SUMMARY – Glaudina Mostert

Banana is considered one of the most important fruit crops in the world, and serves as a staple food to approximately 400 million people worldwide. Cultivated bananas are divided into two types: dessert and cooking bananas. Both types, however, are seedless and therefore clonal in nature, enhancing their vulnerability to attack by diseases and pests. One of the most significant diseases of banana is Fusarium wilt (Panama disease), caused by a soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). Foc is believed to have co-evolved with its host in Southeast Asia, and from there was spread in planting material to banana-growing regions around the world. Foc consists of three races, of which Foc race 1 attacks Gros Michel, Silk and other dessert bananas, Foc race 2 Bluggoe and other cooking bananas, and Foc race 4 Cavendish and Foc race1- and 2-susceptible bananas. The fungus is further divided into eight to ten clonal lineages dependent on the technique used for identification, and 24 vegetative compatibility groups (VCGs). Most of the lineages and VCGs are found in Asia, where a great diversity of bananas is grown. However, it is the presence of Foc 'tropical' race (TR) 4 in some Asian countries which is of particular concern. The objectives of this thesis, therefore, were to assess the diversity and distribution of Foc in Asia, to identify new Foc VCGs found in the continent, and to use next generation sequencing (NGS) to discover single nucleotide polymorphisms (SNPs) for development of putative markers for Foc VCGs.

Vegetative compatibility has been used extensively to analyse fungal diversity and investigate Foc spread internationally. The technique is based on the generation of nitrate non-utilizing auxotrophic (*nit*) mutants for Foc isolates on toxic chlorate medium and to then determine their phenotypic diversity by measuring complementation patterns among the mutants. A collection of 537 Foc isolates from nine Asian countries, representing all banana-producing regions and cultivar diversity in each country, were identified by VCG analysis in this study. Two VCG complexes dominated the Asian population of Foc; VCG 0124/5 in the Indian subcontinent, Vietnam, Cambodia and Thailand; and VCG 01213/16 found in Cavendish producing countries such as Indonesia, Malaysia, The Philippines, mainland China and Taiwan. Two VCGs were reported from Asia for the first time; VCG 01221 in Cambodia and Vietnam and VCG 01222 in Bangladesh, Cambodia, India and Vietnam. VCG diversity of Foc in Bangladesh, Cambodia and Vietnam has been reported for the first time.

Knowledge generated in this study could be used for the geographical employment of resistant banana varieties for Fusarium wilt control.

Several Foc isolates did not pair with existing VCG tester strains were found in Asia. When paired with each other, five new VCGs and eight single member VCGs (SMVs) were found. Isolates within these VCGs and SMVs were then tested for pathogenicity to banana plantlets, along with several unidentified Foc genotypes described in an earlier study. All the new VCGs, SMVs and unknown genotypes proved to be pathogenic, confirming their status as Foc members. A few of the genotypes, however, paired with known VCG tester strains. Multi-gene sequencing of new VCGs, SMVs and genotypes showed that they were mostly related to existing VCGs, with the exception of one genotype from Mexico. These results indicate that Foc in Asia is more diverse than previously anticipated and that new VCGs might still be found or evolve from existing ones.

The assignment of isolates to VCG is a time-consuming exercise, which unnecessarily delays the identification of Foc strains associated with new outbreaks of banana Fusarium wilt. Un Until recently, DNA technologies proved insufficient for the development of molecular markers that could distinguish the different Foc VCGs. However, NGS provides an opportunity to address such shortcomings by means of genome-wide SNP identification. Therefore, the genomes of Foc isolates representing all VCGs were compared by Illumina sequencing, and SNPs unique to each isolate identified. Primer sets have been developed, based on the presence of these SNPs, for future testing of the putative molecular markers to identify individual VCGs. The availability of molecular markers for each VCG would significantly reduce the time, effort and cost associated with Foc identification.