SUMMARY – Noelani van den Berg

Fusarium wilt of banana has a long and devastating history in many of the world's banana producing countries. The most pronounced damage caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*), the Fusarium wilt pathogen, occurred during the 20th century in Central America, where tens of thousands of virgin forests were lost to further banana production. No control strategy is effective against Fusarium wilt other than replacement of susceptible by resistant varieties. It is, therefore, important to develop or identify resistant replacements that would not only be able to resist the pathogen, but also be acceptable to consumers.

Resistance in wild banana varieties has been identified, and hybrids have been developed by breeding programmes with good resistance to Fusarium wilt. These varieties, unfortunately, appear not to be acceptable replacements for Cavendish bananas, the sweet desert banana variety that serves as the primary export banana and constitutes almost 40% of all bananas planted in the world today. A field selection, GCTCV-218, now proved to be the Cavendish plant with the most resistance to *Foc* 'tropical' race 4 (VCG 0121) has saved the Cavendish-based banana industry in Taiwan from devastation. In this thesis, GCTCV-218 has been evaluated against *Foc* 'subtropical' race 4 (VCG 0120), the primary variant of the pathogen in subtropical banana-producing countries such as South Africa, Australia and the Canary Islands. Defence-associated genes that are differentially expressed and that were up-regulated early in the defence response against the pathogen were isolated and identified.

Greenhouse and field trials conducted at the research facilities of the Forestry and Agricultural Biotechnology Institute, University of Pretoria and in Kiepersol, South Africa, respectively, showed that GCTCV-218 had a significantly higher level of disease tolerance against *Foc* 'subtropical' race 4 (VCG 0120) when compared to the commercially grown Williams cultivar. Phenolic assays revealed that total phenolics and cell-wall bound phenolics were expressed at higher levels in GCTCV-218 after pathogen attack and seemed to play an important role in the tolerance of GCTCV-218. It was, therefore, proposed that GCTCV-218 could be considered a replacement for other Cavendish banana varieties planted in South Africa.

The genetic basis of defence mechanisms in banana to *Foc* is unknown. In this investigation, Suppression Subtractive Hybridisation (SSH) was used to construct a cDNA library, containing banana genes that were up-regulated early (3 & 6 hours after infection), in the GCTCV-218/*Foc* interaction. The efficiency of the procedure was confirmed by PCR

amplification of a known defence gene (endochitinase) present in the subtracted tester material, as well as analysing the reduction of a known housekeeping gene, actin, in the subtracted material compared to unsubtracted material. Southern blot data further provided confidence in the subtraction process. A cDNA library containing 736 gene fragments was constructed and then subjected to a screening procedure to remove false positives that escaped the subtraction process.

The screening of a banana cDNA library for defence-related genes involved the development of a high-throughput cDNA microarray technique. This novel technique removed all false positives, such as housekeeping genes that escaped the subtraction as well as clones representing rDNAs. Seventy-nine genes differentially expressed in GCTCV-218 and not in Williams were selected, sequenced and subjected to BLASTX, BLASTN and DBest searches. Of these, several gene fragments showed homology to defence-associated genes, and 20 unique genes fragments were identified. These include two different peroxidases, response regulator 6, catalase 2, metallothionein, pectin acetylesterase (PAE), two different unknown proteins, salt stress, trypsin inhibitor, unspecific monooxygenase cytochrome P450, Bowman Birk proteinase inhibitor, root control, xylanase inhibitor, inhibitor CII, hypothetical protein, putative senescence-associated protein, pathogenesis-related protein 1 (*PR1*) and ribosomal protein S3a.

The significance of the defence reaction to Fusarium wilt diseases in agricultural crops depends on the tempo of plant response. When a host plant is able to respond early to pathogen invasion the pathogen is successfully contained, preventing further spread throughout the plant. The expression of genes with antimicrobial activity, such as endochitinase, suggests an induced biochemical defence response against *Foc.* The expression of PAE and *PR1* results in the deposition of lignin and callose production for cell wall strengthening. Four defence associated genes (catalase 2, pectin acetyl esterase (PAE), *PR-1* and endochitinase) were selected for expression profile analysis using Real-time reverse transcriptase PCR, with TaqMan® and Light Cycler technology. All four genes were shown to be differentially expressed in GCTCV-218 at 3 and 6 hrs after infection, confirming SSH results. *PR-1* and PAE were induced very early (3 hrs after infection) in the GCTCV-218, while *PR3* and catalase 2 followed with a significant induction at 6 hrs after infection. This study concludes that GCTCV-218 is able to respond rapidly in response to *Foc* infection by activating both a biochemical and structural defence mechanism.