PhD Research Progress Report (2019)

Title: Evaluation of African bananas for resistance to *Fusarium oxysporum* f. sp. *cubense* race 1

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Timeline of study: 2015-2020

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Research Objectives

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

Achievements

1. <u>The PhD dissertation completion</u>
   - The PhD dissertation was submitted for examination in December 2019.
   - It is scheduled be completed by April 2020.
2. <u>Develop molecular markers specific to Foc Lineage VI</u>
   - 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 Breeding Better Bananas Project (BBB).
   - An article has been submitted in July 2018 for publication.
3. <u>Develop a rapid screening method of bananas for resistance to Foc</u>
   - The optimization of the screening method is completed.
   - The results will be published as book chapter.
4. <u>Evaluate Mchare and NARITA for resistance to Foc lineage VI</u>
   - Eight Mchare cultivars were evaluated for Foc race 1 resistance in the field and in screen house.
   - Eighteen NARITA and 60 Matooke hybrids were evaluated for Foc race 1 resistance in screen house.

Background/Introduction

East African Highland Bananas (EAHB) and plantains are the main groups produced in Africa, and make up around 70% of banana production. EAHB dominate cultivation in East and Central Africa (ECA) and consist of cooking and beer bananas. Plantains are mostly produced in the humid forests of West and Central Africa, and are exclusively used for cooking. Other bananas grown in Africa include cooking bananas such Bluggoe and Mchare, and beer/dessert bananas such Gros Michel, Cavendish, Sukari Ndizi, Kisubi, Pisang Awak, Red, Yangambi Km 5 and FHIA hybrids. 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 local varieties grown in the region such Pisang Awak, Sukari Ndizi and Gros Michel are susceptible. Foc race 1 also affects Mchare bananas, cooking bananas grown in some regions in Tanzania and Kenya. Foc race 1 is still spreading throughout the region because of the use of susceptible cultivars.

Fusarium wilt of banana can be effectively managed by planting resistant cultivars. 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 methods to screen local varieties and breeding materials against all Foc forms can speed up the process, but have to reflect field results. The identification of banana varieties that can be planted in small-grower fields, or used in breeding programs to develop Foc race 1-resistant EAHB is needed.

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.

Summary of the study

Banana is a staple food and source of income for millions of smallholder farmers in East and Central Africa (ECA). Consumption per capita in countries such Uganda, Burundi and Rwanda ranges from 120 kg to more than 400 kg per year, which is six to 20 times the global average consumption per capita. Bananas cultivated in ECA consist of cooking varieties, such as East African Highland bananas (EAHB), Bluggoe, and juice/sweet dessert varieties such as Pisang Awak, Sukari Ndizi, Gros Michel and Cavendish bananas. EAHB

include diploid bananas such Mchare, Muraru, Mlali and Paka (mostly cooking types), whereas EAHB triploids include Matooke (a cooking type) and Mbidde (a juice/beer type).

Fusarium wilt of banana, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), is present in most banana-growing regions of ECA. Foc comprises three races based on their pathogenicity to a group of differential cultivars, with Foc race 1, race 2 and race 4 causing disease to Gros Michel, Bluggoe and Cavendish bananas, respectively. All three races are present in Africa, but only Foc races 1 and 2 occur in ECA. Foc races 1 and 2 strains in ECA group consist of six vegetative compatibility groups, which cluster together as Foc Lineage VI. In this study, molecular markers specific to Foc Lineage VI were developed from the DNA-directed RNA polymerase III subunit (RPC2) gene region. The primer set was combined in a multiplex PCR assay with the primer set FocLin6bF/R, which was developed from the translation elongation factor-1 alpha (TEF-1α) gene. The multiplex PCR assay was validated on a worldwide population of 623 known Foc isolates, other *formae speciales* and non-pathogenic isolates of *Fusarium oxysporum*. The multiplex PCR can be used as an accurate diagnostic tool for Foc Lineage VI strains.

Effective management of banana Fusarium wilt can be achieved by planting banana varieties resistant to Foc. Resistant bananas, however, require many years of breeding and field-testing under multiple geographical conditions. Field evaluation is reliable but time consuming and expensive, and not feasible for quarantine strains. Small plant screening methods are, therefore, needed to speed up the evaluation of banana varieties for Foc resistance. To this end, a small plant screening method for resistance to banana Fusarium wilt was optimized by investigating the effect of inoculum concentration, inoculation method and plant age on disease development, and the value of phenolic compounds and Foc DNA as indicators of disease resistance. The method, which consisted of planting 2- to 3-month-old banana plants in soil amended with 2-10 g Foc-colonised millet seeds per kg of potting soil, was reliable, and qPCR and rhizome discoloration were suitable for evaluating and ranking the disease response of banana varieties. Phenolic compounds were, however, not consistent in differentiating cultivars' resistance when the same genotypes were inoculated with Foc race 1 and subtropical race 4 (STR4), and cannot be considered a reliable indicator of resistance. The optimized millet seed technique is useful in mass screening of newly developed genotypes for resistance to Foc, and can be used in the screening for Fusarium wilt resistance against quarantine variants of Foc in quarantine facilities.

EAHB triploid banana cultivars are resistant to Foc race 1 and 2 in ECA, but dessert varieties in the region are susceptible. Resistance of diploid Mchare, Muraru and Mlali bananas, as well as newly developed diploid and triploid EAHB hybrids, is largely unknown.

Therefore, in this study eight Mchare cultivars and 19 NARITA hybrids were evaluated for resistance to Foc race 1 in the field and screen house in Tanzania and in Uganda. Eight Muraru cultivars, 27 Mchare hybrids and 60 Matooke hybrids were also screened in pot trials in screen house. Mchare and Muraru cultivars were all susceptible to Foc race 1, whereas the response of Mchare, NARITA and Matooke hybrids ranged from susceptible to resistance. Triploid hybrids were not expected to be susceptible as their pedigrees were resistant to Foc race 1. This suggest that resistance in banana is multi-gene controlled and heterozygous, and that the genes segregated during meiosis into gamete cells leading to a loss of resistance.

This study generated valuable information towards the management of Fusarium wilt in ECA region. Molecular markers that were developed are reliable and affordable to research centres and extension services in the region, and can speed up the diagnosis of Foc Lineage VI strains. The screening method developed in this study will improve the reliability of small plant testing, and will reduce time and cost associated with field evaluation of new varieties.

Next steps

- Complete the PhD dissertation by addressing the comments from examiners, which are expected at end of January 2020.
- Finalize the publications and the book chapter by 2020.