PhD Research Progress Report

TITLE: Distribution and genetic diversity of Pseudocercospora spp. associated with banana Sigatoka diseases in East Africa

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Timeline of study: 2016-2019

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Research Objectives

1. To identify species associated with Sigatoka diseases in Uganda and Tanzania

2. Map the distribution of Sigatoka pathogens and severity in different agro-ecologies in Uganda and Tanzania

3. To determine the genetic diversity, population structure and mating type distribution of *Pseudocerscospora fijiensis* isolates from Uganda and Taanzania

4. Identify natural sources of Sigatoka resistance and evaluate NARITAs hybrids response to Sigatoka under multiple environments.

Achievements

➢ Results from objective one and two have been published as,
"Distribution of Pseudocercospora species causing Sigatoka leaf diseases of banana in Uganda and Tanzania" in Plant pathology journal

➢ Final thesis compilation ongoing.

Background

Banana and plantain are important staple crops in the African Great Lakes region where millions depend on the crop for food and income. Yields in this region are however low due

to biotic and abiotic constraints. Sigatoka leaf spots lead to yield losses >50%. Black Sigatoka is particularly a greater concern due to its widespread nature and by being more aggressive. Management through use of fungicides is impractical for smallholder farmers in the region thus improvement of locally adapted cultivars would greatly benefit farmers. Developing durable resistance is dependent on availability of resistance sources and an understanding of pathogen distribution and population structure. The current distribution and severity of Sigatoka pathogens is unknown. Furthermore, the genetic diversity and population structure maintained by the predominant pathogen in the region is not well characterised. Additionally, response of improved EAHB hybrids (NARITA) to Sigatoka under different environments need to be determined to reveal presence of genotype x environment interactions.

> **Summary of the study**

- Higher disease severity was recorded at mid altitudes 1400-1600 m asl; in Uganda and indication these areas have become conducive for disease.
- Species associated with Sigatoka leaf disease were effectively identified using polymerase chain reaction (PCR). *P. fijiensis* is the most prevalent pathogen in the region and was detected up to 1877 m asl signifying an expansion in pathogen habitat into higher altitudes. *P. fijiensis* adaptation to high altitude areas is attributed to a changing climate where the high altitudes have become warmer and more conducive for the pathogen. *P. fijiensis* being the more aggressive pathogen has effectively displaced *Pseudocercospora musae,* causal pathogen for yellow Sigatoka that was previously associated with the high altitudes. These areas may also have become too warm for survival of *P. musae*. *P. eumusae* causing eumusae leaf spot was not detected in the study areas. Continuous monitoring is required to ensure an incursion is detected early enough.
- Presence of *P. fijiensis* in Kilimanjaro and Arusha (Tengeru) appears to be highly influenced by prevailing environmental conditions; and management practices that involve intense leaf cutting for fodder. This may explain failure to detect the pathogen during the survey. However, subsequent monitoring the pathogen has been recovered and confirmed from Tengeru and Machame areas. However, *Mycosphaerella musae* that causes mycosphaerella speckle was detected in Kilimanjaro region.
- *Parapallidocercospora colombiensis* and *P. thailandica* pathogens of eucalyptus and acacia respectively were detected on banana leaves in Mbeya and Kagera Tanzania probably co-occurring with *P. fijiensis*. Pathogenic significance of these pathogens is unknown and

require to be investigated. This is the first report of these species in banana from Eastern Africa

- A pathogen collection of 369 single spore isolates from seven sites in Uganda and Tanzania has been established and confirmed as *P. fijiensis*. These have been transferred to long term storage on glycerol in Kawanda, Dar-es-Salaam and Arusha labs.

- All the isolates were characterised for mating type idiomorphs using mating type specific primers. both mating types were present in isolates collected in all regions except Mbeya and Bukoba. The frequency of MAT1-1 was 49.1%, MAT1-2 was 36.1. The MAT gene distribution in clone-corrected data was 54% for MAT1-1 and 46% for MAT1-2 in Uganda, while in Tanzania the frequency was 81% MAT1-1 and 19% for MAT1-2. Distribution of MAT idiomorphs conformed to 1:1 hypothesis in Uganda, revealing possibility of sexual reproduction and emergence of new pathogen types. An exception to the 1:1 distribution was however recorded in Mbeya where all isolates were MAT 1 and in Arusha and Sendusu where MAT 2 idiomorph was under represented. This may have been because of sampling strategy where one genotype was repeatedly sampled, or the population originated from a single introduction event. The other possible explanation is that one mating type is more virulent hence capable of proliferating at the expense of the other.

- Further genetic characterisation using simple sequence repeat markers (SSR) was conducted on 319 isolates. A total of 270 multi-locus genotypes (MLGs) were recovered and only six were shared between sub-populations. The populations had a moderate level of genetic diversity (Hexp = 0.12 – 0.31; mean 0.29). Index of association tests showed that all the populations were at linkage equilibrium (I_A and rBarD P > 0.05) consistent with random sexual reproduction. These findings confirmed that both sexual and asexual reproduction are occurring and important in *P. fijiensis* life cycle, and this has important implications in using host resistance to manage the diseases. *P. fijiensis* populations under study revealed poor regional differentiation with 90% of the variability occurring within individuals in a population. This suggests that isolates with different virulence profiles are likely to exist within a population. Requiring the use of different isolates representing different pathotypes should be used to screen potential hybrids and identify the hybrids resistant to most pathotypes.

- To evaluate the response of NARITA hybrids (the first improved East Africa highland bananas) to black Sigatoka and examine the role of genotype x environment interaction, these hybrids were established in five sites, three in Tanzania and two in Uganda.

- Results indicate that the sites differ in disease severity with Kawanda having more disease pressure (AUDPC 181.9) while TACRI had the lowest disease pressure. The environment has the greatest influence on cultivar response to Sigatoka contributing 53.1% of the observed variation of response to Sigatoka while 7.9% of the variation was attributed to the

genotype x environment interaction. This revealed possible differences in *P. fijiensis* populations and virulence in the different environments.

- Most of the hybrids performed significantly better than the local EAHB check (Mbwazirume) meaning that conventional improvement of land races by introgression of Sigatoka resistance from Calcutta 4 a wild diploid confers Sigatoka resistance. The best performing NARITA hybrids across the sites are NARITA 2, NARITA 4, NARITA 7, NARITA 8, NARITA 14, NARITA 21, NARITA 22 and NARITA 23 while NARITA 10 and NARITA 18 were susceptible.

- Mbarara was identified as the ideal site for screening banana for response to Sigatoka because it could discriminate the different hybrids and was representative. This means that materials selected in Mbarara will perform similarly in other locations thus reducing the need for multi-location screening which is expensive.

- To identify additional potential sources of resistance to *P. fijiensis*, 95 banana accessions were evaluated for resistance to *P. fijiensis* under field conditions in Sendusu, Uganda. These accessions were from *Musa acuminata* (41 diploids, 24 triploids, four tetraploids, seven improved diploids *and M. balbisiana* (4 diploids, 14 triploids and one tetraploid*). Three groups of bananas were identified: Those highly resistant to *P. fijiensis* were less affected by Sigatoka and symptom development stopped at early lesion development (Stages 2); partially resistant accessions that allowed the pathogen to sporulate but with a lower disease severity score and susceptible accessions where most leaves were necrotic with high disease severity. The largest number of resistant accessions belong to *Musa acuminata* ssp*. malacensis*, *M. acuminata* ssp*. zebrina* and *M. acuminata* ssp*. burmannica*. Twenty-nine accessions showed either high or partial resistance to *P. fijiensis*. In addition to Calcutta 4, Long tavoy, Pahang, Pisang KRA, 0074 Malaccencis, M.A Truncata, Tani and Balbisiana symptom development stopped at early lesion stage (Stage 2). These accessions are potential sources of resistance to *P. fijiensis*.

Conclusion / next steps

This study has revealed that *P. fijiensis* is the most predominate species causing Sigatoka leaf spot. The pathogen also shows high evolutionary potential thus the pathogen can easily adapt and overcome developed resistance, thus disease control strategies designed need to be durable for example by having different resistance genes in one cultivar. This study has also shown the success of conventional breeding in developing Sigatoka resistant banana cultivars. It is however important to characterize the pathogenic profiles of genetically diverse isolates and understand natural resistance mechanisms observed on different cultivars.