

SUMMARY – Gerda Fourie

Fusarium oxysporum Schlecht. is a cosmopolitan species complex that consists of both pathogenic and non-pathogenic members. The pathogenic members are subdivided into *formae speciales*, based on virulence to specific host species. More than 150 *formae speciales* have been described, of which *F. oxysporum* f.sp. *cubense* (E.F.Smith) Snyder and Hansen (*Foc*), causal agent of Fusarium wilt of banana, is regarded as one of the economically most important and destructive. According to phenotypic and genotypic markers, *Foc* has been classified into three races and 24 vegetative compatibility groups, and can be divided into a number of clonal lineages that roughly correspond with VCG groupings. In this thesis, we investigated the evolutionary relationships among VCGs using multi-gene sequencing and *MAT* genotyping. A PCR-RFLP fingerprint discriminating the *Foc* lineages and a PCR primer that identified *Foc* 'subtropical' race 4 isolates, was developed. Nine microsatellite markers (SSRs) were applied to a global population of *Foc* in order to investigate diversity not always detectable using sequencing data.

Phylogenetic analysis of isolates representing *Foc*, various other *formae speciales* of *F. oxysporum* and non-pathogenic *F. oxysporum* of the genes encoding the translation elongation factor-1 α (TEF), the mitochondrial small subunit (MtSSU), ribosomal RNA (rRNA), the repeated region encoded on the mitochondrion (MtR) and the intergenic spacer (IGS) gene regions separated these isolates into four clades, two of which included *Foc*. Within these two clades, *Foc* separated into six lineages that broadly corresponded to VCGs, while the non-pathogenic isolates of *F. oxysporum* grouped together in only one of the two clades, with an unknown *Foc* VCG isolate. The mating type of all isolates was determined and crosses were attempted between isolates harbouring *MAT*-1 and *MAT*-2 genes, without success. Cultural, morphological and pathogenic variation among isolates of *Foc* was unable to identify lineages as species. The separation of *Foc* isolates into two clades suggested that the banana pathogen evolved during two unrelated events. Factors such as horizontal gene transfer, however, might also have played a part in the pathogen's evolution, as was evident from the divergent placement of some VCGs and lineages within the phylogenetic trees constructed. The inclusion of other *formae speciales* of *F. oxysporum* and non-pathogenic *F. oxysporum* isolates illustrated the great diversity that exists within the *F. oxysporum* complex. The inclusion of the *Foc* isolate of an unknown VCG suggests that the genetic diversity of *Foc* might be far greater than what is known and what was revealed in this study. The opposite mating types found in the respective lineages indicate a sexual origin for the Fusarium wilt fungus that could account for its polyphyletic nature.

Within South Africa, *Foc* 'subtropical' race 4 is regarded the most important constrain to banana production. Conventional control practices for Fusarium wilt of banana are ineffective, and disease management relies heavily on the use of clean planting material and the early detection and isolation of the pathogen, in order to restrict spread to unaffected areas. Identification of *Foc* typically involves vegetative compatibility assays and pathogenicity testing using a set of differential host cultivars. The development of a PCR-based method for the rapid and accurate identification of *Foc* 'subtropical' race 4 will, therefore, be of great importance. The lack of morphological variation between lineages of *Foc*, and between pathogenic and non-pathogenic members, as well as the unreliability in race identification in *Foc*, makes the use of molecular tools a viable alternative. Following DNA isolation, PCR and sequencing of the MtR, the DNA sequence data revealed an 8-bp insertion that was subsequently targeted for the design of a *Foc* 'subtropical' race 4-specific primer. Isolates were positively identified as *Foc* 'subtropical' race 4 with the amplification of an 800-pb fragment. The development of the *Foc* 'subtropical' race 4 primer will aid in rapid and accurate detection of the Fusarium wilt pathogen of banana.

The population structure defined according to SSR data of a global population of 239 *Foc* isolates resembled the structure defined according to multi-gene phylogeny, with some exceptions. Measures of gene and genotypic diversity unequivocally supported the opinion that Asia is the centre of origin of *Foc*. The presence of unique genotypes in all geographically-defined *Foc* populations could potentially indicate their evolution outside the centre of origin, although this is highly unlikely. The absence of certain genotypes from the Asian population was either due to insufficient and selective sampling, or it demonstrated the effects of clonal selection in combination with adaptation to the forces of geographic isolation and environmental changes over time. The worldwide collection of *Foc* mostly consisted of six over represented genotypes, thereby providing support for a clonal genetic structure. It was, however, not possible to reject the hypothesis of a recombining population for the populations representing isolates of Lineage V. The implication of recombination within some *Foc* lineages may be due to unobserved sexual reproduction in nature or the historical association with a sexual ancestor. When one considers diversity within and among genotypes, a specific genotype was mostly associated with only one or two *Foc* VCGs, therefore indicating that vegetative compatibility determination, in combination with phylogenetic analyses, is a powerful tool for characterizing isolates causing Fusarium wilt of banana. Results from this study, in combination with the multi-gene phylogeny, clearly indicated the presence of unrelated lineages that most probably represent cryptic species.