SUMMARY – Patrick Karangwa

Bananas (*Musa* spp.) are among the most important crops in east and central Africa (ECA), where they are a major staple and constitute the main source of income for millions of livelihoods. Yet, banana yields in this region are among the lowest in the world due to a wide range of abiotic, biotic and socio-economic causes. Amongst biotic causes, banana Fusarium wilt, a vascular wilt caused by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc) is a major threat to investments in commercial production of bananas for export and/or post-harvest processing, which have the potential to uplift millions of resource-poor livelihoods in the region.

Banana Fusarium wilt is notoriously difficult to control. The use of resistant cultivars is the most effective option, but the breeding of banana is severely hampered by the crop's low levels of fertility, high levels of heterozygosity and a triploid genome in most cultivars. An integrated disease management approach involving the breeding of resistant cultivars and preventive measures, such as the use of disease-free planting materials in disease-free soils and rigorous quarantine regulations, could significantly contribute to banana Fusarium wilt management, but would necessitate the knowledge of the pathogen's variability and the capacity for its accurate and early detection. Therefore, in the current study, the distribution of banana Fusarium wilt and the diversity and population structure of Foc in five ECA countries (Rwanda, Burundi, Uganda, Tanzania and the Democratic Republic of Congo-DRC) were investigated, and a molecular diagnostic for specific detection of Foc VCGs found in this region was successfully developed.

The distribution and incidence of banana Fusarium wilt in ECA were investigated as related to cropping systems, edapho-climatic and socio-economic factors. Banana Fusarium wilt incidence was found significantly lower in cultivar mixtures consisting of Foc-susceptible cultivars and Foc-resistant east African Highland bananas (EAHB, AAA genome) than in monocultures ($p < 0.01$). In addition disease incidence was found significantly lower at higher altitudes (>1600 masl) and high at lower altitudes, suggesting that high temperatures encourage the development of Fusarium wilt caused by Foc race 1. This study reports for the first time the occurrence of Foc race 2 in Rwanda and Burundi, and suggests that strategies for banana Fusarium wilt management in ECA should include raising farmers' awareness on pathogen spread and enhancing their access to disease-free planting materials.

The diversity of Foc in ECA was investigated using vegetative compatibility group (VCG) analysis, PCR-RFLPs of the ribosomal DNA's intergenic spacer region, as well as phylogenetic analysis of the elongation factor-1α gene. Ninety percent of the strains in the region were found to belong to one lineage (Foc VI), which includes VCGs 0124, 0125,

0128, 01212, 01220 and 01222. The distribution of Foc VCGs was relatively equivalent among countries, but biased within countries to the predominance of VCGs 0124 and 01222, which represented 71% of all tested isolates. VCGs 0128 and 01220 were reported for the first time in the five countries studied, while VCG 01212 was first reported in Rwanda and the DRC. Additionally, isolates which did not belong to any known VCGs were found in Tanzania and Rwanda, and may represent novel VCGs. The presence of Foc strains in ECA that mostly belong to a single lineage (Foc Lineage VI) implies that effective disease management strategies could be developed by targeting the genetic variation shared exclusively by members of this lineage.

Fungal vegetative compatibility analysis constitutes a very useful strain identification tool, but a rudimentary marker for population structure investigations. Further investigations into the amount and the distribution of genetic variation of Foc within and between ECA countries were conducted with microsatellite markers developed in the current study. Analysis of molecular variance (AMOVA) indicated a low level of differentiation between the five populations studied (Φ_{st} = 0.032, p<0.001), and a high level of gene flow within the region (Nm = 14.935). The low level of population differentiation was consistent with VCG analyses, which found a relatively equivalent Foc distribution among countries. The high level of gene flow was consistent with findings on the distribution of banana Fusarium wilt in ECA, which indicated an extensive exchange of planting materials across the region. Although population differentiation of Foc was found to be low in the region, genetic diversity estimates indicated a higher Foc genetic diversity in Ugandan and Tanzanian populations, and a correlation between geographic distance and genetic diversity. This suggests that Foc entered the region through Tanzania or Uganda, from where it was subsequently disseminated in the region through exchange of infected planting materials. This inference was consistent with VCG analyses which found a higher diversity in Tanzania and the history of banana Fusarium wilt in ECA, which indicates an east-western spread. The current study investigated the mode of reproduction and the likelihood of occurrence of genetic recombination in Foc ECA population, and indicated high levels of linkage disequilibrium and allelic associations which were consistent with an asexual reproduction mode (I_A = 2.596; p <0.001), but could not reject the hypothesis of random association of alleles for the Burundian population (I_A= 0.0367; p = 0.366). Results from this study suggested that Foc has a high epidemiological risk potential in the region, and disease management strategies should include restriction of the movement of infected planting materials to limit gene flow and growing cultivar mixtures to lower epidemiological risk.

A molecular marker for the detection of Foc Lineage VI was successfully developed from single nucleotide polymorphisms (SNP) in the translation elongation factor 1α DNA gene which distinguishes it from Foc isolates representative of other Foc Lineages. Primers

developed for Foc Lineage VI were designed and tested on a collection of 113 *F. oxysporum* isolates including representative of all known 24 Foc VCG from diverse geographic areas, as well as other *formae speciales* and non-pathogenic *F. oxysporum* isolates from the soil. The molecular diagnostic marker FocLin6bF/R developed in this study consistently exhibited specificity to the VCGs in Lineage VI, and could detect Foc DNA as low as 0.1 ng/µl in presence of 50 ng/µl banana DNA. This diagnostic could significantly contribute to the management efforts of banana Fusarium wilt in ECA and elsewhere where Foc races 1 and 2 occur by enabling accurate and rapid diagnosis. It could also be used with existing diagnostic markers with specificity to Foc tropical race 4 to curtail Panama disease spread worldwide.

The current study provided valuable insights into the distributions of banana Fusarium wilt in Africa and the distribution of Foc genetic variation in the region, which could significantly assist efforts to manage the disease in the region. In light of results from this study, an integrated banana Fusarium wilt management strategy is needed, and should include measures to restrict the movement of planting materials, which constitute the major Foc gene flow vector. The molecular diagnostic developed in this study will be valuable to research centres and extension services in the region, to assist with early and rapid diagnosis of Foc lineage VI, to which Foc strains in the region belong. Additionally, microsatellite markers developed in the current study will be valuable to Foc researchers worldwide, given the high level polymorphism they exhibited on a population of highly related strains.