

SUMMARY – Claire Munro

Bananas (*Musa* sp.) serve as a staple diet and source of income for millions of people worldwide. The crop, however, is vulnerable to several important diseases such as Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*). During the mid-20th century, thousands of acres of export banana plantations were lost in Central America due to Fusarium wilt. The epidemic was brought under control only by replacing the highly susceptible Gros Michel banana with Cavendish cultivars. Cavendish bananas, however, are susceptible to *Foc* race 4, a highly virulent variant of the pathogen that also causes disease to banana varieties susceptible to *Foc* races 1 and 2. Only this time, no resistant replacement varieties acceptable to the commercial market exist, while cultural and/or chemical control strategies proved to be ineffective for disease control. The only sustainable solution, thus, would be the improvement of existing banana varieties that are acceptable to consumers for resistance to Fusarium wilt.

An understanding of how plants defend themselves against pathogens is an imperative first step towards the development of disease-resistant plants. Two broad defence mechanisms against pathogen attack exist in plants: Constitutive defence mechanisms that are pathogen non-specific and induced plant defence responses following recognition of specific pathogen features. A primary objective of this study was to elucidate induced banana defence responses. Induced defence responses may include the induction of regulator and antifungal proteins, the production of active-oxygen species or products from the phenylpropanoid pathway. A few studies have attempted to elucidate the genetic factors involved in the banana defence response but to date no clear answer has arisen. Forward and reverse genetics encompassed approaches by which plant defence responses can be studied; a particular technique of interest which can be classified under both forward and reverse genetics is cDNA-Amplified Fragment Length Polymorphism (AFLP)

In a field trial conducted in an *Foc*-infested field in Kiepersol, South Africa, cultivars Rose and Calcutta IV proved resistant, and FHIA-17 tolerant to *Foc* 'subtropical' race 4 (VCG 0120) when compared to the highly susceptible Cavendish cultivar 'Williams'. The genetic basis of defence to *Foc* in FHIA-17, Rose and Calcutta IV was verified using quantitative real-time reverse transcriptase PCR (qRT-RT-PCR). *Catalase*, *POX* and *PAE* were strongly up-regulated in the tolerant and resistant banana varieties. These genes are involved in the oxidative burst and secondary metabolism leading to the phenylpropanoid pathway and cell wall strengthening. Resistance to *Foc*, 'subtropical' race 4, thus seems to depend on the

early recognition of the pathogen and subsequent blocking of its progress into the plant's vascular system.

The response of resistant and tolerant banana varieties to *Foc* was elucidated by analysing the banana transcriptome 6 and 72 hours post inoculation (hpi) using cDNA-AFLP. Seventy-six differentially expressed transcript derived fragments (TDFs) were isolated, sequenced and subjected to BLASTX and BLASTN searches. Many of the sequences were not significantly similar to any other sequences in the databases, but several genes fragments showed homology to defence-related genes. TDFs representing genes such as *S-adenosylmethionine synthase* (SAMS) and *isoflavone reductase*, which are potentially involved in the production of cell wall strengthening compounds such as lignin, were identified. Expression patterns of selected TDFs as seen on the cDNA-AFLP gels were confirmed using qRT-PCR. As additional endogenous controls, two TDFs which displayed constitutive expression on the cDNA-AFLP gels, were isolated, sequenced and were optimised for use as endogenous control genes for the normalisation of the qRT-PCR data. In most cases, the expression patterns seen on the cDNA-AFLP gels were replicated by qRT-PCR. This study concludes that the tolerant (FHIA-17) and resistant (Rose and Calcutta IV) banana varieties induced defence-related genes upon attack by *Foc* and that the cDNA-AFLP technique was further effective in identifying additional defence-related genes.

Looking to the future, the greatest understanding of the defence responses induced during the banana/*Foc* interaction would be obtained by using additional molecular approaches or techniques. It is in some cases sufficient to look exclusively at transcriptomic i.e. cDNA-AFLP and microarray data to study the plant's response, however, a look at differential protein and metabolite expression would complement transcriptomic data and add insight into the fate of certain expressed genes. This would possibly speed up the identification of the defence pathways used by resistant bananas to resist infection by *Foc*, once identified these pathways can be manipulated in the susceptible plants and thus the generation of a *Foc* resistant banana could finally become a reality.