**STELLENBOSCH UNIVERSITY PROGRESS REPORT**

**Project Title:** “Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa”

**Reporting Period:** 01 October 2016- 30 March 2017 (Period 5)

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# EXECUTIVE SUMMARY

This report summarizes the progress and outputs achieved by Stellenbosch University (SU) in the 6-month period from 1 October 2016 to 30 March 2017. SU is a member of WP2 and focuses their activities on Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Foc is one of the pests and diseases targeted in the project “Improvement of banana for smallholder farmers in the Great Lakes region of Africa”.

**Results summary**

Outcome 2: Accelerated Matoke and Mshare banana breeding through early identification of material resistant to Fusarium wilt, Sigatoka, nematodes and weevils.

Intermediate outcome 2.1:*Determine the relative importance of Foc,* Mycosphaerella *spp., nematodes and weevil populations as targets for selection breeding in East Africa*

Fifty-six Foc isolates previously collected from Mbeya and Arusha have been characterised to VCG level. In addition, 88 cultures newly collected in Arusha, Mbarara and Kawanda were isolated and characterized. Six VCGs; namely 0124, 0125, 0128, 01212, 01220 and 01222; were present in Tanzania and Uganda. Cultivars affected at the five screening sites by Foc were Pisang Awak, Sukari Ndizi, Mshares, Pisang Kepok, Embu, Safet Velchi and MV1F1.

Intermediate outcome 2.2: *Screen selected EAHB hybrids and Mchare diploids and NARITA hybrids for resistance to Fusarium wilt, Sigatoka, nematodes and weevils and determine influence of plant development, environmental conditions and season on disease severity*

Training in data collection onNARITA hybrids was conducted in September 2016. Data collected thus far indicated that Fusarium wilt development in NARITA hybrids was limited. One of the two samples collected at the Arusha trial site was identified as a Foc Lineage VI member and its VCG identity is being analysed. The second sample was not a *Fusarium* sp. Mshare plants are being multiplied in Arusha and Kawanda to evaluate nine cultivars in the greenhouse and field. The trials will be established in March and April 2017.

Intermediate outcome 2.3: *Rapid and precise screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed for use in breeding programs*

Millet seed inoculated with Foc works best for the screening of banana varieties in the greenhouse and provides a consistent and slow infection of plants compared to dipping and drenching methods. The method will be further assessed on 19 banana varieties that are being multiplied at SU.

Intermediate outcome 2.4: *Train staff in banana disease evaluation, resistance screening and pathogen and pest identification with focus on Fusarium wilt, Sigatoka, nematodes and weevils*

A training manual was developed for field and greenhouse identification of Fusarium wilt and other pest and diseases of banana. This manual was used to train data collectors during a workshop held at NARO, Uganda on 19-23 September 2016.

# PRIMARY OUTCOMES, INTERMEDIATE OUTCOMES, OUTPUTS AND MILESTONES

**Table 1.** Framework and Results Trackerfor Stellenbosch University.

|  | **Primary Outcomes** |  | **Interm Outcomes** |  | **Outputs** | **Targets/ Milestones** | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  | **YEAR 1** | **YEAR 2** | **YEAR 3** | **YEAR 4** | **YEAR 5** |
| **2** | **Accelerated Matoke and Mchare banana breeding through early identification of material resistant to Fusarium wilt, Sigatoka, nematodes and weevils.** | 2.1 | Determine the relative importance of *Fusarium oxysporum* f.sp *cubense*, *Mycosphaerella* spp., nematodes and weevil populations as targets for selection breeding in East Africa | 2.1.1 | Pests and diseases characterised in breeding and testing sites and screening banana cultivars for resistance to Fusarium wilt, Sigatoka, nematodes and weevils | "Assess impact (disease score) for at least 20 plants for each site of target pest and disease species on different banana varieties, across different environments and collect representative samples  'Appoint and train PhD student for *Mycosphaerella* spp and another for Fusarium wilt" | "Determine distribution and impact of target pests and diseases across sites in Tanzania and Uganda  using morphological and molecular diagnostics and pathogenicity/virulence tests " | "Catalogue distribution and impact of pest and pathogens for different banana varieties across different environments of Tanzania and Uganda  Characterise species and populations of target pests and diseases recovered from visits to screening trials on station and at regional testing sites using morphological and molecular diagnostics and pathogenicity/virulence tests " | "Develop GIS maps to show prevalence of pests and pathogen species at breeding and testing sites, their impact on banana varieties being cultivated and links to environmental conditions  Characterise species and populations of target pests and diseases recovered from visits to screening trials on station and at regional testing sites using morphological and molecular diagnostics and pathogenicity/ virulence tests “ | "Develop and analyse database to define the variance of pest and pathogen populations and influence of environment and season, banana variety and plant development  Develop 1 peer reviewed publication " |
|  |  |  |  | 2.1.2 | Collect and preserve Fusarium wilt, Sigatoka, nematodes and weevils from each breeding and testing site for characterization and rapid screening of bananas | "Recover from each breeding and testing sites sample pest and disease isolates and culture into pure stocks for each accession  For each accession preserve using appropriate methods for long term storage" | Establish reference collections at NARO, IITA and SUN and wherever possible replicate accessions across these collections | Recover from each sample collected from screening trials on station and from regional testing trials pest and disease isolates, culture into pure stocks and preserve each accession | Recover from each sample collected from screening trials on station and from regional testing trials pest and disease isolates, culture into pure stocks and preserve each accession | "Establish reference database detailing accessions held in reference collections at NARO, IITA and SUN" |
|  |  |  |  | 2.1.3 | Rapid and accurate methods to determine the identity and fitness of Fusarium wilt, Sigatoka, nematodes and weevils developed and validated | Fully document (characterise and preserve) at least 25 accessions from each target pest and pathogen acquired from surveys of Tanzania and Uganda | Fully document (characterise and preserve) at least 6 accessions from each target pest and pathogen acquired from on station trials and from regional test sites | Fully document (characterise and preserve) at least 6 accessions from each target pest and pathogen acquired from on station trials and from regional test sites | Fully document (characterise and preserve) at least 6 accessions from each target pest and pathogen acquired from on station trials and from regional test sites | Characterise and compare the diversity of populations for each pest and pathogen rom surveys and from on station screening sites and regional testing trials.  Develop peer reviewed publication. |
|  |  |  |  | 2.1.4 | Map the distribution of Fusarium wilt, Sigatoka, nematodes and weevils of banana and using GIS link to climatic and environmental data | Survey and Secondary data used to develop models to account for economic value of pests and diseases in Matoke and Mchare | Selection weights assigned to pests and diseases based on economic value of pests and diseases in bananas | Analyse data catalogued from surveys of Tanzania and Uganda on distribution and impact of pest and pathogens for different banana varieties across different environments Selection Index for pest/disease resistance progressively incorporated into selection of Matoke and Mchare hybrids at PYT | Analyse data catalogued from screening sites on station and from regional test sites on distribution and impact of pest and pathogens for different banana varieties across different environments Selection Index for pest/disease resistance progressively incorporated into selection of Matoke and Mchare hybrids at PYT | "Develop GIS maps to show prevalence of pests and pathogen species at survey sites, their impact on banana varieties being cultivated and links to environmental conditions” |
|  |  | 2.2 | Screen selected EAHB hybrids and Mchare diploids and NARITA hybrids for resistance to Fusarium wilt, Sigatoka, nematodes and weevils and determine influence of plant development, environmental conditions and season on disease severity | 2.2.1 | Map natural populations of Fusarium wilt, Sigatoka, nematodes and weevils on station and at regional field testing sites in Uganda and Tanzania and determine influence of plant development, environmental conditions and season on disease severity | Map distribution and impact (disease score for at least 20 plants per site and banana variety) of target pest and pathogen populations collected from surveys | Map distribution and impact (disease score for at least 20 plants per site and banana variety) of target pest and pathogen populations collected from visits on station and to regional test sites | Map distribution and impact (disease score for at least 20 plants per site and banana variety) of target pest and pathogen populations collected from visits on station and to regional test sites | Map distribution and impact (disease score for at least 20 plants per site and banana variety) and variance of target pest and pathogen populations collected from surveys and from visits on station and to regional test sites | Complete map to show distribution, impact and variance of isolates of target pest and pathogen populations across Tanzania and Uganda for selected banana varieties |
|  |  |  |  | 2.2.2 | Evaluate resistance of EAHB hybrids on station and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils | Trials being prepared | Complete biannual visits on station to assess impact of target pest and disease species on EAHB hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on EAHB hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on EAHB hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete annual visit on station to assess impact of target pest and disease species on EAHB hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations  Catalogue distribution and impact of pest and pathogens for EAHB hybrids on station and determine influence of plant development, environmental conditions and season on disease severity |
|  |  |  |  | 2.2.3 | Evaluate resistance of selected Mchare dipoids on station and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils | Trials being prepared | Complete biannual visits on station to assess impact of target pest and disease species on Mchare diploids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on Mchare diploids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on Mchare diploids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on Mchare diploids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations  Catalogue distribution and impact of pest and pathogens for Mchare diploids on station and determine influence of plant development, environmental conditions and season on disease severity |
|  |  |  |  | 2.2.4 | Evaluate resistance of 27 NARITA hybrids under varied field conditions at 3 regional field test sites and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils | trials being prepared | Complete biannual visits to regional test sites to assess impact of target pest and disease species on 27 NARITA hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits to regional test sites to assess impact of target pest and disease species on 27 NARITA hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits to regional test sites to assess impact of target pest and disease species on 27 NARITA hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits to regional test sites to assess impact of target pest and disease species on 27 NARITA hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations  Catalogue distribution and impact of pest and pathogens for Mchare diploids on station and determine influence of plant development, environmental conditions and season on disease severity |
|  |  | 2.3 | Rapid and precise screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed for use in breeding programs | 2.3.1 | In vitro screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed to increase the speed and number of samples that can be evaluated in breeding programs | "MSc student recruited to characterize and partition resistance to nematodes and weevils in Musa  Existing Biossays for *Fusarium oxysporum* f.sp *cubense*, *Mycosphaerella* spp., nematodes and weevils catalogued Develop test runs to assess survival of banana material in vitro using different methods and to ensure suitability across banana varieties” | "Test suitability of in vitro methods across target pests and pathogens for at least 10 selected banana varieties  Bioassays for *Fusarium oxysporum* f.sp *cubense*, *Mycosphaerella* spp., nematodes and weevils adapted/developed for high throughput screening" | Challenge selected banana varieties with representative populations of target pests and pathogens (from surveys) and document results Factors determining resistance to target pests and diseases in selected cultivars determined (such as stomatal characteristics and epicutular wax for *Mycosphaerella* spp.) | Challenge selected banana varieties with representative populations of target pests and pathogens from surveys and from on station and regional test sites using in vitro methods and compare results. | Analyse data and develop peer reviewed publication through combination with results from challenging whole plants compared to in vitro methods. |
|  |  |  |  | 2.3.2 | Compare the reliability of young plant resistance testing compared to whole plant testing in field for evaluations against Fusarium wilt, Sigatoka, nematodes and weevils | Establish cultivation of plantlets of selected banana varieties | Manage cultivation of plants of selected banana varieties | Challenge whole plants of selected banana varieties with representative populations of target pests and pathogens from surveys | Challenge whole plants of selected banana varieties with representative populations of target pests and pathogens from surveys and from on station and regional test sites | Compare results generated for each selected banana variety using whole plants and within vitro methods in terms of resistance rating, speed and number of varieties that can be assessed |
|  |  | 2.4 | Train staff in banana disease evaluation, resistance screening and pathogen and pest identification with focus on Fusarium wilt, Sigatoka, nematodes and weevils | 2.4.1 | Train staff on survey and culturing of Fusarium wilt, Sigatoka, nematodes and weevils | Some institutions are lacking trained personnel to carryout survey for mapping the occurrence, distribution and abundance of identified key pests and pathogens | Field manuals developed to support the recognition of target pests and diseases and protocols for sample collection | On station training provided in both Tanzania and Uganda during visits | Training provided to staff at regional test sites during visits | Training provided to staff at regional test sites during visits |

# RESULTS TO DATE

**Table 2:** Progress made from 1 October 2016 to 30 March 2017 at Stellenbosch University.

| **Primary Outcomes** |  | **Intermediate Outcomes** |  | **Outputs** | **Targets/ Milestones** | | | **Progress** | **Variance** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | **YEAR 1** | **YEAR 2** | **Year 3** |
| **Accelerated Matoke and Mchare banana breeding through early identification of material resistant to Fusarium wilt, Sigatoka, nematodes and weevils** | 2.1 | Determine the relative importance of *Fusarium oxysporum* f.sp. *cubense*, *Mycosphaerella* spp., nematodes and weevil populations as targets for selection breeding in ECA | 2.1.1 | Pests and diseases characterised in breeding and testing sites and screening banana cultivars for resistance to Fusarium wilt, Sigatoka, nematodes and weevils | Assess impact (disease score) for at least 20 plants for each site of target pest and disease species on different banana varieties, across different environments and collect representative samples  Appoint and train PhD student for Fusarium wilt and *Mycosphaere*lla spp. | Determine distribution and impact of target pests and diseases across sites in Tanzania and Uganda  using morphological and molecular diagnostics and pathogenicity/ virulence tests | Catalogue distribution and impact of pest and pathogens for different banana varieties across different environments of Tanzania and Uganda  Characterise species and populations of target pests and diseases recovered from visits to screening trials on station and at regional testing sites using morphological and molecular diagnostics and pathogenicity/ virulence tests | * Sampling has been completed at the five screening sites. * Characterization of 208 isolates has been completed up to VCG level. * The different varieties being affected by Foc has been catalogued. | 5% variance.  Reason: 26 Foc isolates still need to be VCG characterised. Results should be available in the next reporting period. |
|  |  |  | 2.1.2 | Collect and preserve Fusarium wilt, Sigatoka, nematodes and weevils from each breeding and testing site for characterization and rapid screening of bananas  Stellenbosch univ (Fusarium), Sigatoka (IITA), weevil (NARO), nematodes (ARI-Tengeru) | Recover from each breeding and testing sites sample pest and disease isolates and culture into pure stocks for each accession  For each accession preserve using appropriate methods for long term storage | Establish reference collections at NARO, IITA and SUN and wherever possible replicate accessions across these collections | Recover from each sample collected from screening trials on station and from regional testing trials pest and disease isolates, culture into pure stocks and preserve each accession | * 208 Foc samples collected in Mbeya, Arusha, Mbarara and Kawanda were added to the culture collection. | 0% variance |
|  |  |  | 2.1.3 | Rapid and accurate methods to determine the identity and fitness of Fusarium wilt, Sigatoka, nematodes and weevils developed and validated | Fully document (characterise and preserve) at least 25 accessions from each target pest and pathogen acquired from surveys of Tanzania and Uganda | Fully document (characterise and preserve) at least 6 accessions from each target pest and pathogen acquired from on station trials and from regional test sites | Fully document (characterise and preserve) at least 6 accessions from each target pest and pathogen acquired from on station trials and from regional test sites | * All isolates collected were fully documented. * Markers were developed for the rapid identification of Foc Lineage VI strains. * The markers were used to identify samples collected at the screening sites. * A paper on markers has been prepared for publication. | 0% variance |
|  |  |  | 2.1.4 | Map the distribution of Fusarium wilt, Sigatoka, nematodes and weevils of banana and using GIS link to climatic and environmental data | Survey and secondary data used to develop models to account for economic value of pests and diseases in Matoke and Mchare | Selection weights assigned to pests and diseases based on economic value of pests and diseases in bananas | Analyse data catalogued from surveys of Tanzania and Uganda on distribution and impact of pest and pathogens for different banana varieties across different environments Selection Index for pest/disease resistance progressively incorporated into selection of Matoke and Mchare hybrids at PYT | * All data on the distribution and impact of Fusarium wilt in Uganda and Tanzania has been collected. | 10% variance  Reason for variance:  Mapping will be completed once data of all diseases and pests are available. |
|  | 2.2 | Screen selected EAHB hybrids and Mchare diploids and NARITA hybrids for resistance to Fusarium wilt, Sigatoka, nematodes and weevils and determine influence of plant development, environmental conditions and season on disease severity | 2.2.1 | Map natural populations of Fusarium wilt, Sigatoka, nematodes and weevils on station and at regional field testing sites in Uganda and Tanzania and determine influence of plant development, environmental conditions and season on disease severity | Map distribution and impact (disease score for at least 20 plants per site and banana variety) of target pest and pathogen populations collected from surveys | Map distribution and impact (disease score for at least 20 plants per site and banana variety) of target pest and pathogen populations collected from visits on station and to regional test sites | Map distribution and impact (disease score for at least 20 plants per site and banana variety) of target pest and pathogen populations collected from visits on station and to regional test sites | * Two samples from NARITA plants at Arusha have been received and were identified * The identification of Foc isolates from trial sites will continue for the next 2 years | 0% variance |
|  |  |  | 2.2.2 | Evaluate resistance of EAHB hybrids on station and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils | Trials being prepared | Complete biannual visits on station to assess impact of target pest and disease species on EAHB hybrids Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on EAHB hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | * Not relevant to Fusarium wilt, as it does not cause disease to EAHB. | 0% variance |
|  |  |  | 2.2.3 | Evaluate resistance of selected Mchare diploids on station and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils | Trials being prepared | Complete biannual visits on station to assess impact of target pest and disease species on Mchare diploids. Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits on station to assess impact of target pest and disease species on Mchare diploids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | * Screening and field trials are being established at Kawanda in Uganda and Arusha in Tanzania. | 90 % variance  Reason for variance:  A delay in the multiplication and planting of Mshare diploids. Trials to be established in March and April 2017. |
|  |  |  | 2.2.4 | Evaluate resistance of 27 NARITA hybrids under varied field conditions at 3 regional field test sites and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils | Trials being prepared | Complete biannual visits to regional test sites to assess impact of target pest and disease species on 27 NARITA hybrids Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | Complete biannual visits to regional test sites to assess impact of target pest and disease species on 27 NARITA hybrids  Collect samples of target pests and diseases for preservation in reference collections and to characterise populations | * Data collected from NARITA hybrids started in October 2016. * At this early stage hybrids have not been affected by Fusarium wilt. | 10% variance  Reason for variance:  The first data on Fusarium wilt was made available only in March 2017. |
|  | 2.3 | Rapid and precise screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed for use in breeding programs | 2.3.1 | *In vitro* screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed to increase the speed and number of samples that can be evaluated in breeding programs | MSc student recruited to characterize and partition resistance to nematodes and weevils in Musa  Existing biossays for Foc, Mycosphaerella spp., nematodes and weevils catalogued Develop test runs to assess survival of banana material in vitro using different methods and to ensure suitability across varieties | Test suitability of in vitro methods across target pests and pathogens for at least 10 selected banana varieties  Bioassays for Fusarium oxysporum f.sp cubense, Mycosphaerella spp., nematodes and weevils adapted/developed for high throughput screening. | Challenge selected banana varieties with representative populations of target pests and pathogens (from surveys) and document results  Factors determining resistance to target pests and diseases in selected cultivars determined (such as stomatal characteristics and epicutular wax for *Mycosphaerella* spp.) | * The technique for a small plant screening test has been optimised using four Cavendish selections and strains of Foc STR4. * A study to optimize inoculum levels and inoculation method has been performed, but results will only be available by the end of April 2017. * The greenhouse screening test with Foc race 1 will be performed on 19 banana varieties received from ITC to determine its reliability. * Six phenolic compounds were identified in banana, including gallic acid, caffeic acid, ferulic acid, p-coumaric acid, vanillic acid and sinapic acid, as well as four unknown constitutive and three unknown induced phenolic acids. * All known phenolic compounds suppressed Foc at a concentration of 2.5 mM | 70% variance  Reason for variance:  The 19 cultivars from ITC arrived in March 2017. They will now be multiplied, and it is expected that the trial will be complete by June 2018. |
|  |  |  | 2.3.2 | Compare the reliability of young plant resistance testing compared to whole plant testing in field for evaluations against Fusarium wilt, Sigatoka, nematodes and weevils | Establish cultivation of plantlets of selected banana varieties | Manage cultivation of plants of selected banana varieties | Challenge whole plants of selected banana varieties with representative populations of target pests and pathogens from surveys | * Mshare bananas will be evaluated in greenhouse and field. Results will be correlated to assess the reliability of young plant screening compared to field evaluations. | 80% variance  Reason for variance: Trials will be planted in April and May 2017 |
|  | 2.4 | Train staff in banana disease evaluation, resistance screening and pathogen and pest identification with focus on Fusarium wilt, Sigatoka, nematodes and weevils | 2.4.1 | Train staff on survey and culturing of Fusarium wilt, Sigatoka, nematodes and weevils  Stellenbosch univ (Fusarium), Sigatoka (IITA), weevil (NARO), nematodes (ARI-Tengeru). | Some institutions are lacking trained personnel to carryout survey for mapping the occurrence, distribution and abundance of identified key pests and pathogens | Field manuals developed to support the recognition of target pests and diseases and protocols for sample collection | On station training provided in both Tanzania and Uganda during visits | * A manual has been developed for identification of Foc and other pest and diseases. This has now been published. | 0% variance |

# PROGRESS REPORT

Intermediate outcome 2.1: *Determine the relative importance of Foc,* Mycosphaerella *spp., nematodes and weevil populations as targets for selection breeding in East Africa*

2.1.1: **Pests and diseases characterized in breeding and testing sites, and screening banana cultivars for resistance to Fusarium wilt, Sigatoka, nematodes and weevils**

Determine distribution and impact of target pests and diseases across sites in Tanzania and Uganda using morphological and molecular diagnostics and pathogenicity/virulence tests

Significant progress on the characterization of isolates collected during surveys for Fusarium wilt in Tanzania and Uganda has been achieved in the past 6 months. A total of 56 isolates previously collected in Mbeya and Arusha were characterized to VCG level (Annex 1). Another 88 cultures collected from symptomatic plants at Arusha, Mbarara and Kawanda in August 2016 were isolated and characterized. Of these, 70 isolates were Lineage VI members and 18 were non-members. The 18 isolates were identified as *F. sacchari*, *F.* *oxysporum* and non-*Fusarium* species (Annex 1). Of the 70 Lineage VI isolates, 57 were characterized as VCGs 0124, 0125, 0128, 01222 and complexes thereof, while the VCG identity of 13 isolates still need to be determined (Table 3, Annex 1).

**Table 3:** VCG groups of *Fusarium oxysporum* f. sp. *cubense* of isolates collected from Kawanda, Mbarara and Arusha.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sites | VCG group/Complex | | | | | | |
| **0124** | **01222** | **0124/22** | **0124/8** | **0124/8/22** | **0124/5/8/22** | **Total** |
| Uganda  (Mbarara and Kawanda) | 2 | 1 | 10 | 1 | 11 | 4 | **29** |
| Tanzania  (Arusha) | 2 | 3 | 18 | 0 | 5 | 0 | **28** |
| Total | **4** | **4** | **28** | **1** | **16** | **4** | **57** |

Catalogue distribution and impact of pest and pathogens for different banana varieties across different environments of Tanzania and Uganda

Six VCGs, namely 0124, 0125, 0128, 01212, 01220 and 01222, were found in Uganda and Tanzania (Table 4). Complexes formed among the six VCGs, such as VCG complexes 0124/22, 0124/8/22, 0125/8, 0124/8, 0128/20, 0124/5/8/22, 0125/8/20/22 and 0124/5/20/22. VCG 0124, as well as complexes 0124/22 and 0124/8/22, were found in all five collection sites in Uganda and Tanzania. The complexes 0124/22 and 0124/8/22 were dominant in Mbarara, Kawanda, Kagera and Arusha. VCG 01212 was not found in Uganda, but was most dominant in Mbeya, Tanzania. Fungal isolates other than Foc were isolated from banana in all the screening sites.

Fusarium wilt affected various banana varieties across the five screening sites. Sukari Ndizi and Pisang Awak were affected in all the sites. Mshare bananas, which dominate banana cultivation in Arusha, were also susceptible to Fusarium wilt (Table 4). Other varieties affected by Fusarium wilt include Pisang Kepok, Embu, Safet Velchi and MV1F1. These varieties were found in a banana field in Mbarara and at the breeding site in Arusha. Fusarium wilt was not observed on East African Highland Bananas (EAHB), which is the dominant banana grown in East and Central Africa. The disease was also not observed on Cavendish bananas grown by small scale farmers in mixture with EAHB.

**Table 4:** VCG groups or VCG complexes of Foc identified in the five screening sites and varieties affected.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Variety | | VCG group/Complex | | | | | | | | |
|  |  | | **0124** | **0125** | **0128** | **01212** | **01220** | **01222** | **0124/22** | **0124/5/8/22** | **Total** |
| Kawanda | Pisang Awak | |  |  |  |  |  |  |  | 1 | **1** |
| Sukari Ndizi | | 1 |  |  |  |  |  | 5 | 5 | **11** |
| Mbarara | Safeti Velchi | |  |  |  |  |  |  | 1 |  | **1** |
| Embu | | 1 |  |  |  |  |  |  |  | **1** |
| Sukari Ndizi | |  |  |  |  |  | 1 | 4 | 10 | **15** |
| Arusha | Mshares | | 3 |  |  | 2 |  |  | 15 | 5 | **25** |
| Sukari Ndizi | | 1 |  |  | 1 |  | 2 | 7 |  | **11** |
| Pisang Awak | |  |  |  |  |  | 2 | 9 |  | **11** |
| Pisang Kepok | |  |  |  | 2 |  |  |  | 2 | **4** |
| MV1F1 | |  |  |  |  |  | 1 |  |  | **1** |
| Mbeya | Sukari Ndizi | | 2 |  | 1 | 44 |  |  | 1 | 5 | **53** |
| Pisang Awak | |  |  |  | 8 |  | 1 | 3 | 5 | **17** |
| Kagera | All cultivars | | 11 | 5 | 4 | 6 | 1 | 9 | 13 | 6 | **55** |
| Total | | **19** | | **5** | **5** | **63** | **1** | **16** | **58** | **41** | **206** |
| Percentage |  | | **9.2** | **2.4** | **2.4** | **30.6** | **0.5** | **7.8** | **28.2** | **18.9** | **100.0** |

Characterise species and populations of target pests and diseases recovered from visits to screening trials on station and at regional testing sites using morphological and molecular diagnostics and pathogenicity/virulence tests

Two samples collected at the NARITA trial at Arusha were received in December 2016 and analyzed. The samples were collected from NARITA 9 plants showing Foc symptoms. One isolate (CAV 3972) tested positive for Foc Lineage VI, while the other (CAV 3973) was not a *Fusarium* species. Pathogenicity testing is needed to confirm the ability of isolate CAV 3972 to cause disease to NARITA bananas.

**2.1.2: Collect and preserve Fusarium wilt, Sigatoka, nematodes and weevils from breeding and testing sites for characterization and rapid screening of bananas**

Activity completed. All the 208 fungal isolates described in the section 2.1.1 are stored at -80oC in the collection of University of Stellenbosch’s Department of Plant Pathology (USPP).

**2.1.3: Rapid and accurate methods to determine the identity and fitness of Fusarium wilt, Sigatoka, nematodes and weevils developed and validated**

Activity completed. The development of a rapid and accurate marker has been reported on in March 2016, and an article on this work is in review prior to submission for publication.

**2.1.4: Map the distribution of Fusarium wilt, Sigatoka, nematodes and weevils of banana and using GIS link to climatic and environmental data**

Mapping has not been completed because data from all diseases need to be combined and the relevance of each disease calculated. All GPS coordinates for Fusarium wilt are available (Annex 2).

Intermediate outcome 2.2: *Screen selected EAHB hybrids, Mshare diploids and NARITA hybrids for resistance to Fusarium wilt, Sigatoka, nematodes and weevils and determine influence of plant development, environmental conditions and season on disease severity*

**2.2.1. Map natural populations of Fusarium wilt, Sigatoka, nematodes and weevils on station and field testing sites in Uganda and Tanzania and determine influence of plant development, environmental conditions and season on disease severity**

Two samples from NARITA plants at Arusha have been received and were identified. The work on the isolation and identification of Foc from trial sites will continue for the next 2 years

**2.2.2. Evaluate resistance of EAHB hybrids on station and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils**

EAHB are not susceptible to Foc Lineage VI VCGs present in East and Central Africa.

**2.2.3. Evaluate resistance of selected Mchare diploids on station and determine influence of plant development, environmental conditions and season on disease severity caused by Fusarium wilt, Sigatoka, nematodes and weevils**

Mshare cultivars have been multiplied and hardened off in Arusha and Kawanda, and will be evaluated in screening house and field trials to be established in March and April 2017, respectively. For greenhouse trials, three replications of 10 plantlets each will be used, while five replications of 20 plantlets each will be planted for field trials.

**Table 5**: Mshare and control varieties available for Fusarium wilt evaluation at Kawanda and Arusha.

|  |  |  |  |
| --- | --- | --- | --- |
| Arusha | Plantlets available | Uganda | Plantlets available |
| 1. Makyughu II (ITC.1446) | 115 | 1. Kahuti | 177 |
| 2. Makyughu I (ITC.1454) | 103 | 2. Hutishamba | 120 |
| 3. Mshale Mlelembo (ITC.1455) | 150 | 3. Mshale Laini | 28 |
| 4. Ijihu Inkundu (ITC.1460) | 130 | 4. Muraru | 127 |
| 5. Kahuti (ITC.1468) | 132 | 5. Njuru | 90 |
| 6. Huti green bell (ITC.1559) | 150 | 6. Mlelembo | 156 |
| 7. Huti-white | 113 | 7. Kamunyila | 45 |
| 8. Mshare laini | 84 | 8. Nshonowa | 23 |
| 9. Akondro Mainty (ITC0281) | 12 | 9. Huti | 120 |
| 10. Gros Michel | 80 | 10. Sukari Ndizi | 150 |
| 11. Nshonowa | 50 | 11. Mbwazirume | 87 |

**2.2.4. Evaluate resistance of 27 NARITA hybrids under field conditions at 3 regional testing sites and determine influence of plant development, environmental conditions and season on Fusarium wilt, Sigatoka, nematodes and weevils**

Data on NARITA hybrids are currently being collected at five screening sites in Tanzania and Uganda. Early results do not indicate much Fusarium wilt symptomology.

Intermediate outcome 2.3: *Rapid and precise screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed for use in breeding programs*

**2.3.1. *In vitro* screening methods for Fusarium wilt, Sigatoka, nematodes and weevils developed to increase the speed and number of samples that can be evaluated in breeding programs**

Bioassays for *Fusarium oxysporum* f. sp *cubense* adapted/developed

A technique for small plant screening has been optimised. Four Cavendish selections were inoculated with strains of Foc STR4 while we awaited the arrival of banana cultivars from ITC. Foc TR4 was used because it is endemic in South Africa and could thus be used in our greenhouse facility, and because of its pathogenicity to Cavendish bananas. The three inoculation methods were compared. These 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. The millet seed inoculation method proved to be the best method to screen cultivars for Fusarium wilt in the greenhouse, and caused consistent and slow infections compared to the dipping and drenching methods. Studies to investigate the effect of inoculum level and inoculation method is ongoing using Gros Michel. The drenching method consisted of 50 ml suspensions of 102, 104 and 106 Foc spores/ml poured onto the surface of the potting soil. For the millet seed method 1, 2, 5 and 10 g of inoculated millet seeds were mixed with 1 kg of soil, and 20-30 cm high bananas planted in these infested soils. The combined method consisted of dipping plantlets in 102, 104 and 106 spores/ml for 5 min before replanting in sand infested with 2 g of millet seeds per kg sands. Rhizome discolouration will be used to evaluate disease severity. Results will be available by April 2017.

Challenge selected banana varieties with representative populations of target pests and pathogens (from surveys) and document results

Nineteen banana cultivars have been selected to assess the ability of the small plant screening test to differentiate cultivars’ response to Fusarium wilt (Table 6). These cultivars have been ordered from ITC in 2016, and was delivered in April 2017.

**Table 6:** Banana varieties ordered for resistance screening in the greenhouse against *Fusarium oxysporum* f. sp. *cubense* race 1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **NO** | **Name of cultivar** | **Genome group** | **Subgroup** | **Synonym** | **ITC Reference** |
| 1 | Igisahira Gisanzwe | AAA | EAHB | Inyamunyu | ITC 0083 |
| 2 | Igitsiri | AAA | EAHB | Intuntu, Entundu | ITC0081 |
| 3 | Williams | AAA | Cavendish | - | ITC0570 |
| 4 | Gros Michel | AAA | Gros Michel | - | ITC 484 |
| 5 | Sukari Ndizi | AAB | Apple bananas | Kamaramasenge | ITC127 |
| 6 | Silk | AAB | Apple bananas | True Apple | ITC 348 |
| 7 | Pisank Awak | ABB | Cooking banana | Kayinja, Gisubi | ITC0213 |
| 8 | Bluggoe | ABB | Cooking banana | - | ITC0010 |
| 9 | FHIA 17 | AAAA | Hybrid | - | ITC 1264 |
| 10 | FHIA 01 | AABB | Hybrid | Goldfinger | ITC 0504 |
| 11 | FHIA 18 | AAAB | Hybrid | - | ITC 1319 |
| 12 | Calcutta 4 | AA | Wild type | - | ITC0249 |
| 13 | SH-3460 | AAAB | FHIA Hybrid | - | ITC 1307 |
| 14 | Akondro Mainty | AA | Mchare | - | ITC 0281 |
| 15 | Guyod | AA | Cultivar | - | ITC.0299 |
| 16 | Borneo | AA | Wild type | - | ITC.0253 |
| 17 | Huti Green Bell | AA | Subgroup Mshare | - | ITC1559 |
| 18 | NARITA 17 | AAA | EAHB Hybrid | - | NARO-IITA |
| 19 | NARITA 7 | AAA | EAHB Hybrid | - | NARO-IITA |

Test suitability of *in vitro* methods across target pests and pathogens for at least 10 selected banana varieties

The rapid *in vitro* screening techniques will be used to evaluate at least 10 banana varieties to determine the suitability of these techniques across target pests, using Foc race 1. This work has not yet started.

Factors determining resistance to target pests and diseases in selected cultivars determined (such as stomatal characteristics and epicutular wax for *Mycosphaerella* spp.)

Several studies have shown that phenolic compounds can be used to distinguish banana varieties for resistance to Foc (De Ascensao and Dubery, 2000; 2003; Van den Berg, 2006). A pilot study was, therefore, conducted in greenhouse to investigate the potential use of constitutive and induced phenolics to discriminate plant response among different Cavendish clones. These clones included Williams (susceptible), GCTCV-119 (resistant), Asdia (susceptible) and DPM-25 (intermediate). The fungal strain used as inoculum was Foc subtropical race 4 (CAV 095). Banana roots were collected 0, 8, 20, 32 and 40 hrs after inoculation using a modified version of the method of De Ascensao and Dubery (2000). Total soluble phenolics were quantified using the Folin-Ciocalteau Reagent and expressed as ng of ferulic acid/g fresh weigh. The precipitate was dried and used to extract ester-bound cell wall phenolic acids. Phenolic profiles were identified by HPLC. Provisional results indicated the presence of total soluble phenolics in all four accessions. Six known phenolic compounds were identified, including gallic acid, caffeic acid, ferulic acid, p-coumaric acid, vanillic acid and sinapic acid, as well as four unknown constitutive and three unknown induced phenolic acids.

The ability of vanillic acid, trans-ferulic acid, caffeic acid, p-coumaric acid, sinapic acid and protocatechuic acid to suppress Foc growth *in vitro* was studied. The phenolic compounds were dissolved in 100% ethanol, except for sinapic acid (100% methanol), to a stock concentration of 100 mM and evaluated at three concentrations (0.5 mM, 1.5 mM, and 2.5 mM). For the controls, non-amended PDA was used. Mycelial plugs of 7-day-old Foc cultures were then placed at the centre of the Petri dishes and incubated at 25°C. The diameter of developing colonies was measured on days 2, 4 and 6. The average surface area (𝑆𝐴 =1/4 𝜋 𝐷2) was calculated from five repeats, and the % suppression calculated. Suppression of 100% indicated that the Foc isolate was effectively suppressed, while a negative value indicated that phenolic acid promoted growth of Foc.



**Figure 1**: Mean percentage suppression of *Fusarium oxysporum* f. sp. *cubense* by plant phenolic compounds.

All phenolic compounds were suppressive Foc at a concentration of 2.5 mM, except sinapic acid which promoted fungal growth at 0.5 mM, 1.5 mM and 2.5 mM. Trans-ferulic acid at a concentration of 2.5 mM was most active, and suppressed Foc growth by 51.2% (Figure 1). Protocatechuic acid was relatively suppressive at all three concentrations. Sinapic acid did not show any suppression against *Foc* at all concentrations, and increased mycelial growth (Fig. 1).

**2.3.2. Compare the reliability of young plant resistance testing to whole plant testing in field for evaluation against Fusarium wilt, Sigatoka, nematodes and weevils**

Mshare bananas will be evaluated in the greenhouse and in field in Uganda and Tanzania. Planting will be done in April 2017.

Intermediate outcome 2.4: *Train staff in banana disease evaluation, resistance screening and pathogen and pest identification with focus on Fusarium wilt, Sigatoka, nematodes and weevils*

A training manual was developed for field diagnosis of Foc and other pest and diseases, sample collection and trial evaluations. This manual has been published as a book.

**REFERENCES**

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# CHALLENGES ENCOUNTERED

* The multiplication of banana plantlets was delayed by 2 months at Kawanda and Arusha because plant hardening corresponded with end of year holidays.
* The delivery of 19 banana varieties ordered from ITC was delayed by 6 Months. This has slowed down activities related to the development of a rapid screening technique for Fusarium wilt.
* A lack of communication from laboratory staff about progress on multiplication has caused delays in the establishment of Mshare trials.

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# LESSONS LEARNED

Report regular progress on the multiplication and plants availability for a better planning of activities.

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# WORK PLAN

* Finish the identification and characterisation of Foc at the screening sites by end of 2017.
* Establish Mshare trials in Uganda and Arusha in April and May respectively.
* Multiply bananas in 2017 to be used for rapid screening technique development.

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# OTHER RELEVANT INFORMATION

* Travelling will become more extensive and expensive than originally anticipated to visit trial sites and other related activities such plant multiplication, field planting and data collection,
* EAHB hybrids appear not to be affected by Fusarium wilt. Trials will thus be limited to the screening of NARITA hybrids and Mshare diploids.

# LIST OF ANNEXES

**Annex 1.** Progress of the Foc characterisation of fungal isolates collected from Mbeya, Arusha, Mbarara and Kawanda

**Annex 2.** GPS data for mapping Fusarium incidence in the five screening sites in Tanzania and Uganda