; Luce 1959) as recently implemented in the R package PlackettLuce (Turner et al. 2018). This package has been already proved to be useful for crop varieties ranking in a relatively small experiment (van Tilborg 2018; Turner et al. 2018).

The following steps will be made:

1. Collect data from banana varieties trials databases: Data will be gathered from trials repositories such as the AgTrials database (CCAFS 2013) and MusaBase (BTI et al. 2018; Fernandez-Pozo et al. 2015). If available, on-station trials from the "Breeding Better Bananas" project will be also included. This step involves substantial data cleaning and data quality assessment, especially in terms of incomplete location data. In that case, data will be completed using geocoding.
2. Convert performance data to rankings, based on one or more traits of interest.: Variety rankings will be based on individual traits or a combination of different traits. For example, varieties A, B and C could be ranked as B > C > A based on their resistance to a given disease. However, a combination of high disease resistance and high yield would be a desired combination and therefore the ranking would be the result of evaluate the most resistant variety with the higher average yield. As it is likely that a combination of traits might end up being contradictory, a multi-objective optimization approach (Hermoso et al. 2015; Chiandussi et al. 2012) will be developed.
3. Collect environmental data from open-access databases for each trial site: To produce location-specific rankings of varieties, environmental data will be added as covariates. The selection of the environmental covariates will be based on existing evidence of their influence on banana growth, like the effect of temperature (Turner and Lahav 1983) and altitude (Sivirihauma et al. 2016). The environmental data will be gathered from open databases such as Climate Hazards Group InfraRed Precipitation with Station data (CHIRPS) (Funk et al. 2015), Hole-filled SRTM for the globe Version 4 (Jarvis et al. 2008) and SoilGrids (Hengl et al. 2017).
4. Fit the Plackett-Luce tree model using the environmental data as explanatory variables.
5. Model assessment using cross-validation (Arlot and Celisse 2010).

**Methods to address RQ2**

At this stage, the method developed for answering RQ1 will be extended to use on-farm trials data that will be collected from on-farm trials established by two different methods: "mother-baby trials" design (Weltzien and Christinck 2017) and crowdsourcing participatory variety selection (Van Etten et al. 2016). The former is the method currently used by national programs in Uganda and Tanzania, and the latter will be introduced within the variety evaluation process in both countries by the "Breeding Better Bananas" project as an alternative approach.

In addition to the environmental covariates included in the first stage, socioeconomic data collected through participatory rural appraisal tools (PRA)will be included as explanatory variables in Plackett-Luce tree models. Furthermore, end user preferences data will be collected and included as response variable in the ranking models. As in the previous stage, this could bring up the challenge of ranking construction based on multiple traits. Again, a multi-objective optimization approach will be considered.

The proposed methodology to answer RQ2 includes the following steps:

1) On-station and on-farm trials data gathering and cleaning; data are provided by the "Breeding Better Bananas" project. This data includes agronomic performance, sensory evaluations and preference rankings by farmers.
2) Construction of rankings based on selected traits
3) Collect environmental data for on-farm trials locations
4) Socioeconomic data cleaning and transformation to be included into the model as covariates + responses from end-user evaluations.
5) Fit Plackett-Luce tree models using environmental and socioeconomic data
6. Model assessment using cross-validation (Arlot and Celisse 2010).

**Methods to address RQ3**

To answer RQ3, I propose to develop an inventory of actors and their perceptions within the banana varieties releases processes, to understand how they work and to identify potential constraining factors to incorporate new information products, like the proposed within this project. This will be done using Social Network Analysis (Haythornthwaite 1996), focusing on the identification of actors participating in the variety release process and how the information flow between them. Social network analysis and their associated measurement techniques are grounded on graph theory (Haythornthwaite 1996). Therefore, a social network analysis typically involves the construction of a graph, where the actors are represented by nodes and their relationship by edges. Along with the graph, a matrix containing the information about the network is used as input to calculate a set of measurements to assess the network in terms of cohesion, structural equivalence, prominence, range and brokerage (Haythornthwaite 1996).

The approach proposed here will be based on previous experiences using social network analysis on spatial data infrastructures, such as the developed by Omran and van Etten (2007) and Georis-Creuseveau et al. (2017).

The proposed methodology includes the following steps:

1) Design of questionnaire for identified actors, asking about their perceptions of information products and information flows.
2) Collect data through survey instruments designed in previous step.
3) Construction of the social network graph, with identified actors as nodes and their relationship as edges.
4) Calculate network measures (e.g., centrality, degree and betweenness.)
5) Data analysis and results interpretation, particularly to identify information usage and potential missing links.

**Methods to address RQ4**

The challenge is to identify effective ways to effectively present the information that will be generated with the methods to address RQ1 and RQ2, to actors identified with methods to address RQ3, considering their perceptions and information requirements. Information visualization aims to produce insights from complex datasets, combining aesthetics and functionality (Chen 2010). This aim slightly differ from the one of Statistical Graphics, more focused on statistical properties (Kosara 2013). Figure 2 shows a tree produced by the PlackettLuce R package (Turner et al. 2018), which can be effective for expert users with some knowledge of the statistic model behind it, but perhaps would not be effective communicating the information to other audiences.

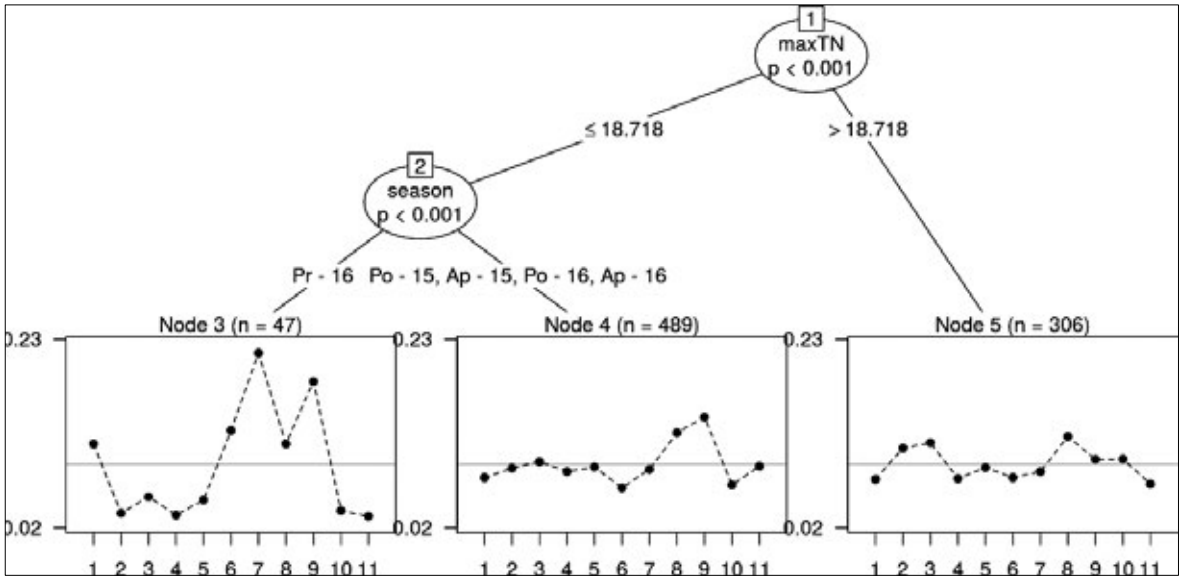



Figure 2 – PlackettLuce Tree (Turner et al. 2018) showing two splits based on maximum temperature at night (maxTN) and a subsequent based on planting season (Pr=primera, Po= Postrera and AP=Apante). The end nodes shows the worth (log) of each variety on the "y" axis and variety number on the "x" axis.
Retrieved from https://cran.r-project.org/web/packages/PlackettLuce/vignettes/Overview.html

The proposed methodology involves the following steps:

1) User requirements will be collected by interviews with stakeholders, identified in the previous stage.
2) Design of interactive information product prototypes, considering information visualization aspects like aesthetics and functionality (Chen 2010).
3) Presenting the prototypes to users to request feedback from them. After each feedback round, the information product prototype will be updated accordingly to the user requirements.
4) Final assessment of the product prototypes by stakeholders.

Interaction with stakeholders will include face to face meetings during planned visits to Uganda and Tanzania, along with e-mail and Skype communications.

The idea is to deliver a prototype information product that can next be incorporated into a platform such as ClimMob [1].

**The innovative aspects of this research are:**

- Application of the novel Plackett-Luce modelling with recursive partitioning method on a new data set involving multiple traits and multi-faceted environmental characterization
- Spatially explicit ranking of banana varieties using both environmental data and socio-economic data
- Providing tailored information visualizations supporting crop variety release processes

[1] ClimMob – a software for crowdsourcing climate smart-agriculture https://www.bioversityinternational.org/news/detail/climmob-a-software-for-crowdsourcing-climate-smart-agriculture/

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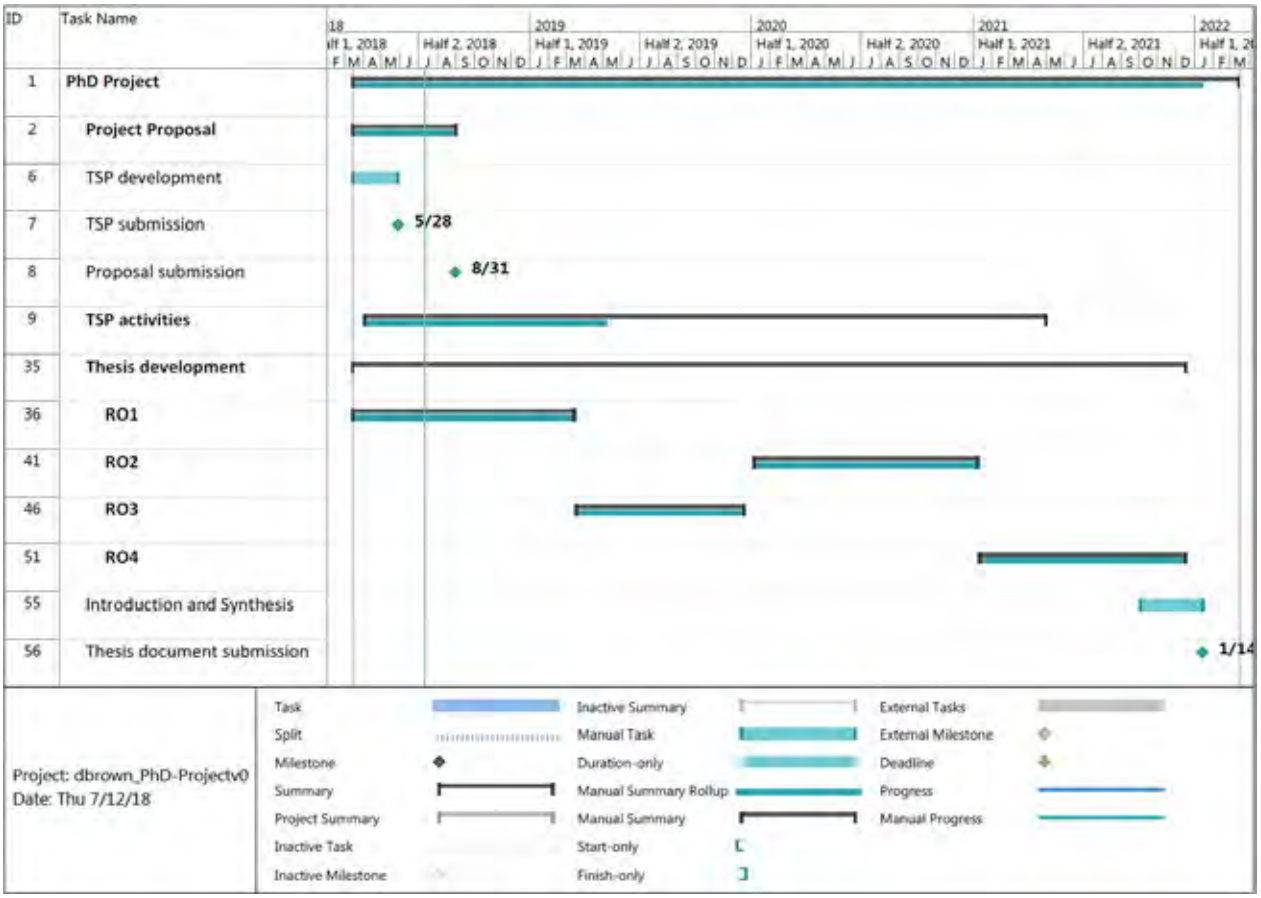
## 8. TIME TABLE OF THE PROJECT AND WORK PROGRAMME




**Supervision**

**Role of each member of the supervision team**

- Dr. Arnold Bregt (promotor) will provide general supervision of the project.
- Dr. Sytze de Bruin (co-promotor and daily supervisor) will provide methodological and technical guidance, especially on spatial analysis.
- Dr. Inge Van den Bergh (daily supervisor) is daily supervisor at Bioversity International and will provide guidance on cultivar evaluation methodologies, linkages with partners on the "Breeding Better Bananas" project and progress evaluation within the Bioversity International framework.
- Dr. Jacob van Etten (advisor) will provide technical and methodological advice, especially on evaluation of crowdsourced data and rank-aggregation methods.

**Progress evaluation**

Regular Skype meetings every two weeks with daily supervisors (both in WUR and Bioversity International) will assure effective progress evaluation. Milestones will also serve as check points for progress evaluation and short progress reports will be delivered to the supervision team.

**Management of changes in the project team and guarantee of supervision**

Supervision is guaranteed both in the GRS group and Bioversity International. While not major changes are expected during the PhD project, in the case of the GRS group, another professor from the same group would take over if necessary. At Bioversity International the current supervisor will hand over supervision to another senior scientist.

**Execution**

**Availability of technical equipment and facilities**

Technical equipment and facilities will be guaranteed by Bioversity International, along with the daily working facilities. During travels to Uganda and Tanzania, appropriate conditions will be also guaranteed by Bioversity International and partners institutions within the "Breeding Better Bananas Project".

**Availability of assistance by technical personnel**

Technical staff working on the "Breeding Better Bananas" project will provide field and logistic support, especially on collecting data and communicating with local stakeholders.

**Risks management (e.g. weather, availability and willingness of third parties, etc)**

The main identified risk is the weather as it highly influences the establishment of on-farm trials. In the case that on-farm trials data were not available to meet the current project schedule, available data from other on-farm trials will be considered to develop the methodology, even if they evaluated other crops.

**Agreements with collaborating organisations**

The current PhD project will be developed within project "Improvement of banana for smallholder farmers in the Great Lakes Region of Africa", also known as the "Breeding Better Bananas" project. The project is led by the International Institute of Tropical Agriculture (IITA) and has been structured in six working packages: 1) Banana Breeding Pipeline; 2) Pest and Disease Control; 3) Leveraging Genetics; **4) Empowering End-User Evaluation**; 5) Harnessing Data; and 6) Governance, Research Oversight and Management. The working package number 4, led by Bioversity International, is where this PhD's activities will be executed.

## 9. SOCIETAL RELEVANCE

Increasing food demands require crop varieties that are well-adapted to local environmental circumstances. Furthermore, adaptation of agriculture to future climates needs quick varietal replacement informed by climatic information (Atlin et al. 2017). Genetic improvement is a key element not only to assure food availability for local consumption but also to sustainable increase of production to generate income through commercialization (Campos and Caligari 2017). Bananas are particularly relevant for Uganda and Tanzania, where the current project aims to contribute to increase banana production and make it more sustainable through the generation of information that enables decision makers to provide better variety recommendations to farmers.

## 10.DATA MANAGEMENT (max. 250 words)

**Data storage**

- Short term: data will be stored on working computer and backed up daily on local external hard drive. Weekly backups of key files will be made on WUR managed storage (M: drive or OneDrive)
- Long term storage: Will be arranged with chair group GRS in agreement with Bioversity International and in consultation with the Wageningen Data Competence Centre (WDCC)
- Data archiving, see: https://www.wur.nl/en/Value-Creation-Cooperation/WDCC/Data-Management-WDCC/Data-policy/Archiving.htm
  https://www.wur.nl/en/Value-Creation-Cooperation/WDCC/Data-Management-WDCC.htm

To make the methodology reproducible, all the models and algorithms will be implemented using the R language and environment for statistical computing (R Core Team, 2018) and the data will be stored in open standards formats, like Comma Separated Values (CSV).

**Data ownership**

Data generated by or within the framework of the Breeding Better Bananas project should be handled adhering to the project guidelines, which will be included into the data management plan.

**Data sharing**

Unpublished data could be accessed only by project members. Access can be granted to others upon request.

*More detailed information can be found in the Data Management Plan document, which will be developed following the data management guidelines of WDCC.*

2015.01 **PAGE * ALPHABETIC E**

| **11.POTENTIAL REVIEWERS** | | | | |
|---|---|---|---|---|
| **#** | **Name + title** | **Organisation** | **Specialisation** | **Email address** |
| **1.** | Gert Kema | WUR | Biointeractions and plant health, Banana | gert.kema@wur.nl |
| **2.** | Robert Hijmans | UC Davis | GIS and crop science | rhijmans@ucdavis.edu |
| **3.** | Ivan Simko | USDA | Statistics and breeding | ivan.simko@ars.usda.gov |
| **4.** | Fred van Eeuwijk | WUR | Biometrics, statistics and quantitative genetics | fred.vaneeuwijk@wur.nl |
| **5.** | Victoria Fast | University of Calgary | Citizen science, and participatory GIS methods | victoria.fast@ucalgary.ca |
| **6.** | Matthew Reynolds | CIMMYT | Plant physiology | m.reynolds@cgiar.org |
| **7.** | Hans-Peter Piepho | University of Hohenheim | Statistics and breeding | piepho@uni-hohenheim.de |
| **8.** | David Turner | University of Western Australia | Banana physiology | david.turner@uwa.edu.au |

| **12.SIGNATURES** (this form needs to be signed by the PhD candidate as well as all supervisors) | | |
|---|---|---|
| **PhD candidate** | **Promotor / Principal Supervisor** | **Supervisor 2** |
| Name: David Brown Fuentes | Name: prof.dr.ir. AK (Arnold) Bregt | Name: dr.ir. S (Sytze) de Bruin |
| Date: | Date: 2-9-2018 | Date:3-9-2018 |
| |  |  |
| **Supervisor 3** | **Supervisor 4** | **Supervisor 5** |
| Name: Inge Van den Bergh | Name: Jacob van Etten | Name: |
| Date: | Date: | Date: |
| | | |

## APPENDIX: EXPLANATION TO THE INDIVIDUAL QUESTIONS

Q 4: Please mention the collaborating organisations in the context of this project. Only mention those collaborations which will result in joint activities such as joint publications.

Q 5: In some projects animals (vertebrates) may be involved or biotechnological research may be involved. In that case ethical guidelines of WU might be applicable.

Q 6: The short summary should be written as an explanation of the title of the research project.

Q 7: Elaborate your project proposal here. This should contain the following elements:

- Introduction, including history and background
- Objectives
- Hypotheses
- Research methodology
- Innovative aspects

Q 8: The PhD candidate should be able to finish the thesis work within 4 years. This means that the reading version of the PhD thesis has to be submitted within 4 years. Within the work programme the following issues should be dealt with:

- In what way is appropriate supervision guaranteed?
  - What is the role of each member of the supervision team?
  - In what way is progress evaluated?
  - If during the project period changes will occur in the project team, in what way will supervision be continued?
- In what way is execution arranged? Please specify:
  - Availability of technical equipment and facilities
  - Availability of assistance by technical personnel
  - Risks (e.g. weather, availability and willingness of third parties, ….).
- Agreements made with collaborating organisations (question 4) and/or other PE&RC groups, as far as important for the execution of the project.

Q 9: What is the societal significance of the proposed research?

Q 10: This section outlines the data management plan and must encompass:

- Data storage (short term and long term storage),
- Data ownership (issues with respect to ownership of data produced in this project or external data used for this project)
- Data sharing (agreement on who will have access to and use your (un)published data)

This section may include references to a more comprehensive (i.e. 2 to 3 pages) data management plan in which elements are outlined in more detail and can also refer to a plan at the level of a research group. Note that the full data management plan does not need to be included in this proposal, and that data collection is also part of a data management plan but is specified in section 7 and 8 of this project proposal.
For more details, see http://www.wageningenur.nl/en/Expertise-Services/Data-Management-Support-Hub/Browse-by-Subject/Data-Management-Planning-1.htm.

Q 11: **The proposal will be sent to 3 reviewers. Please provide the names and contact details of 4-5 potential reviewers who are in no way involved in this project. A balanced representation of men and women from inside and outside the main PE&RC affiliated institute is preferred. It is allowed, and even encouraged to verify the proposed reviewer's willingness to provide an independent review of the proposal. This speeds up the review process.**

**Please submit the signed PDF of the PE&RC PhD Project Proposal by email to the secretariat of the graduate school PE&RC (office.pe@wur.nl) no later than 6 months after the start of the PhD project.**

## 7.5 PhD Research Progress Report (2016 – 2018) no assigned WP

| | |
|---|---|
| **Title:** | Modeling of the Banana cropping structure using the *AquaCrop* growth model |
| **Name of Student:** | **STEVENS BERT** |
| **Supervisor:** | Rony Swennen, Jan Diels, Eline Vanuytrecht, Patrick Ndakidemi, Allen Brown |
| **Timeline of study:** | 2016-2020 |
| **University:** | Katholic University Leuven, Belgium & the Nelson Mandela African Institution of Science and Technology |

### Research Objectives

The project is composed of different work packages as shown in Figure 11

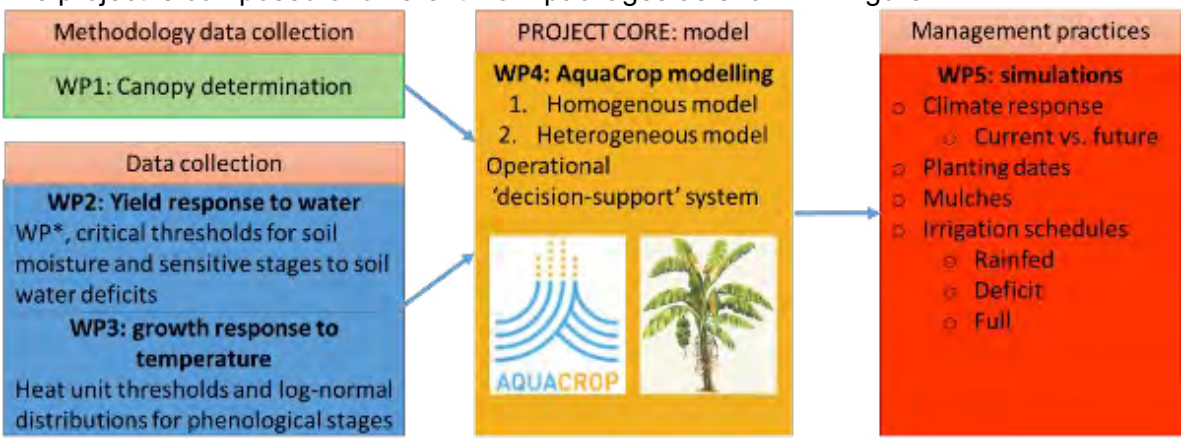



**Figure 11: work packages (Ws in PhD.**

Hypotheses and goal

The project 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.

Following research questions will be addressed in several work packages (WP):

- **Objective 1:** devise a methodology to measure canopy cover (CC) for homogenous banana groups in a field (WP1).
- **Objective 2:** determine the most sensitive stages of different banana varieties (AA and AAA) to drought stress by applying drought stress at different development stages (vegetative and flowering) (WP2). Additionally, use data from WP2 as a database for *AquaCrop* calibration.
- **Objective 3:** determine the timing and development of banana phenology (growth stages) in response to temperature (accumulated heat units) and soil moisture (WP3).
- **Objective 4:** develop an *AquaCrop* model for the banana plant. By using data from WP2 and WP3 the heterogeneous banana population structure will be incorporated in *AquaCrop* through 'cohort population dynamics' developed by Tixier et al.[17] (WP4).

- **Objective 5:** test and use the *AquaCrop* growth model to assess the effect of management practices and irrigation schedules on water use and yield of banana plantations to create guidelines for plantation management (WP5).

## Achievements

**WP1**

- ImageJ protocol for determining CC based on a normal RGB picture.
- Canopy cover curve for a diploid (Mchare- AA) and triploid (Grand Naine-AAA) banana variety for drought stressed and non-stressed banana plants.
    - Canopy cover is ploidy (cultivar) dependent
    - Canopy cover is not stationary but variable in a day due to leaf folding
    - Canopy cover is a good indicator for drought stress, only still not able to pinpoint stress at the moment of onset
    - Canopy cover cannot be separated between mother and daughter

**WP2**

- Growth database of Mchare and Grand Naine under differential irrigation regimes (soil moistures) is being collected. Mchare until harvest P (cycle 1) and Grand Naine until flowering P (cycle 1)
- Pot-trial to determine water productivity (WP*) and critical moisture levels is currently being carried out.

**WP3**

- Growing degree day (heat unit) thresholds for sucker emergence (Mchare) and flowering (Mchare and Grand Naine)
    - Plants flower at different heat unit thresholds and follow a probability function.

**WP4**

- Bananas cannot be correctly modeled with *AquaCrop* (or any other model) before 2 aspects of banana plantations are built in model
    - Heterogeneity between generations: both mother (P) and daughter (R1) generation present at the same time.
    - Heterogeneity within generations: asynchronous suckering, flowering and harvest which increases the variance in a generation (P and R1).

## Short Summary

Currently, banana plantations are not optimally managed concerning 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.

## Objective 1: Canopy cover determination

The *objective* is to devise a methodology to measure CC of the banana plant and separate the CC between Parent generation (P-cycle) and following ratoon crops (R1 = Ratoon 1 and R2 = Ratoon 2). *AquaCrop* makes use of CC to simulate crop development and transpiration, so correct measurement of CC is of vital importance.

***Canopy cover methodology***

In a running irrigation trial at IITA at Arusha, Tanzania, techniques to determine CC and LAI are tested, and a methodology on picture taking and image analysis is developed. The field layout is shown in Figure 12. 2 cultivars (Mchare and grand Naine) are subjected to 2 irrigation treatments (FI= full irrigation and DI= Deficit irrigation).

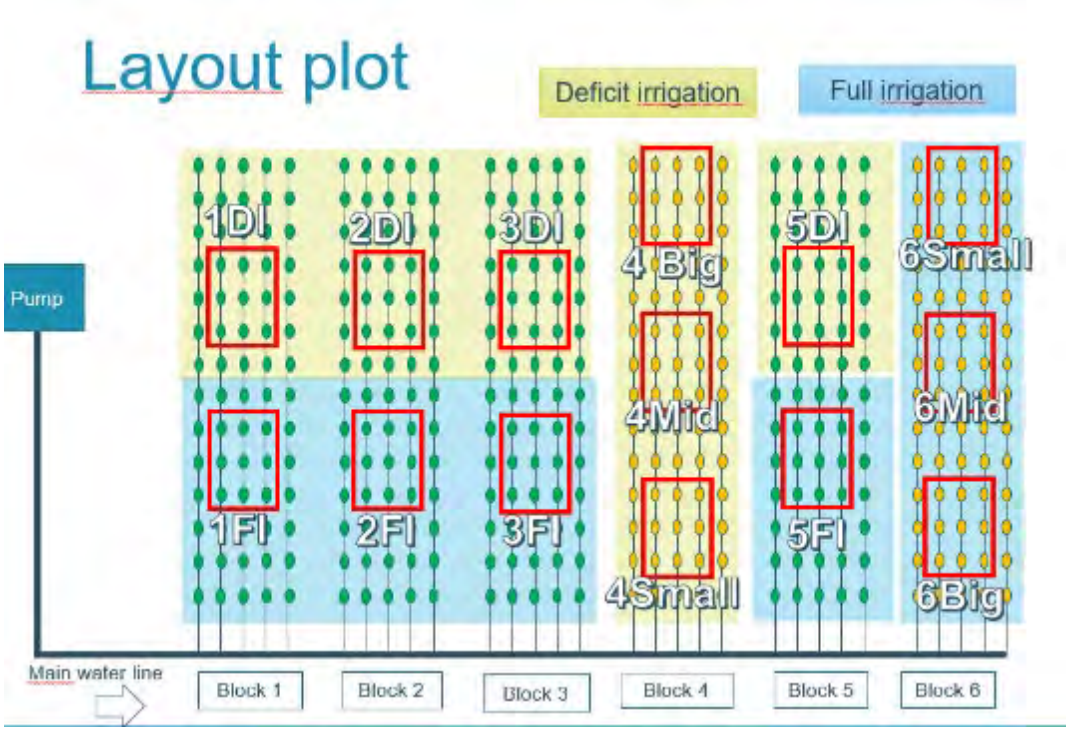



**Figure 12: field layout irrigation trial; the green dots (block 1,2,3,5) note the GN cultivar and the orange dots (block 4 and 6) note the MC cultivar. The red squares note the different regions which are used for data collection and their respective codes (block-treatment for the GN**

**cultivar and block-initial size for the MC cultivar since each block in MC only has 1 irrigation treatment).**

**- CC** (% ground area covered by canopy) in small stands is determined by taking photos of plants with a normal tablet, when plants grow taller a drone (or pole of 10m) is used until the drone permits were in order. From that point onward, drone images were taken on a monthly basis at multiple timepoints (morning, midday and evening) from which the CC was determined for banana stands.

***Outcome: CC evolution***

Canopy cover, CC, (in %) was measured on a monthly basis through image analysis of images taken with a DJI Phantom drone. An example of the image processing is seen in

**Figure 13. Images were captured at minimally 3 time points: morning (9 – 11 AM), midday (11AM – 14PM) and afternoon (14 - 17PM) to capture daily CC variations.**

| Original | Cropped | Green pixel filtering |
|---|---|---|
|  |  |  |

**Figure 13: Canopy cover image determination with the image J protocol**

CC in Figure 14 is taken as an average of the CC over a day. The different lines represent the different plots. For MC, the first time CC starts to diverge significantly between FI and DI is in WAP 23, 4 weeks after shutting off irrigation. For GN, the first time the FI and DI plots start diverging is in WAP 38, the first time CC is measured after shutting off irrigation. CC can therefore be used as an indicator of moisture stress, but only if a baseline CC without moisture stress is present. CC starts showing a reduction approximately 4 weeks after stopping irrigation, so only after the plant is already stressed for 4 weeks. If farmers use CC as an indicator for moisture stress to start irrigating, they are too late.

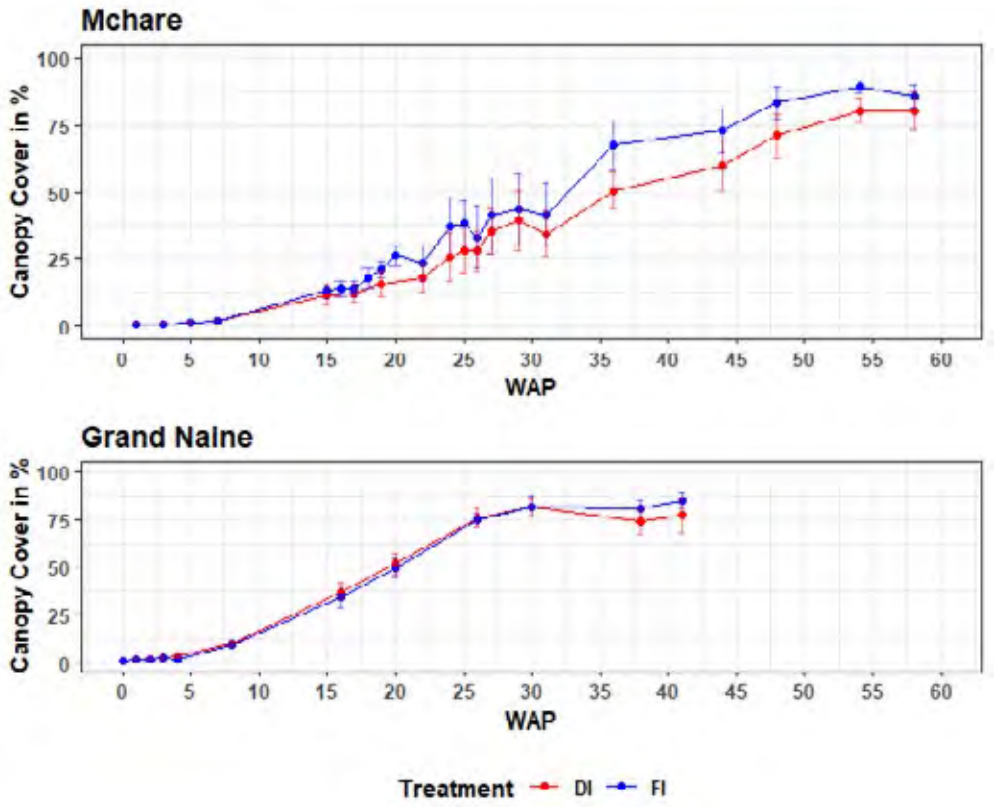



**Figure 14: canopy cover evolution of Mchare and Grand Naine**

Even though they start with a similar CC size, the GN reach their maximum value (CCmax) faster than the MC variety. This is due to the MC variety being a diploid characterised by narrower leaves standing more erect than the GN triploid variety which is characterised by broader, more hanging leaves. Figure 15 shows a comparison of the MC and GN irrigated plots with a similar CCmax of 85%. In the MC plot, the daughters (R1= ratoon 1) are clearly seen from above, while in the GN plot, the daughters are completely covered by the mothers.

| Mchare WAP 44 | Grand Naine WAP 42 |
|---|---|
|  |  |

**Figure 15: canopy cover Mchare March 2018 (WAP 48) and Grand Naine August 2018 (WAP 42).**

Following Figure 14 and Figure 15, different cultivars have different CC curves. The CC is dependent on the ploidy of the cultivar and the respective ages of the mother and daughter.

***Diurnal CC variation***

**Figure 16 and Figure 17 show the diurnal CC variation for all the days when pictures were taken by the drone at different time points (Morning: < 11 AM, Midday 11AM – 14PM, afternoon: 14PM – 17PM).**

The highest CC values were always measured during the morning. Throughout the day, this CC stayed constant or declined until midday. From here on the CC-values increased again whether or not to its morning value. For both MC and GN, the variety in diurnal CC depends on the day of measurement. Some days, there is a clear distinction between CC at midday, and other times of the day. Days with high incoming radiation around noon to have higher folding of the lamina, and this is seen in the CC.

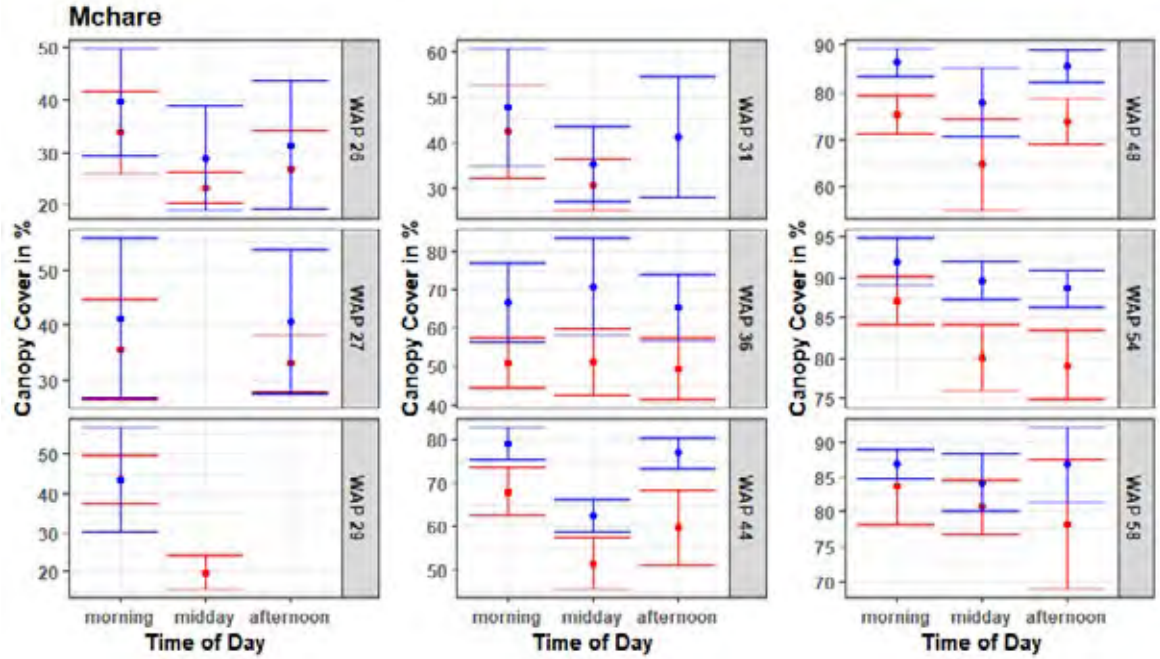



**Figure 16: Canopy cover of Mchare in terms of time of day for different WAP. Canopy cover is taken as an average of the 3 plots per block.**

The same applies for the GN plants. In the GN plots, after shutting off irrigation (WAP 38 and onward), the CC reduction between morning, midday and evening is more pronounced in the DI plots, than in the FI plots, which could indicate drought stressed plants of GN tend to fold more than non-stressed plants. Further data is being collected on this.

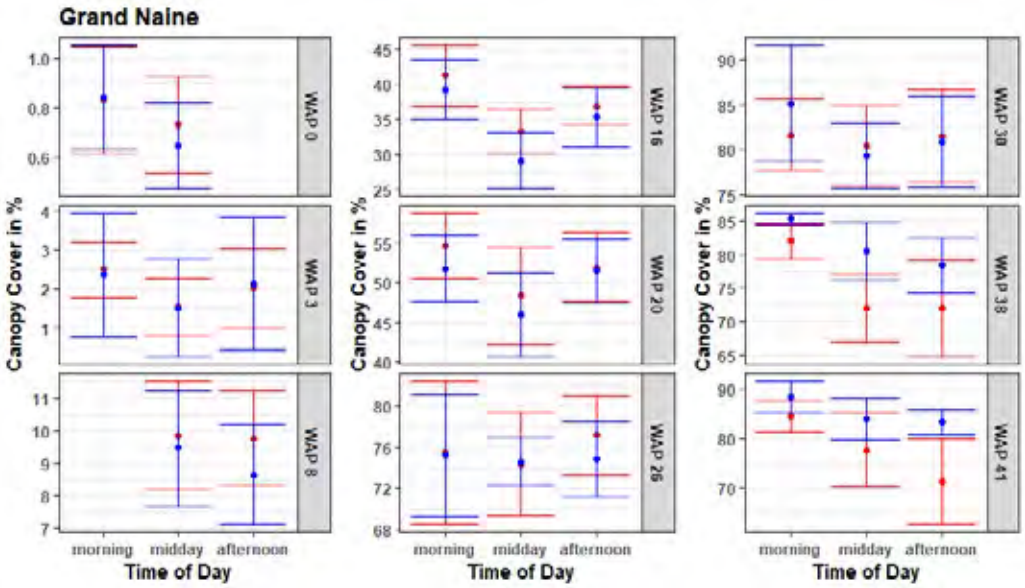



**Figure 17: : Canopy cover of Mchare in terms of time of day for different WAP. Canopy cover is taken as an average of the 4 plots per treatment**

**Conclusion WP1:**

- When doing an *AquaCrop* calibration, a first step should be to make a distinction between Diploid and triploid banana varieties, and the timing of daughter selection should be accounted for as this will have a significant effect on the CC.
- CC varies diurnally, so the ideal baseline CC should be taken in the morning when incoming radiation is minimal and leaves do not yet fold

## Objective 2: Growth response to water and a database for *AquaCrop* calibration

The *objective* is to create a database on the growth response of Banana (MC and GN) to drought stress which is to be used for computer modelling purposes. We aim to follow growth in terms of height, girth, CC, LAI, biomass and yield for all different banana generations (P, R1 and R2) present in the field under differing irrigation treatments (FI and DI). Soil moisture and climate data are also collected daily.

***Soil moisture over course of trial***

Figure 18 shows the soil moisture depletion (in mm) over time for the different plots for both MC and GN. The soil moisture depletion is the amount of water that is depleted below field capacity (FC). If the soil is at FC or above, the depletion is 0. The black line shows the soil moisture depletion below which stresses start occurring. Figure 19 shows the weekly cumulative depletion over the moisture threshold.

In the MC plots the irrigation started to diverge at the end of August 2018 (WAP 19). Originally the DI treatments was shut off and then irrigated again to study the effect of deficit irrigation on growth. Water was applied originally when 50% of the plants showed a physiological indicator of water stress: opening of the cigar leaf before unfurling. This indicator did not prove an easy indicator to spot drought stress as the unfurled cigar lasted even after applying water to FC again. Therefore, the decision was made to shut the irrigation off completely in November 2018 (WAP 27), and compare growth of FI with rainfed conditions. For GN, irrigation was shut off at the end of the rainy season in June 2018 or WAP 29 .In the dry season, water is only given to the DI plots when they are highly visibly stressed, i.e. leaves

start wilting, drying and petioles of the oldest leaves start collapsing. These graphs show the DI treatment has a significantly lower amount of soil moisture than the FI treatment.

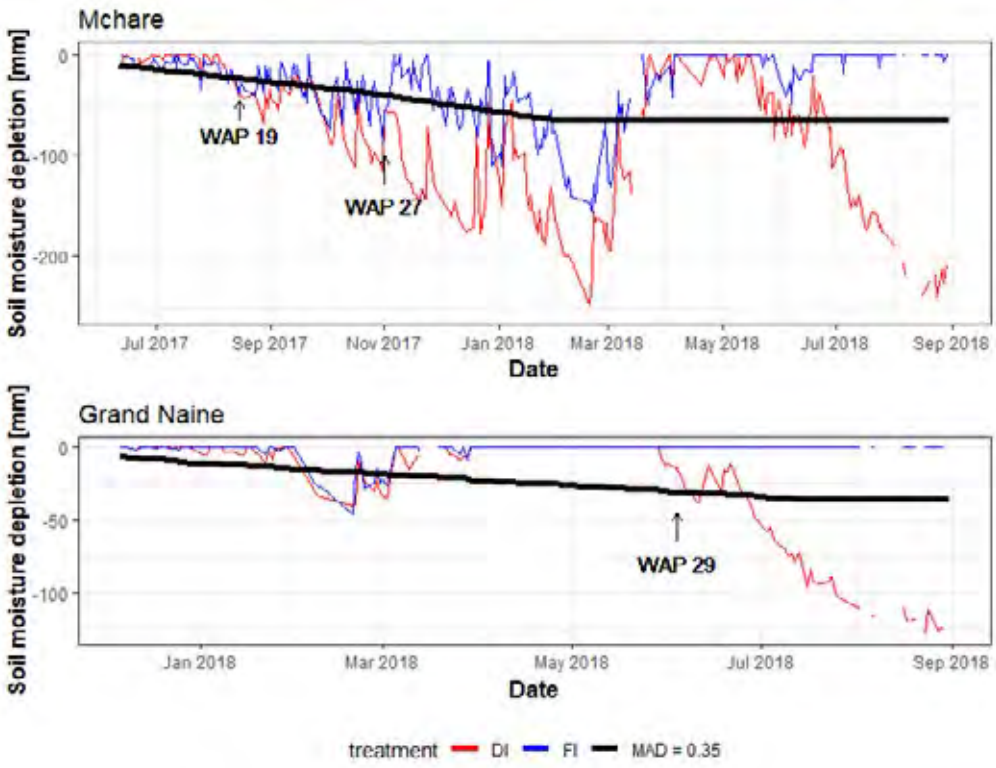



**Figure 18: Soil moisture depletion. Need to stay above the threshold. Due to malfunctioning of the irrigation infrastructure there were some problems in trying to stay above the moisture threshold.**

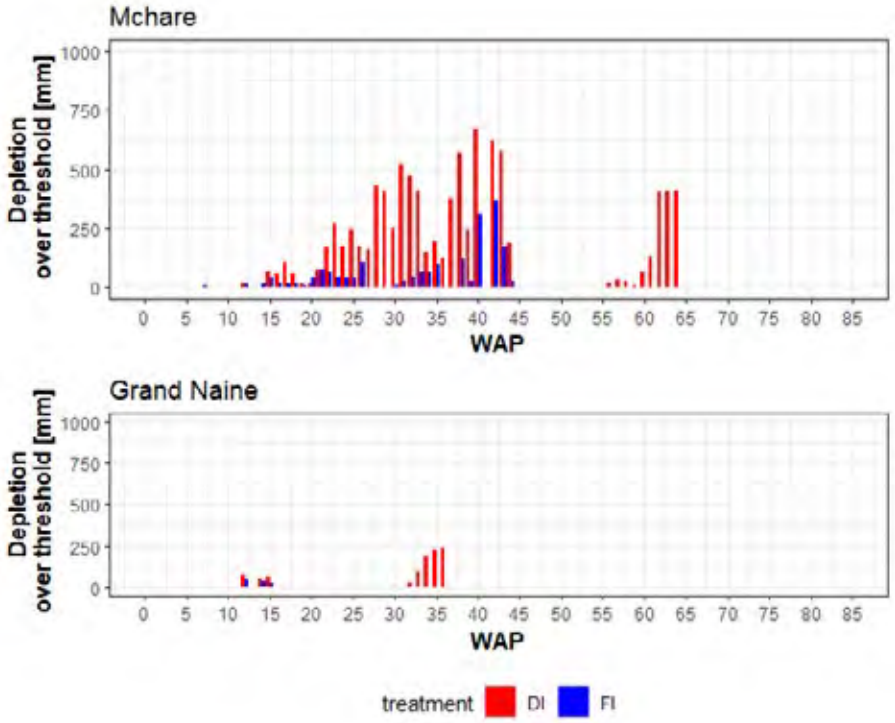



**Figure 19: weekly cumulative depletion (in mm) over threshold.**

***Plant growth in response to WAP and drought***

All growth parameters collected on a monthly basis are very highly correlated (Figure 20). These parameters are all representations of the underlying 'growth' of the banana plant. To limit collinearity of the results, in further sections, we focus on the parameter "height", as an indicator of the growth of the banana plants. Other parameters have similar growth curves. In the section plant growth we will focus on the growth in terms of Height, Canopy cover, biomass and yield.

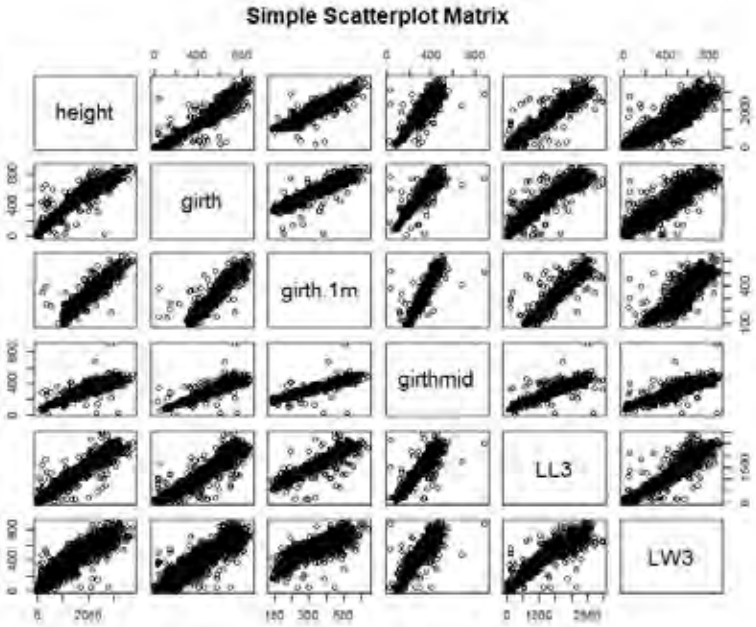



**Figure 20: correlation matrix. All growth parameters which are collected monthly are very highly correlated.**

***Height as an indicator for plant growth***

For MC, irrigation treatments started diverging in the vegetative stage (WAP 19 and WAP 27) leading to a significant effect on plant height and other vegetative growth parameters for both mother and daughter plants. Growth rates of plants in the DI treatment started to be significantly reduced at 25 WAP.

For GN, the treatments started diverging at the approximate time of flowering (WAP 29) leading to a similar size in vegetative growth parameters among the different treatments. From WAP 29, the growth rate slows down for both GN treatments and cycles. This corresponds to the arrival of the cold season in Arusha (June, July), which could explain the decrease in growth rate.

All growth curves follow a normal sigmoidal plant growth curve with a lag phase, an exponential phase and a stagnation at flowering.

It can be seen that for the P-cycle (the mothers) the MC is taller than the GN at flowering, which is not due to a worse growth, but is cultivar dependent. MC plants are taller, thinner and characterised by thin erect leaves (diploïds). GN plants are shorter, thicker and characterised by broad hanging leaves (triploïds).

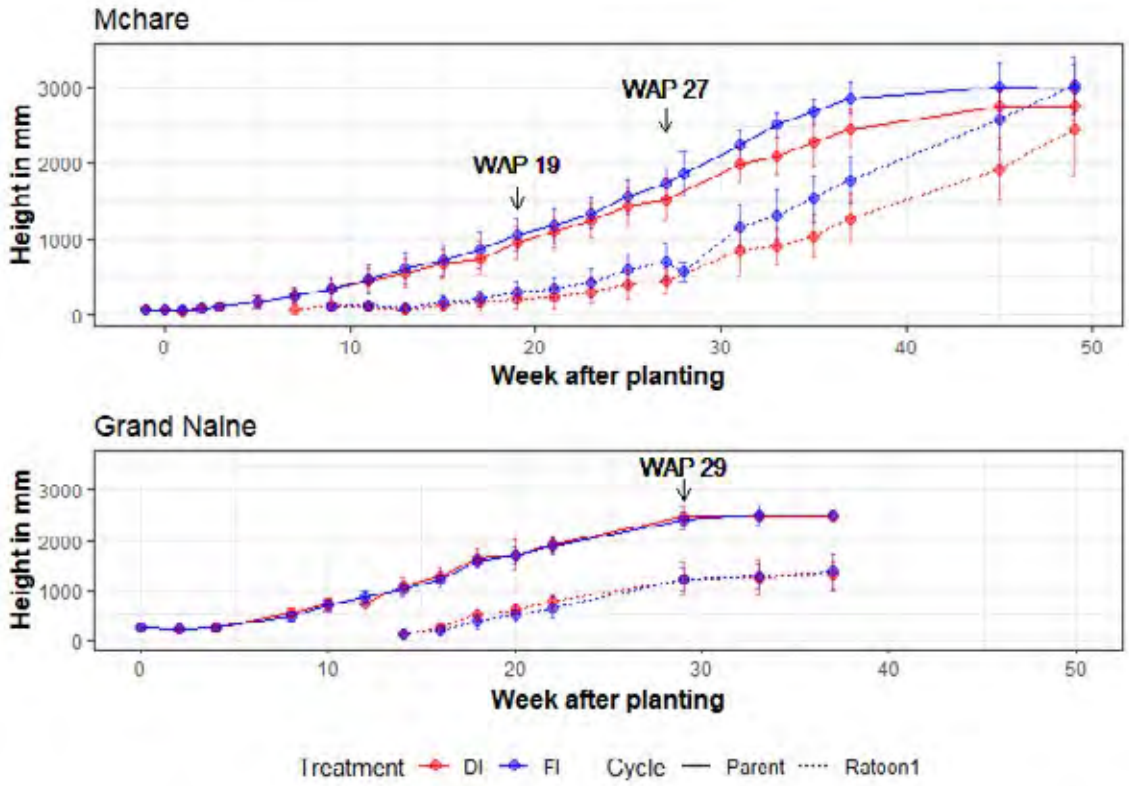



**Figure 21: Growth in terms of WAP. Arrows indicate time points when irrigation started to diverge. For MC, at WAP 19, irrigation was shut off roughly on a weekly basis and replenished to FC, at WAP 27, irrigation was shut off completely. For GN, irrigation was shut off completely at WAP 29**

***Biomass***

Biomass was calculated according to allometric relationships created by Nyombi (Nyombi et al., 2009) based on the non-destructive parameter "girth at base [cm]" for the MC and GN cultivar as shown in Figure 22. Different equations were used for flowering plants and plants in the vegetative state.

$$\boldsymbol{vegetative\ state}: Aboveground\ biomass\ [kg] = 0.0001 * (girth)^{2.35}$$

$$\boldsymbol{flowering}: Aboveground\ biomass\ [kg] = 0.325 * e^{0.036 * girth}$$

It is shown that the biomass for MC is lower than the biomass for GN. Even though the allometric relationships by Nyombi were made for the East African Highland Banana, it cannot be used for MC, as MC is a diploid characterised by a thin long stem and erect leaves. It is taller than most other East African Highland bananas, so an allometric relationship based on girth underestimates the biomass produced. This clearly pinpoints the danger in using allometric relationships developed on other cultivars than our cultivars in the field.

We therefore have to create our own allometric relationships for the MC cultivar based on data from destructive sampling and harvest data. This data is still being collected. If necessary, more plants need to be destructively harvested in farmers plots so an allometric relationship can be obtained for the MC plant and the GN plant. This is part of the Msc. Thesis project undertaken by Erick Kibona of the Nelson Mandela African Institution of Science and Technology.

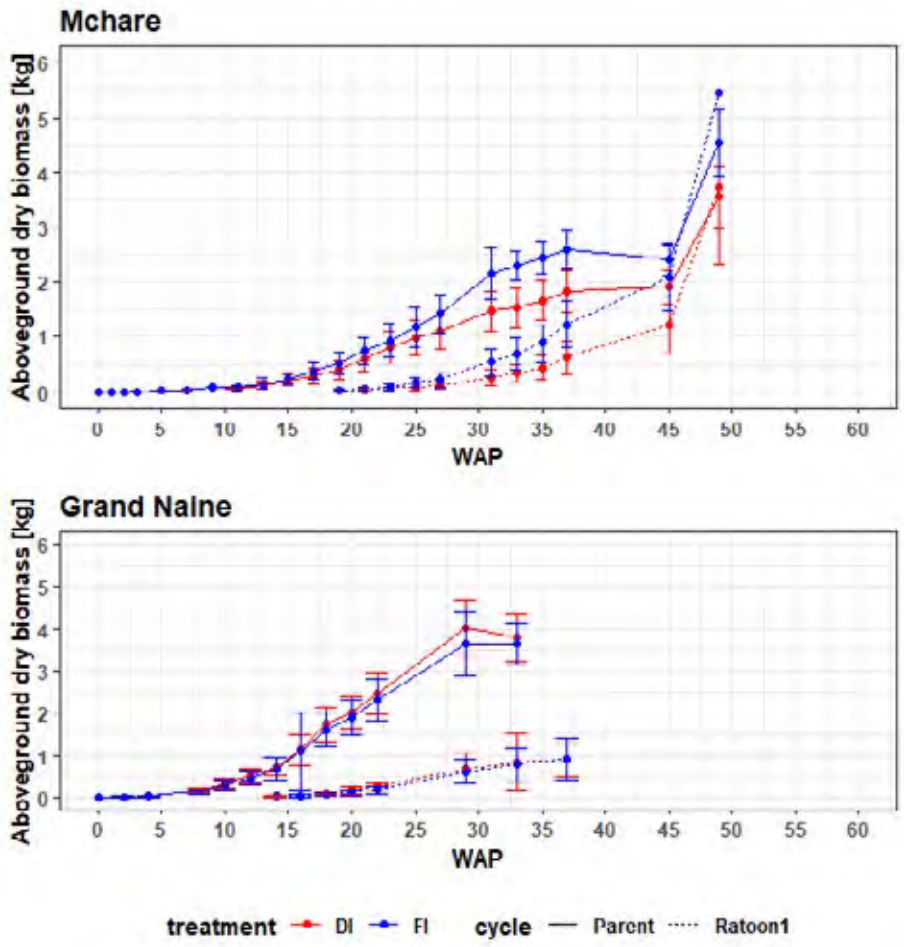



**Figure 22: aboveground dry biomass evolution, based on girth and the allometric relationships by Nyombi.**

***Yield***

The first cycle of the MC plots is currently being harvested. Data collection for the first MC cycle is on its way and expected to be fully collected by the end of September 2018. Total harvest data, both for the second cycle of MC as both cycles of GN is expected to be fully collected around April 2019.

**Conclusion WP2:**

- Drought stress clearly has an effect on growth and is seen approx. 4 weeks after shutting off irrigation
- Database for modelling and effect of drought stress is being collected.
- Existing Biomass allometric relationships cannot be used for our varieties, and need to be adapted and validated.

## Objective 3: Banana phenology (growth stages) in response to temperature (accumulated heat units) and soil moisture (WP3)

***Growing degree days (GDD) as a heat unit***

Growing degree days (or GDD) is a concept that is used to predict when crops will reach crop specific growth stages like flowering and maturity. It is calculated by subtracting a crop specific base temperature (14°C for banana) from the prevalent average temperature of the day. By summing these values over the course of a growing season, plants accumulate heat

units and plants that are not sensitive to photoperiod and vernalisation to induce phonologic events are believed to reach these stages when a certain amount of GDD is accumulated. Figure 23 shows the accumulated GDD for the MC and GN cultivar at the end of each WAP.

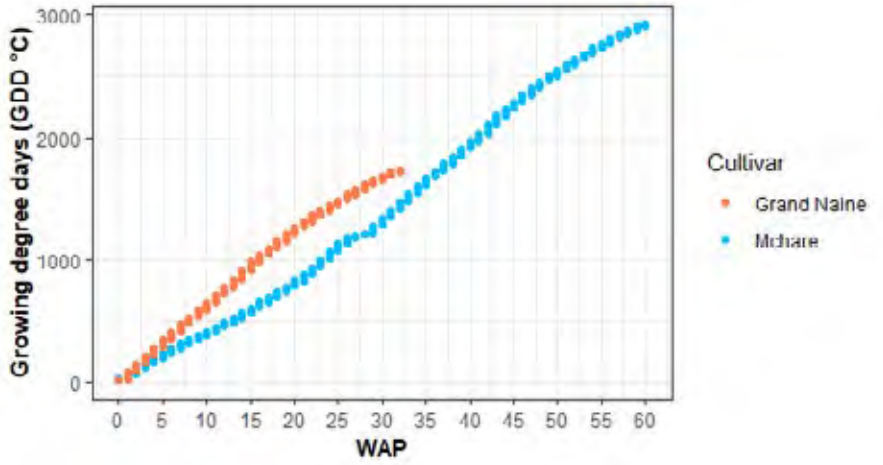



**Figure 23: incorporated GDD at the end of each WAP**

***Banana phenologic development***

One of the key characteristics of a banana plantation is its asynchronous growing behaviour and heterogeneous population structure, indicating multiple generations of plants occur at the same time in a plantation. A banana plantation can be heterogeneous due to natural occurrences (sucker emergence, flowering) or human induced (sucker selection, harvest selection).

In order to correctly model a plantation, these processes need to be accounted for. As a first start of the modelling process, we focus on sucker management. Flowering behaviour is the next indicative step that determines the heterogeneity of a plantation. Thirdly, harvest dynamics create further heterogeneity.

***Sucker emergence per plant***

In the MC variety, suckers developed freely in the first 4 MAP after which a sucker was selected from the south side for the next generation. Suckers emerged under optimal moisture conditions.

Sucker emergence was counted every month for the different plots and shown in Figure 24. Suckers started to appear first in WAP 9 (and multiple suckers developed per plant. Naturally suckers that developed later are smaller than the first suckers. At WAP 21 (or 4 MAP), we chose a sucker at the south side for the next generation and removed all others.

For GN, the sucker appearance rate was also determined, but here, suckers developed due to an excess of growth hormone of the in vitro plant, and suckers emerged much earlier than normal. Suckers were removed monthly and suckers on the south side were let to develop. After 4 MAP, one sucker was chosen for further measurement.

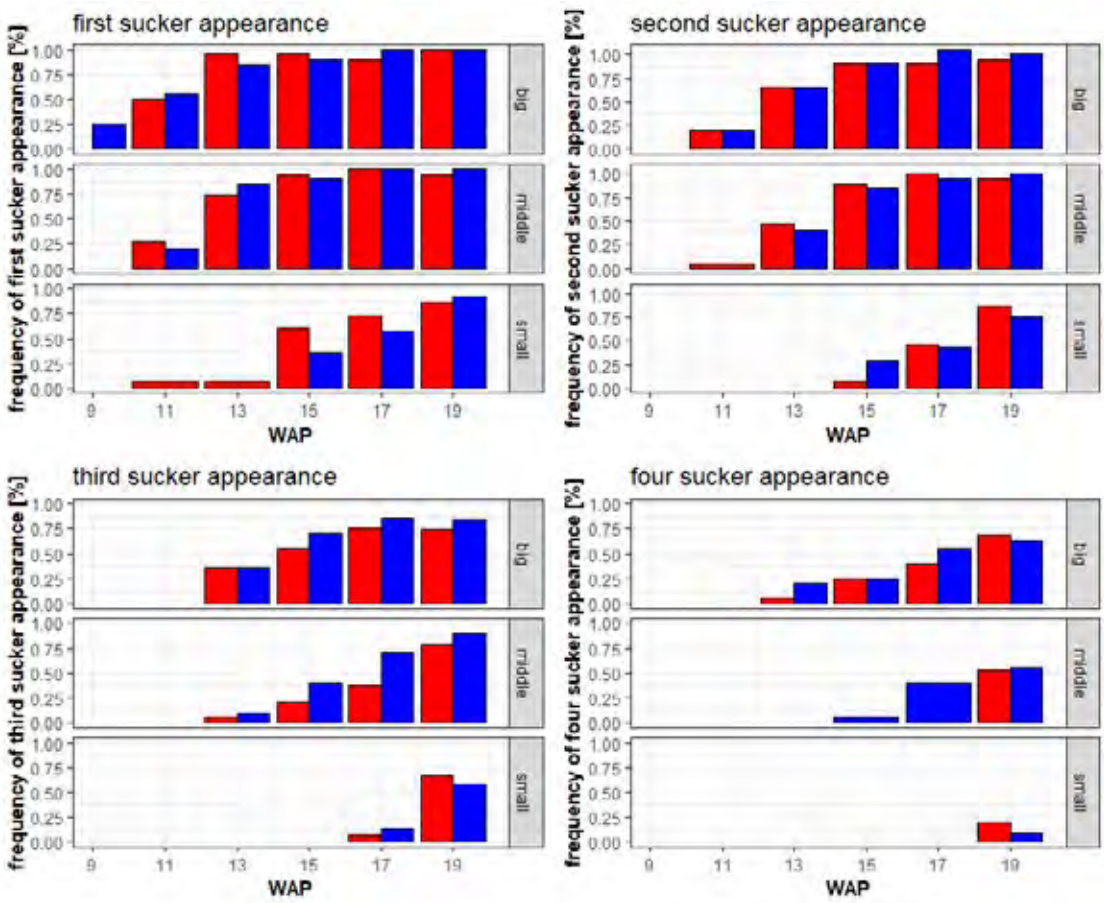



**Figure 24: sucker emergence rate in WAP. Red is DI and blue is FI. At WAP 21, we selected our suckers for the second cycle.**

**Table 21: growing degree days accumulation until suckers start developing.**

| Phenologic event | Big | Middle | small |
|---|---|---|---|
| | GDD | GDD | GDD |
| First sucker appeared | 343.60 | 447.20 | 447.20 |
| Second sucker appeared | 447.20 | 447.20 | 605.15 |
| Third sucker appeared | 482.40 | 482.40 | 694.65 |
| Fourth sucker appeared | 482.40 | 605.15 | 777.25 |

In a banana plantation, sucker selection depends on the individual farmers; and it is this sucker management that will determine the yield and harvest dynamics of a plantation. In our case, suckers are chosen from the same side of the banana plant to keep plant spacing in the field equal in following generations. We aimed to select suckers at the same side of the plant and aimed for them to be in the same growth stage. In that sense, it is not the heterogeneity of sucker emergence that will describe the heterogeneity of the next generation, but the heterogeneity of the selected suckers on the south side.

***Selected sucker size at 4 MAP.***

The initial size of the suckers in the GN plots after 4 MAP is bigger than those selected for MC. For MC, the selected suckers in the DI treatment are slightly smaller than the ones in the FI treatment. At 19 WAP, the irrigation treatments were separated, which could explain the slightly smaller size in the DI treatment.

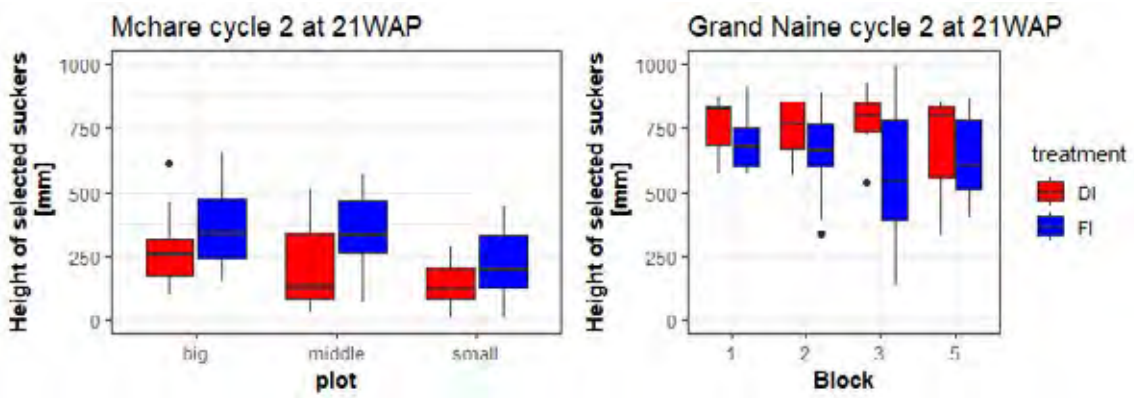



**Figure 25: initial sucker size of second cycle for Mchare and Grand Naine (n=9 for each boxplot).**

For GN, the opposite is true, the selected suckers are slightly bigger in the DI treatment than in the FI treatment. There is also a bigger range among the suckers in the third and fifth block, indicating a bigger starting heterogeneity in these plots. We will see if the increased heterogeneity in size among the suckers will translate to an increase in heterogeneity in growing behaviour and timing of phenologic stages for the R1 crop.

***Flowering***

A second parameter influencing heterogeneity is flowering behaviour. Asynchronicity in flowering also creates a heterogeneous plantation. For MC and GN the frequency of flowered plants per plot is shown in Figure 26 and Figure 27.

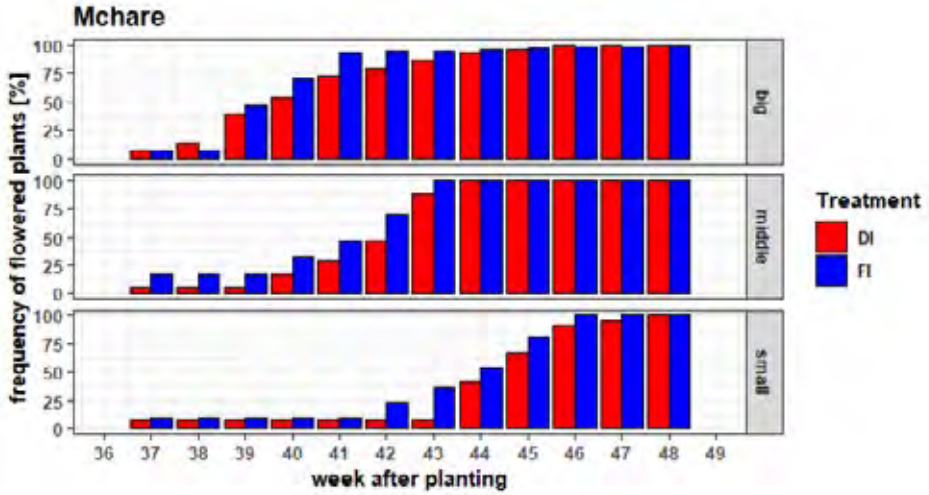



**Figure 26: frequency of flowered plants for the Mchare plots**

There is not a big difference in terms of flowering date between the different irrigation treatments of MC. Plants that are not sensitive to photoperiod and vernalisation can normally use the GDD concept to indicate flower timing. In our case, there is no significant difference between flowering in the DI or FI treatment, so drought does not seem to have a big effect on onset of flowering. Flowering occurs between WAP 37 (1778.1 GDD) and WAP 48 (2452.9 GDD).

The biggest effect on flowering frequency appears to be initial plant size. The plants from the smallest group flower later than plants from the middle and big groups. This indicates the importance of seedling (and sucker size) at planting for modelling.

In each size group there is a difference of 8 to 11 weeks between the first flowered plant, and the last flowered plants. The individual GDD threshold used by *AquaCrop* to indicate the onset of flowering can therefore not be used to model a banana plantation.

For GN, the first plants started flowering in the 31st WAP (1714.6 GDD) and all plants in the plot flowered around WAP 42, indicating flowering spread over 11 weeks or roughly 2 to 3 months, similarly as in the MC plots.
In most blocks, more plants flowered earlier in the DI plants than in the FI plants. The FI plants soon caught up and at WAP 41 to 42 all plants achieved flowering.

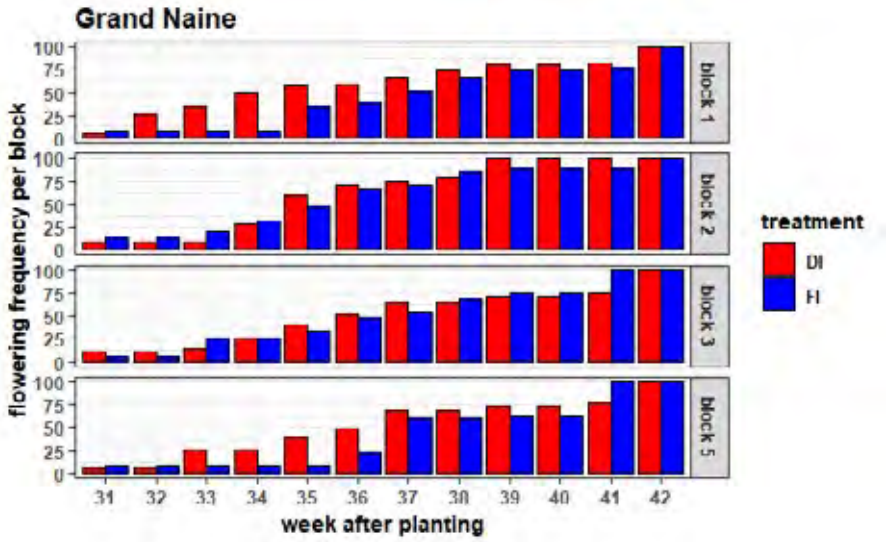



**Figure 27: frequency of flowered plants per treatment per block for the Grand Naine variety.**

What follows from this section is that a single GDD value in *AquaCrop* as a threshold for flowering cannot be used for a banana plantation.
Instead, a probability function in terms of GDD or WAP for flowering needs to be built into the *AquaCrop* model.

***Harvest dynamics***

A third factor influencing heterogeneity is the timing of harvest. A bunch is ready between 11 and 20 weeks after flowering depending on the genotype, prevailing climate and farmer preferences (Yara, 2001). However, given the highland climate in Arusha, it is believed it takes longer as temperatures are lower. Fruit can be harvested fully mature for immediate ripening and consumption in local markets, at 90% maturity for short distance transport and at 75% maturity for long distance transport. Farmers therefore are the ones deciding the timing of harvest (ranging from 75% to fully mature). In Arusha, the aim was to achieve maximum yield, so a bunch was considered harvest ready at the full mature stage when fruits on the bunch show signs of yellowing.

**Conclusion WP3:**

- Sucker emergence, flowering and harvest dynamics do not follow a clearcut GDD threshold, but follow a GDD probability curve
- Time of flowering is dependent on GDD, but did not show to be affected by soil moisture for MC. For GN, plants in the DI treatment started to flower slightly earlier.

## Objective 4: Incorporating the heterogeneous banana growth structure in *AquaCrop*

***Current AquaCrop model***

The *AquaCrop* modeling scheme is composed of 4 steps. Firstly, green canopy cover (CC) is simulated whereby the expansion of CC under non-stressed conditions follows a logistic growth curve determined by the initial CC (CC0), the maximum CC (CCx) and a canopy

growth coefficient (cgc). In the late season stage, the decline of CC is determined by a canopy decline coefficient (cdc). Secondly, crop transpiration (Tr) is simulated based on prevailing weather conditions (ET0) and a crop transpiration coefficient that is proportional to the simulated CC. Next, cumulative crop transpiration is translated to dry aboveground Biomass (B) by means of the normalized water productivity factor (WP*), a conservative factor for each crop species. Lastly, the biomass is used to determine yields (Y) by means of a harvest index (HI). Water stress has an effect on multiple steps in the simulation process as shown in figure 4. After reaching specific threshold water values different processes are affected: (a) for canopy expansion the potential canopy cover (CCpot) is reduced to simulated CC, (b) stomatal closure is simulated by multiplying the potential transpiration with the stress coefficient for stomatal closure (Kssto); (c) Senescence is triggered more early and (d) harvest index and root expansion are also influenced by soil moisture values (Steduto, Hsiao, Fereres, & Raes, 2012).
Schematically the process is shown in Figure 28, and this scheme should be adapted to incorporate the banana population structure.

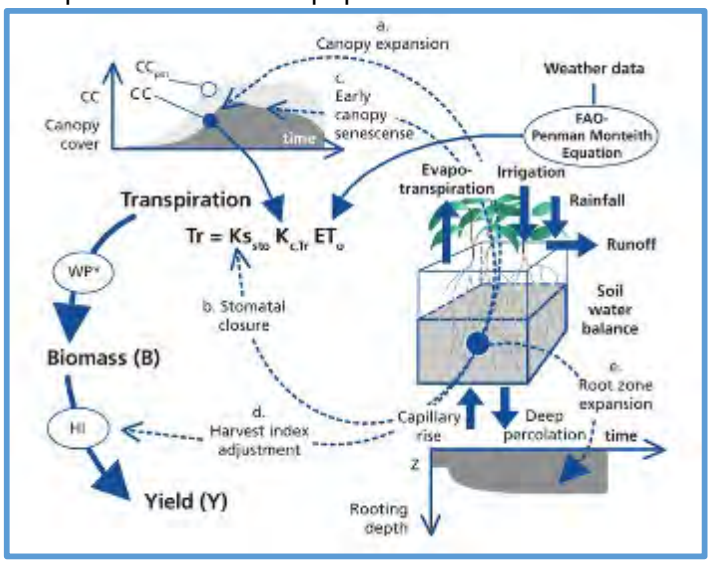



**Figure 28: AquaCrop modelling scheme and influence of water stress on simulation processes (a to e) indicated by dotted lines.**

In view of the *AquaCrop* modelling structure, the emphasis should firstly be on calibrating the CC curve for the banana plant as CC determines the crop transpiration, biomass and yield. *AquaCrop* currently only works for homogenous plantations, like maize, where the population structure is characterised at each moment in the growth season by individuals in the same growth stage. As shown in previous section, this is not the case for a banana plant, as banana plants have heterogeneity between generations P (Parent) and R1 (Ratoon 1), and even within generations P and R1.

***AquaCrop adaptation for the banana plant***

These two characteristics should be included for *AquaCrop* to work for a banana plantation.

1. Heterogeneity between generations: both mother (P) and daughter (R1) generation present at the same time.
2. Heterogeneity within generations: asynchronous suckering, flowering and harvest which increases the variance in a generation (P and R1).

**The first step** should be to assume the P and R1 generation to be homogenous: all P in the same growth stage and all R1 in the same growth stage and model these two generations at

the same time with *AquaCrop*. The heterogeneity is therefore between generations, and not within.

**The second step** should be to increase the heterogeneity within a generation by looking flowering and harvest dynamics.

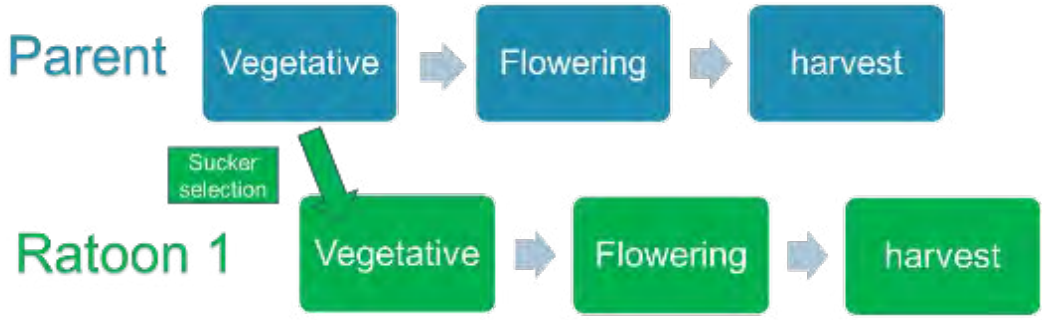



**Figure 29: Simplified model of banana cycles present in a field. Heterogeneity between generations (P vs. R1) and heterogeneity within generations (plants present in vegetative, flowering or harvest state within P and R1).**

***Heterogeneity between generations: homogenous P and R1***

A banana plantation is composed of different cycles (P and R1) present at the same time and position. The resulting CC is composed of different leaf layers of P and R1. Originally the CC is composed solely of the P-layer as it completely blocks the R1 leafs, but as time goes on, the R1 leafs will grow and eventually intermingle with the P leafs and later possibly cover the P leafs. Since the CC is composite, we have to model it as such without separating CC for P and R.

*AquaCrop* uses the CC as an indicator of leaf area of a plantation. Another indicator often referred to is the LAI or Leaf area index of a plantation, which is the one sided total leaf area (in m²) per m² soil area. Where CC is impossible to separate between mother and daughter, LAI can be separated, so this can serve as a possible alternative to CC to be used in *AquaCrop*. We will aim to model LAI for the P and R1 cycle (respectively LAI-P and LAI-R) in terms of GDD and moisture depletion.

$LAI - P = f(GDD + moisture\ depletion)$ and $LAI - R1 = f(GDD + moisture\ depletion)$.

The total LAI is then a summation of the LAI-P and LAI-R1.

We can create a functional relationship between total LAI and CC following (Nielsen, Miceli-Garcia, & Lyon, 2012) who determined the relationship between CC and LAI for wheat, triticale and corn.

$$CC = f(LAI) = f(LAI\ P + LAI\ R1)$$

We expect that in the beginning, the LAI-P is going to influence the CC predominantly and LAI-R1 is negligible. However, when the LAI-R1 continuous to grow, the leaves start intermingling and having a more significant effect on CC. Even after flowering of P, the CC (and total LAI) are growing continually until harvest of the first cycle. At this point, the cycles switch: the P cycle disappears, the R1 becomes the mother and the R2 becomes the daughter. So the LAI R1 then becomes the LAI P in our formula.

The assumption is that only the LAI of the mother and LAI of the daughter influence the CC, as the granddaughter is mostly covered by the mother and daughter. At harvest of the mother, when the daughter becomes the mother and the granddaughter becomes the daughter, the LAI of the granddaughter will start playing a role.

One of the things to build in the *AquaCrop* model for banana is this so called CC-drop after harvest, or the initial CC of the second cycle, when the R1 becomes the mother and R2 becomes the daughter. This depends on 2 factors: 1) timing and size of selected suckers which determines the initial variation and size potential at harvest 2) drought stress during development, which will determine the actual size at harvest.

In the MC plantation, the CC went from 93% before harvesting the first cycle to 87% after harvesting, indicating the R1 generation to be near maximum CC. At WAP 48, the R1 plants

reached a height similar to the P plants. The P plants no longer cover R1 fully and R1 starts taking over most of the CC.

***Heterogeneity within a generation: cohorts***

Heterogeneity within a generation can be modelled by using probability functions for suckering, flowering and harvest, by using cohort population dynamics by Tixier, Malezieux, & Dorel (2004). A cohort is defined as a group where all the plants are in a similar growth stage (pre flowering, flowering and harvest). Each cycle can exist of multiple cohorts (pre flowering, flowering and harvest).

| | Timeline in MAP | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P cycle | Pre flowering | 100% | 100% | 100% | 100% | 100% | 100% | 96% | 50% | 25% | 0% | | | | | | | | | |
| | Flowering | | | | | | | 4% | 50% | 75% | 100% | 100% | 100% | 92% | 70% | 15% | 0% | | | |
| | Harvest | | | | | | | | | | | | | 8% | 30% | 85% | 100% | | | |
| R1 cycle | Pre flowering | | | | 100% | 100% | 100% | 100% | 100% | 100% | 96% | 50% | 25% | 0% | | | | | | |
| | Flowering | | | | | | | | | | 4% | 50% | 75% | 100% | 100% | 100% | 92% | 70% | 15% | 0% |
| | Harvest | | | | | | | | | | | | | | | | 8% | 30% | 85% | 100% |

**Figure 30: hypothetical example of heterogeneity in a generation. % note the amount of plants in the P respectively R1 cycle that are in the pre-flowering cohort, the flowering cohort or the harvest cohort.**

By creating probability functions of flowering and harvest in terms of GDD for both P and R1 plants, we can separate a seemingly homogenous cycle in cohorts.

**Conclusion WP4:**

- *AquaCrop* needs some underlying changes to correctly model a banana plantation
  - Different ratoon crops present at the same time (P, R1 and R2)
  - Heterogeneity within a generation (sucker behavior, flowering and harvest).

## Conclusion

Data collection is on its way, and the first database to start modelling is almost collected. The current *AquaCrop* system has its shortcomings to model heterogeneous plantations, and needs to be adapted to allow heterogenous banana plantations.

**A first step is to model 2 generations at the same time:** mother plants in the same growth stage, and daughter plants in the same growth stages. In order to do this, we aimed to select suckers of similar size for the first ratoon crop. A field is then composed of 2 groups: mother and daughter plants. As indicated, *AquaCrop* uses CC as a key component from which the Transpiration, Biomass and Yield are calculated. It proved impossible to separate the CC into the mother and daughter generation, but another indicator of leaf area, LAI may prove to be used instead.

**A second step is to include inter plant asynchronicity in the same generation.** Timing of flowering and harvest in *AquaCrop* follows a clearcut growing degree day threshold (GDD in °C). For the Banana plant, with its asynchronous growing behaviour, sucker emergence, flowering and harvest follows a logarithmic curve and the GDD threshold is therefore not a clear pinpointed value anymore, but also follows a logarithmic curve. This will lead to a spread in suckering, flowering and harvest, leading to a more heterogeneous plantation.

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## PhD Student Progress Report (2017-2018)

**TITLE:** Nutrient use efficiency in banana-bean intercropping systems in the Upper Pangani Basin, Tanzania

**Name of Student:** **AKIDA IGNAS MEYA**

**Supervisor**: Profs. Roel Merckx, Rony Swennen and Patrick Ndakidemi

**Timeline of study:** February 2014 to December 2019

**University:** KU Leuven, Belgium

**Research Objectives**

List the individual topics of study – objectives or study areas

- To contribute to the understanding of soil variability in the northern Tanzania banana growing areas and its role in increased crop production and to develop a starting point for site specific soil fertility management practices
- To determine the current limiting factors in banana-bean production systems as related to respective soil types and altitudinal gradients on the slopes of Mount Meru and Mount Kilimanjaro
- To evaluate the effect of integrating mineral and organic N sources on banana growth and fruit yield
- To evaluate the nutrient use efficiency of banana cropping system
- To quantify nutrient transfer via maize stover transportation from the maize production belt to highland areas

**Achievements**

Highlight significant achievements – e.g. in bullets

- Full draft of research article "*Integrated soil fertility management: an alternative strategy to optimize banana production in volcanic soils of Northern Tanzania"*
    - From this study it was observed that applications of N fertilizer through complete urea resulted to a substantial increase in vegetative growth and banana yield. Increasing the rate from 153.3 kg to 229.9 kg N hectare$^{-1}$ year$^{-1}$ gave a non-significant effect on plant growth and banana fruits yield. Such indicates that, the optimum fertilizer rate for these soils is 92g N mat$^{-1}$ year$^{-1}$ equivalent to 153.3 kg N hectare$^{-1}$ year$^{-1}$
    - Remarkably, applications of the same rate via urea and cattle manure at 50% per each resulted to larger plant size and higher banana fruits yield relative to other strategies tested in this study. Since animal manure has become a scarce resource, disseminating this strategy to smallholder farmers will significantly reduce the amount of manure needed to address crop nutrient requirements while improving yield and soil quality

**Background/introduction**

Banana is a major food staple and an important cash crop in sub-humid areas of Tanzania. Currently, banana ranks fifth in terms of quantity produced after maize, cassava, sweet potato and sugar cane in the country (FAOSTAT, 2015). In the typical banana farming systems, almost all households grow bananas for food and cash (Byabachwezi and Mbwana, 1999). In these areas, the crop ranks 1st as a major food staple and 2nd or 3rd as a source of income (Nkuba *et al*., 2003). The current yield level attained under farmer's conditions is far below the potential yield. This is attributed to the decline in soil fertility as a consequence of the continuous nutrient mining originating from continuous production without proper nutrient replenishment. Generally, crop nutrient requirements in banana farming systems are addressed using sole cattle manure which has become a scarce resource. Land scarcity originated from high human population is forcing smallholder farmers to convert grazing lands to farms (e.g. Baijukya et al., 2005; Mbonile, 2005) to produce food to feed more people. As a consequence, smallholder dairy farmers have been forced to reduce the number of animals due to limited amounts of available fodders. This has negatively impacted the accessible amounts of animal manure. In turn, farmers apply small quantities which do not meet crop nutrient demand. Thus, supplementation with mineral sources is necessary. Yet, studies by Kaihura et al. (2003), Gallez et al. (2004), Baijukya et al. (2005) and Maro et al. (2014) reveal that most banana growers in Tanzania are unwilling to use mineral fertilizers. Such reluctance has been attributed to lack of knowledge related to their appropriate use perceived by farmers. Previous works (e.g. by Mizota et al., 1988; Kaihura et al., 1998; Kaihura et al., 2003; Baijukya et al., 2005; Pabst et al., 2013; Maro et al., 2014; Raeymaekers and Stevens, 2015) indicate that low soil N is the most limiting nutrient for crop production in most areas of Tanzania.

This study aims to investigate the potential of organic resources on improving mineral-N use efficiency in banana-legume cropping systems in the Upper Pangani catchment areas as an alternative strategy to improve and manage soil fertility of the system while minimizing land degradation. The overall objective of the study is to optimize integrated soil fertility management (ISFM) practices as an alternative strategy to manage soil fertility.

**Planned activities v/s current implementation status as per respective specific objective**

**Specific objective 1:** To contribute to the understanding of soil variability in the northern Tanzania banana growing areas and its role in increased crop production and as a starting point for site specific soil fertility management practices.

**Activity 1:** To conduct soil survey in banana producing farms in Rombo District located on the northeast slopes of Mount Kilimanjaro to investigate nutrient gradients, and examine the capacity of the soils to supply nutrient requirements for banana production.

**Implementation status:** The survey was successfully conducted in January-February 2018. Samples were transported to Belgium for analysis in soil and water division laboratory in end of June 2018. Unfortunately, I could not manage to analyse the samples while in Belgium. Therefore, samples will be analysed in March-May 2019 during my next visit.

**Specific objective 2:** To determine the current limiting factors in banana farming systems as related to respective soil types and altitudinal gradients on the slopes of Mount Meru and Mount Kilimanjaro.

**Activity 1:** To collect banana leaf samples from banana producing farms in Rombo District located on the northeast slopes of Mount Kilimanjaro to investigate the current limiting nutrients for banana production.

**Implementation status:** Collection of banana leaf samples and growth measurements on sampled plants was successfully done in January-February 2018. Plant samples were analysed for nutrient contents in the Soil and Water Division Laboratory of the KU Leuven in May-June 2018. Currently, I am compiling the information obtained from plant tissue analyses to investigate the current limiting nutrients in the banana farming systems across the sites.

**Specific objective 3:** To evaluate the effect of integrating mineral and organic N sources on banana growth and fruit yield.

**Activity 1:** Performance evaluation of different N sources and fertilizer application strategies on plant growth and banana fruits yield.

**Implementation status:** The field experiment consisted of 8 N treatments. These included (nutrient rates expressed in kg per hectare$^{-1}$ per year$^{-1}$): T1 = 0 (control), T2 = 76.7, T3 = 153.3, T4 = 229.9 kg mineral N, T5 = 153.3 kg N of which 50% of mineral N was substituted by cattle manure, T6 = 153.3 kg N but this time mineral N was substituted by 100 % cattle manure, T7 = 76.7 kg mineral N plus biological nitrogen (BN), and T8 = 100 % BN from inoculated legume intercrop. Banana growth and yield data were assessed in 9 plants from central rows. Growth observations were made based on the following parameters: (i) plant height from soil level to the last 2 leaf curvatures using a tape measure, (ii) stem girth at 100 cm above the soil using a tape measure, (iii) leaf area of the 3 functional leaves using the formula given in equation 1 below (Obiefuna and Ndubizu, 1979), and (iv) crop cycle. Observations on parameter i-iii were made at 6 (except girth), 9 months after planting (MAP), and at shooting. Crop cycle was estimated by summation of number of days from planting to shooting. Similarly, yield parameters were assessed, and these included: (i) finger size using tape measure and weigh scale, (ii) number of fingers per hand and per bunch, (iii) number of hands per bunch, (iv) bunch weight using weigh scale, and (v) total yield per hectare calculated using the formula given in equation 2 below. Bunches were harvested when banana fingers attained horticultural maturity.

*Leaf area=leaf length x leaf width x 0.8 ……...1*

$$Yield = (Bunch\ weight\ x\ number\ of\ bunches\ per\ hectare\ x\ 365) \div days\ to\ harvesting \ldots\ldots\ldots 2$$

We have a complete data set (growth, yield and foliar nutrient contents) for cycle 1 from all 3 study sites. Currently, I am finalizing a draft of research article to be titled: ***Integrated soil fertility management: an alternative strategy for improved Mchare banana production in the volcanic soils of Northern Tanzania.***

**Observations made:**

a. **Relationship between selected growth and yield characteristics and attained banana fruits yield**

Almost all N fertilizer treatments had a significant positive influence ($p \leq 0.001$) on plant growth and banana fruits yield. The results of this study indicate that high banana fruits yield was linked to plant size, crop cycle and maturity, number of fingers per bunch and finger weight. Best treatments resulted in a large plant volume (Fig. 1), early flowering (Fig. 2), early crop maturity (Fig. 3), more fingers per bunch (Fig. 4), and heavy fingers (Fig. 5).

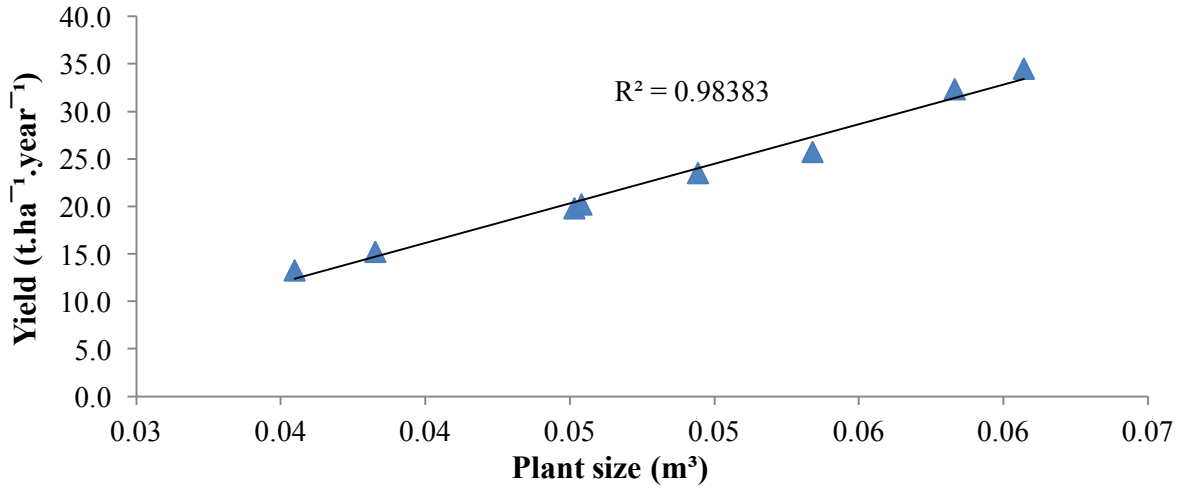



**Figure 1:** The observed relationship between plant size at flowering and banana fruits yield.

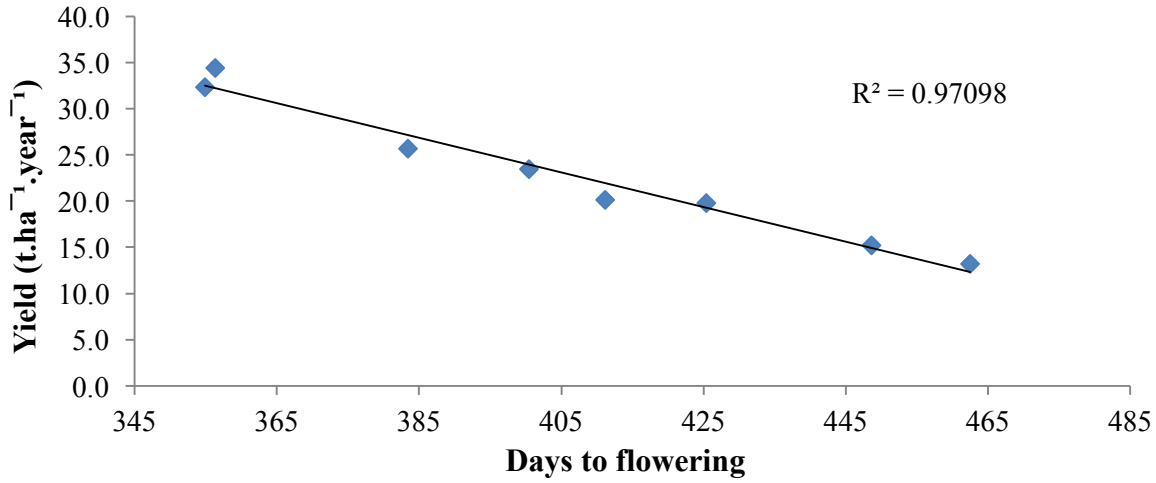



**Figure 2:** The observed relationship between days to flowering and banana fruits yield.

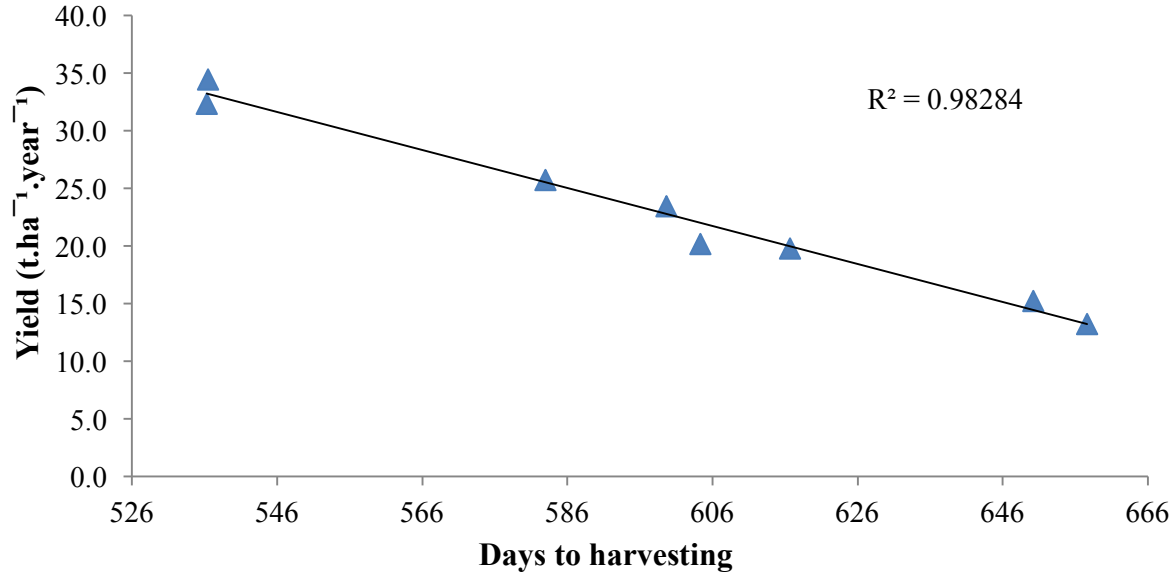



**Figure 3:** The observed relationship between days to crop maturity and banana fruits yield.

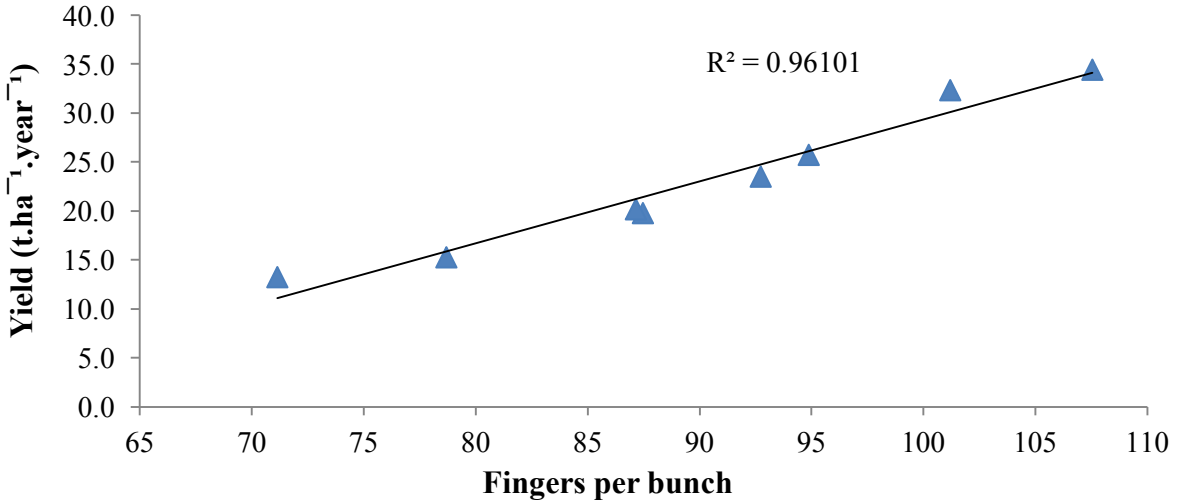



**Figure 4:** The observed relationship between number of fingers per bunch and banana fruits yield.

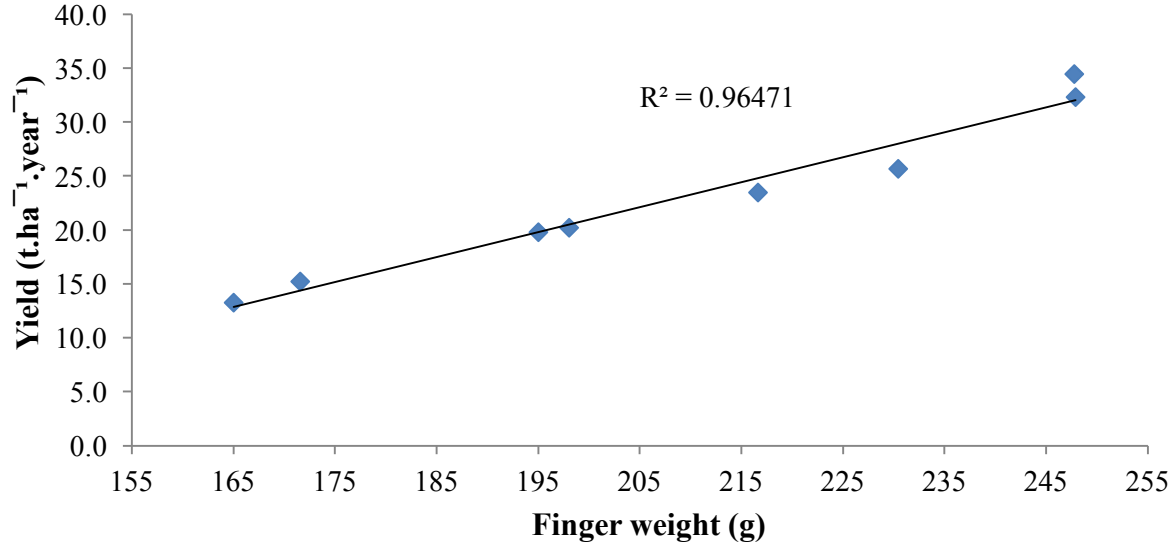



**Figure 5:** The observed relationship between finger weight and banana fruits yield.

### b. Relationship between nutrient uptake by banana plants and total fruits yield

#### Macronutrient uptake by banana plants

Banana plants in the best treatments contained large amounts of N and K (Fig. 6 and 7 respectively). Increased uptake of K in plants system appeared to reduce the uptake of Ca and Mg (Fig. 8). This relationship was suggestive for antagonistic interaction between K and the other 2 nutrient elements. The observed wider K:Mg, K:Ca, K:(Ca+Mg) and N:K ratios (data not presented) explains further that plants in the best treatments contained larger quantities of K. This confirms that banana has larger requirements for K relative to other nutrients. Foliar concentrations of N, P, K, Ca and Mg were compared with the Compositional Nutrient Diagnosis (CND) norms for the East African Highland Bananas (EAHB) developed by Delstanche (2011) to identify foliar nutrient shortage and toxicity. The author established 2.35-2.81, 0.13-0.18, 3.23-4.12, 0.49-0.80 and 0.32-0.45% as sufficiency ranges for N, P, K, Ca and Mg in the critical leaf. To that end it was found that, plants in the best treatments (T5 and T6) contained adequate amounts of N, P, K, Ca and Mg. In addition, N:K ratio in plants grown in the best treatments was found to be 1:1.09. The observed ratio falls well within the proposed N:K ratio of 1:1.0-1:1.1 considered suitable for optimum bunch development (Reuter and Robinson, 1997). As such, high banana fruits yield reflected in number of fingers per bunch and finger weight in the best treatments partly can be linked to the increased uptake of N and K.

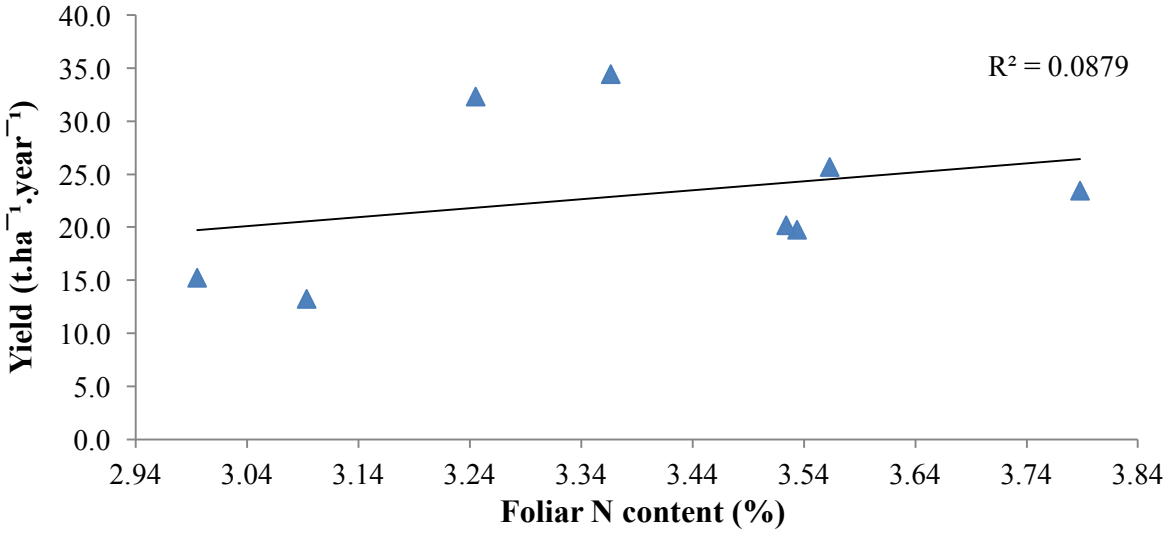



**Figure 6:** The observed relationship between N uptake by banana plants during active growth and total fruits yield.

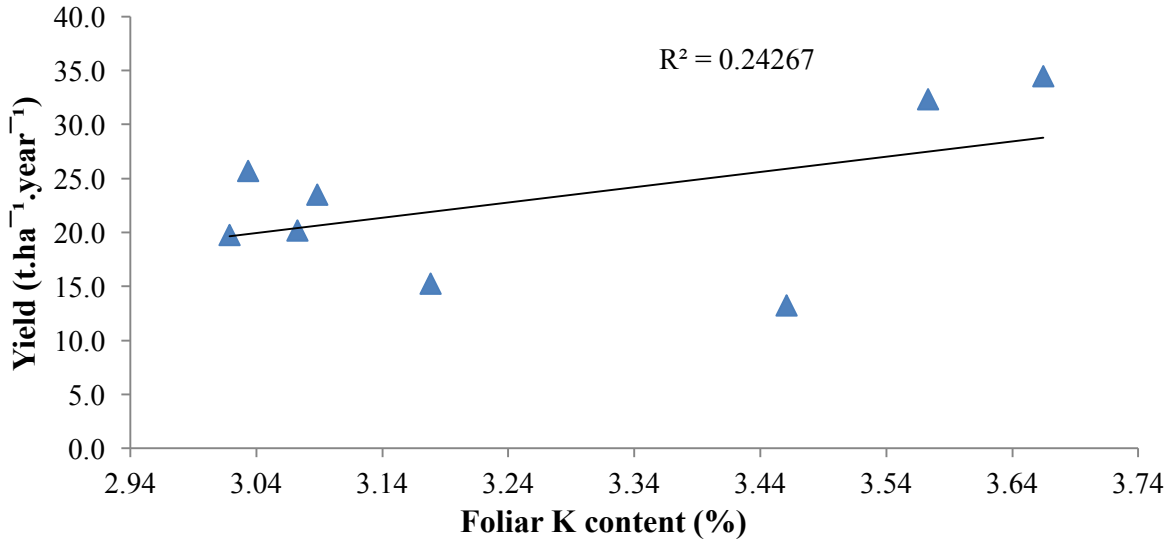



**Figure 7:** The observed relationship between K uptake by banana plants during active growth and total fruits yield.

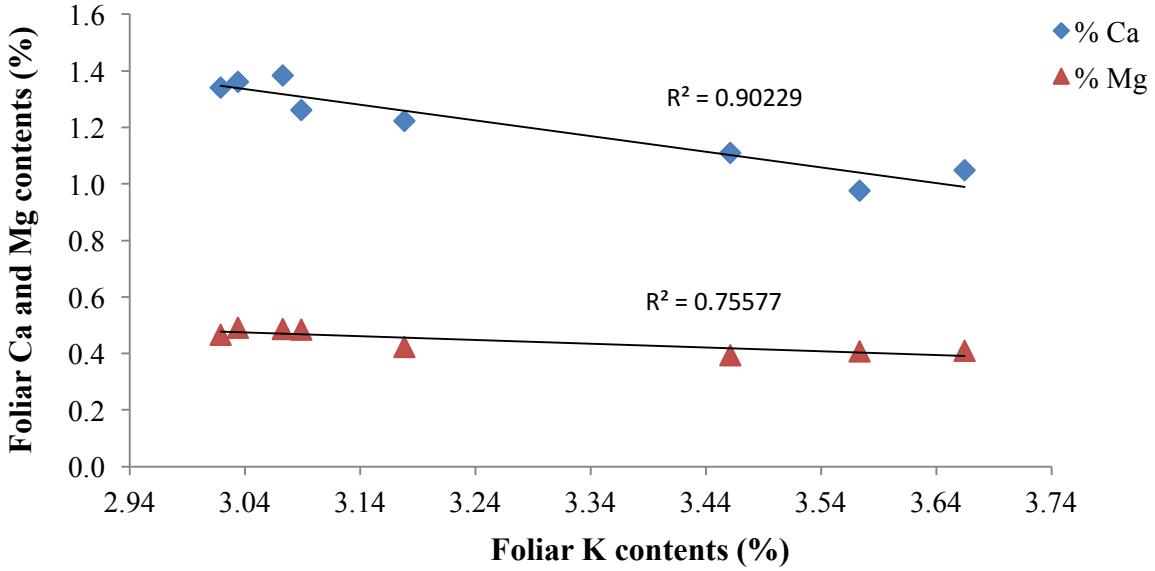



**Figure 8:** The observed relationship between macronutrients uptake by banana plants during active growth and total fruits yield.

## Micronutrients uptake by banana plants

Banana plants grown in the best treatments appeared to contain large amounts of Cu, Zn and Mo (Fig. 9, 10 and 11 respectively). Foliar concentrations were compared with the critical levels published in Reuter and Robinson (1997) to identify nutrient shortage or toxicity. Plants grown in the best treatments were found to contain adequate quantities of Cu, Mn, and Fe but there was a shortage of Zn (across the sites), B (at Lyamungo) and Mo (at Tarakea). Zink seemed to be the most important micronutrient constraint in banana production across the sites followed by B (at Lyamungo) and Mo (at Tarakea). The attained yield in this study partly could have been affected significantly by the shortage of the aforesaid micronutrients. Zinc and B deficiencies have been reported to cause considerable yield reduction in both annual

and perennial crops all over the world. As such, addition of Zn and B in fertilizer programs in deficient areas should be given special consideration.

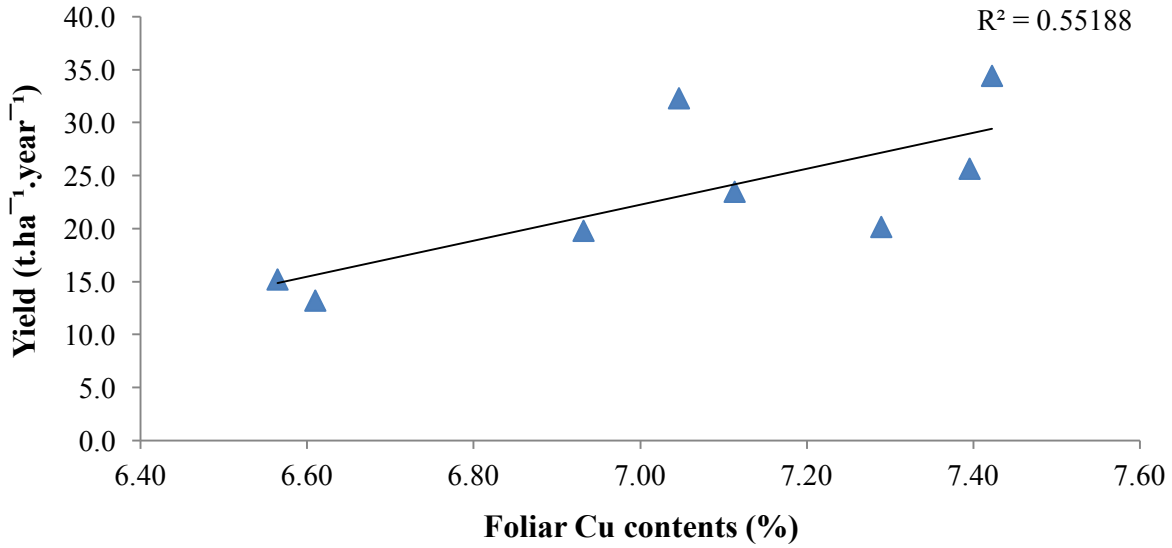



**Figure 9:** The observed relationship between Cu uptake by banana plants during active growth and total fruits yield.

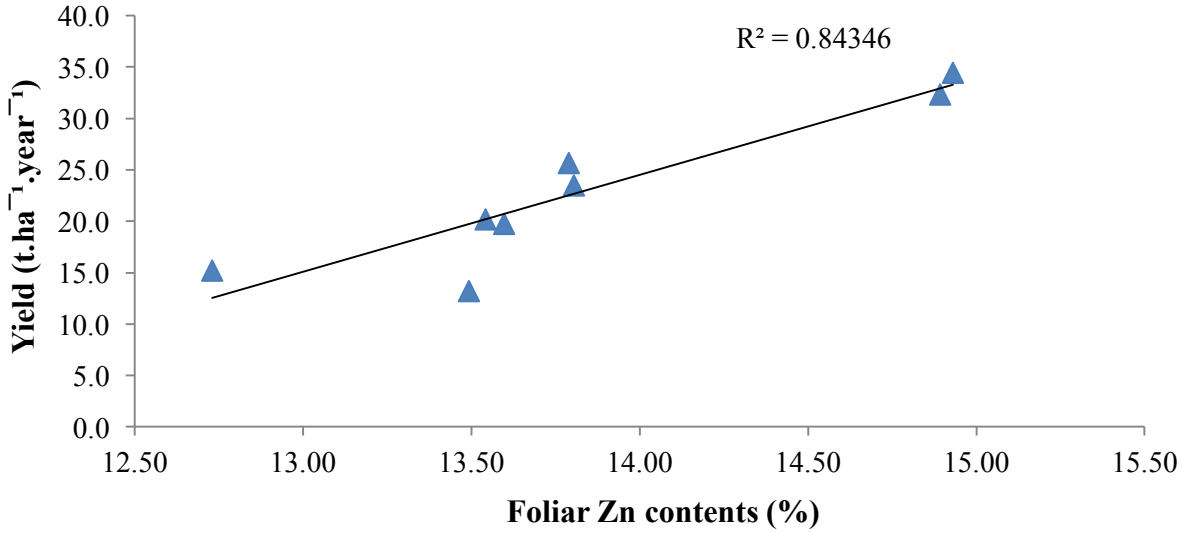



**Figure 10:** The observed relationship between Zn uptake by banana plants during active growth and total fruits yield.

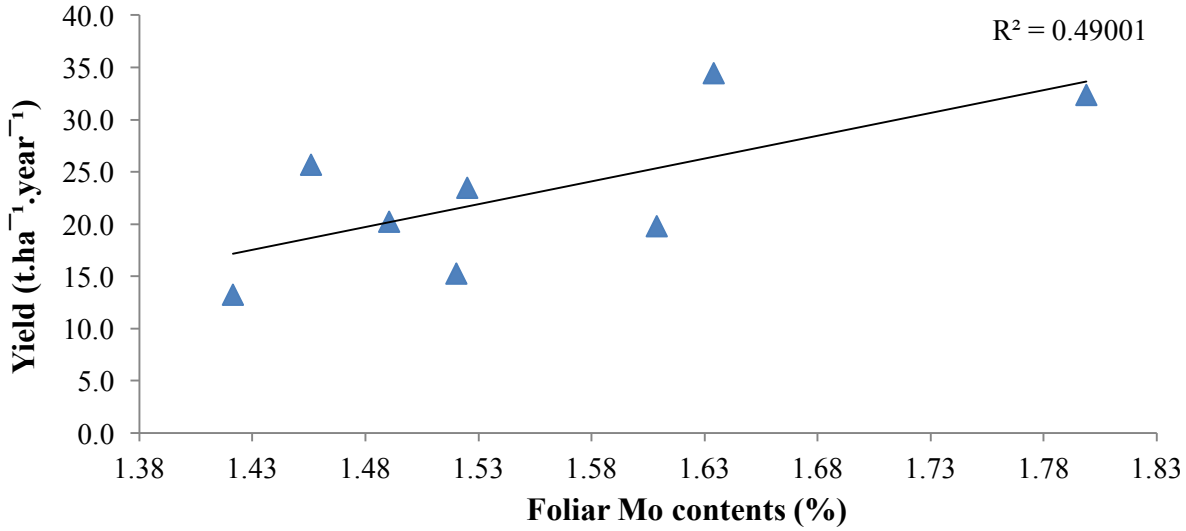



**Figure 11:** The observed relationship between Mo uptake by banana plants during active growth and total fruits yield.

### c. Effects of N fertilizer treatments on banana fruits yield

Nitrogen fertilizer treatments resulted in significant ($p < 0.001$) increases in banana fruits yield across the sites. Applications of sole mineral N at 153.3 kg ha-1.year-1 (T3) increased banana fruits yield up to 40.9 t.ha-1.cycle-1 (25.7 t.ha-1.year-1) higher than those attained in the other mineral N treatments (T1, T2 and T4) (Fig. 12). Substitution of 153.3 kg mineral N ha-1.year-1 by organic N at 50% per each component (T5) significantly increased the yield up to 50.7 ta.ha-1.cycle-1 (34.5 t.ha-1.year-1). The findings of this study demonstrate that high banana fruits yield was recorded in T5 followed by T6. Yet, the performance of these 2 treatments did not differ ($p = 0.05$) significantly based on Tukey test. In addition, the study endeavoured to evaluate the contribution of BN from legume intercrop (T7 and T8) to improve banana production. Treatment 7 produced significantly ($p < 0.001$) higher yield relative to T8. Still, the contribution of BN to improve banana production was negligible. Sole BN (T8) was inferior to all other N fertilizer treatments. Since all banana plants were blanketed with K and P, high yield recorded in T5 can be linked to increased N uptake by banana plants during active growth. On the other hand, increased uptake of K, Cu, Zn and Mo together with improved physical soil properties resulted from decomposing animal manure increased the use efficiency of the applied mineral N fertilizer. The observed initial total soil organic carbon (TOC) and soil N (TN) at Tengeru and Tarakea research farms before the establishment of this experiment was below the proposed minimum critical values of 20 and 2.0 g/kg respectively for most crops (NSS, 1990). This implies that soils in these 2 sites are unable to satisfy crop demand for N. These observations therefore, justify the need for proper N fertilizer amendments to satisfy crop nutrient requirements for optimum growth and yield.

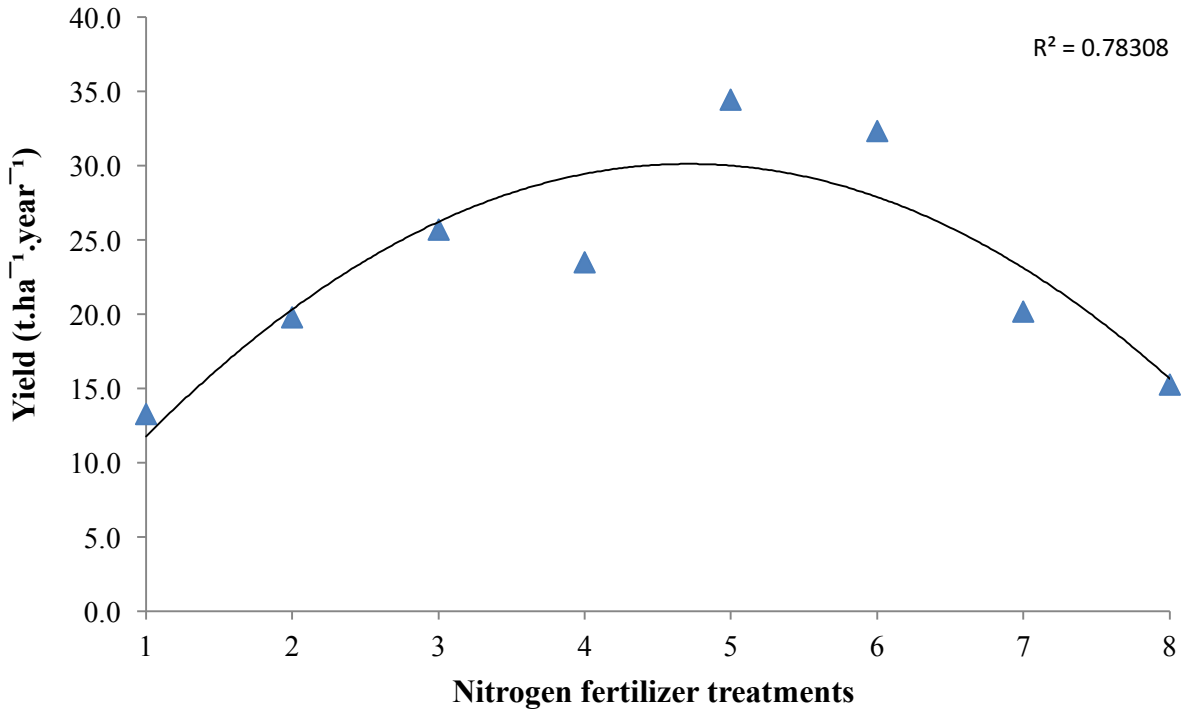



**Figure 12:** The observed effects of N fertilizer treatments on banana fruits yield. Dots in the graph represent the attained yield for each respective treatment (T1-T8).

**Specific objective 4:** To determine the contribution of bean intercrop on improving soil fertility and nutrient use efficiency of banana.

**Activity:** To plant common bean intercrop, quantify the amount of bean residues and evaluate its effects on growth and yield of Mchare bananas.

**Implementation:** The study hypothesized that banana-legume intercropping improves soil fertility and the overall cropping system as such. Instead, the performance of bean intercrop treatments used in this study was relatively inferior to almost all other nitrogen fertilizer treatments. Probably, the amount of biologically generated N was smaller than the fertilizer amounts needed.

**Specific objective 5:** To evaluate the nutrient use efficiency of banana cropping system

**Implementation:** We already have all necessary information for this objective. Will start working on it immediately after submission of our first research article for publication.

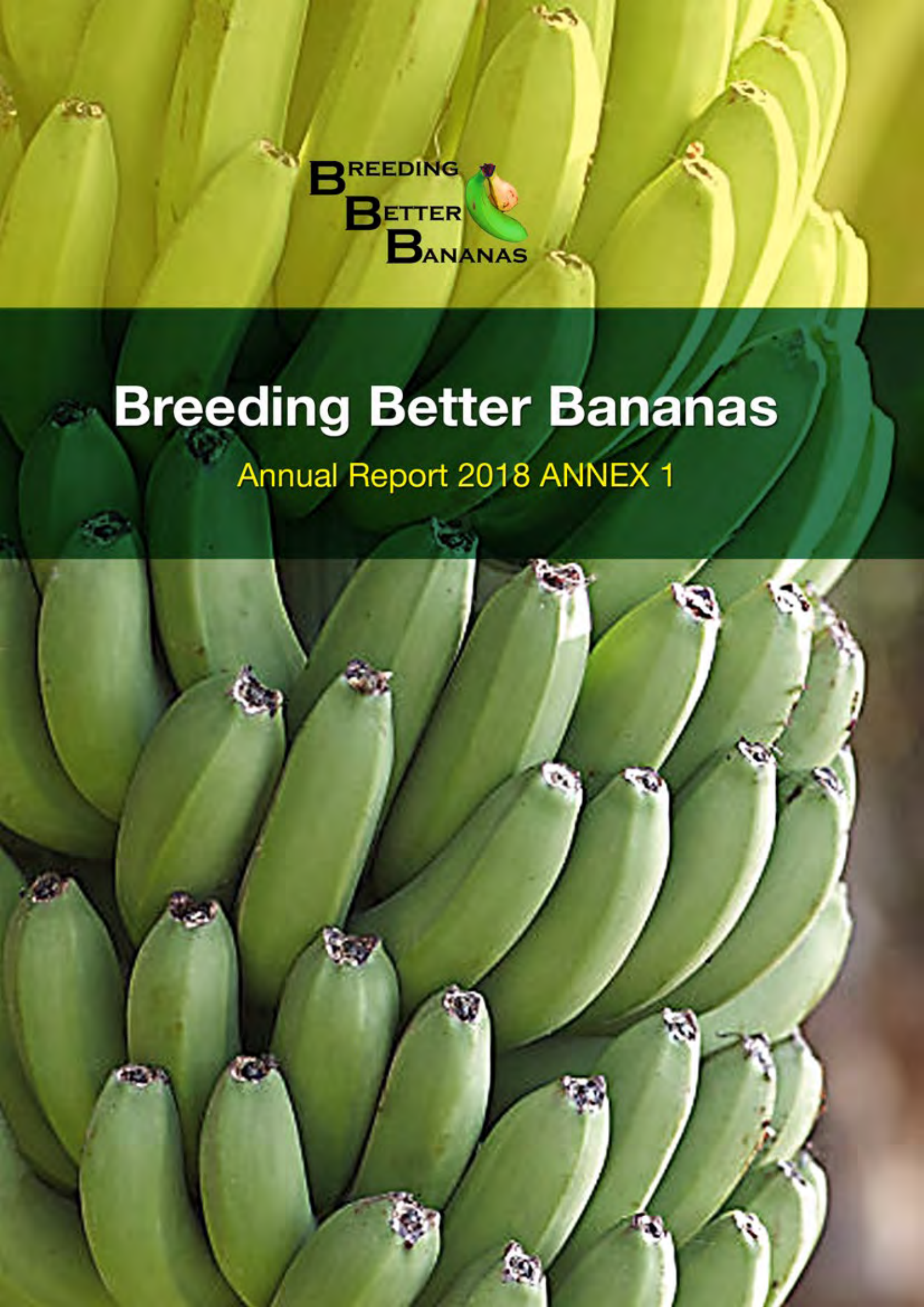
**Specific objective 6:** To quantify nutrient transfer via maize stover transportation from maize production belt to highland areas

**Activity 1:** To conduct soil survey in the maize production belt of the lowlands and to quantify the amount of maize stover transported to the highlands. It is a common practice for smallholder dairy farmers to transport fodders including maize stover from low altitude zone to Mid and Upper altitude zones to feed cattle. In view of the fact that the obtained manure is used in the banana-based farming systems of high altitude zones, a significant nutrient transfer across the landscape occurs at the expense of nutrients from the lowland soils. Therefore, investigation on nutrient transfers as related to the aforesaid practice and devising appropriate crop residue management strategy in the lowlands of the study sites receive special attention in this PhD research.

**Implementation:** Soil and maize stover samples were collected from Hai and Siha Districts in Kilimanjaro Region in September 2017. Samples will be analysed in March-May 2019 in Soil and Water Division Laboratory of the KU Leuven.

**Conclusion / next steps**

- ✓ Conclude the use efficiency of the soil fertility management strategies used in our study
- ✓ Compare the performance of the tested strategies on different sites with varying soil types and contrasting weather conditions.
- ✓ Collect growth and yield data for cycle 2
- ✓ Quantify nutrient export by crop harvest.




# Breeding Better Bananas

## Annual Report 2018 ANNEX 1