ABSTRACT

Pseudocercospora fijiensis (P. fijiensis), causal agent of the black Sigatoka disease (BSD) on banana, has spread globally since its discovery in Fiji 1963 in all the banana and plantain growing areas across the globe. It is economically important disease in this crop. Several control measures including use of chemicals have been used against the disease. However, these control measures have not been effective. There is an indication that *P. fijiensis* is undergoing evolutionary changes by moving from low land warm climate to high land cool climate. This may furthermore complicate control of BSD.

Mitogen Activated Protein Kinase (MAPK) signal transduction pathways are involved in a wide dimensions of cell regulation. In yeast cells *Saccharomyces cerevisiae*, four MAPK pathways are well known in growing cells, these are; filamentous - invasion growth, mating, cell wall integrity and high osmolarity pathways. Currently, there is lack of information for MAP kinase signalling pathways and their related genes which regulate fungal growth and development, pathogenicity or other phenotypes in ascomycete *P. fijiensis*. This is one of the major challenges affecting the development of management strategies for BSD. There is no information on molecular mechanism of *P. fijiensis*- host interaction particularly genes associated with virulence.

The research presented in this thesis provides novel insights into the RNA interference mediated gene silencing in an ascomycetes *P. fijiensis*, *Agrobacterium tumefaciens* mediated transformation system, identification and characterisation of MAPK genes in *P. fijiensis*. This is an effort to knowledge contribution towards potential control of BSD in the East African Highland bananas and plantains.

To identify genes regulating virulence in *P. fijiensis*, MAPK *Hog1*, *Slt2* and *Fus3* sequences of *Mycosphaerella graminicola* were used as the reference pathogen gene sequence in homology Blastn search for *P. fijiensis* target sequences. These genes were isolated from *P. fijiensis* genomic DNA by PCR amplification and cloned in pGEM-T easy vector for Sanger sequencing. In this study, *in silico* analysis, functional gene prediction and annotation, protein structure model prediction and evolutionary analysis were used in the identification and characterisation of the *P. fijiensis* MAPK isolated sequences. The *PfHog1, PfSlt2* and *PfFus3*

sequences were confirmed to be MAP kinase encoding genes that belong to Serine/Threonine catalytic domain. The functional analysis confirmed that the *PfHog1* is important in hyper osmotic stress regulation, *PfSlt2* involved in cell wall integrity, morphogenesis, cell shape maintenance, and *PfFus3* regulates cell growth, cell proliferation and cell progression. Finally, protein structure model prediction indicated that both protein and nucleotide sequences for these genes are >50% identical to MAPK structure of the *Saccharomyces cerevisiae*. Evolutionary analysis confirmed *PfHog1, PfSlt2* and *PfFus3* sequences are > 90% identical to their respective MAPK *Hog1, Slt2* and *Fus3* of strictly fungal pathogens. These findings are in line with the research question of virulence in *P. fijiensis* is regulated by MAP Kinase encoding genes.

Genetic transformation procedure was developed for *P. fijiensis* and transformants expressing siRNA for *PfHog1, PfSlt2* and *PfFus3* were characterized for efficiency of RNAi mediated gene silencing. *Agrobacterium tumefaciens* mediated transformation (ATMT) was done by co- cultivation of *AGL1* strain and mycelia fragments. The ATMT of *P. fijiensis* using mycelia proved to be efficient method, characterised by stable genetic transformation of several sub- culturing, can be used for virulence assay and easily available for phenotype studies. ATMT provide the most suitable and effective method in functional genetics. This research demonstrated a novel RNAi-mediated gene silencing method in *P. fijiensis* through ATMT of mycelia fragments. Results from this study revealed that *A. tumefaciens* strain *AGL1* is capable of transforming filamentous fungi *P. fijiensis*. This study confirmed that the research hypothesis of *Agrobacterium tumefaciens* mediated transformation is an alternative method of introducing transgenes in *P. fijiensis* mycelium fragments is true. The efficiency of RNAi silencing in *P. fijiensis* by the vector pKOIISD1 was demonstrated by low expression of the target genes in silenced strains as compare to wild type *P. fijiensis*. Silencing of the target genes was achieved by more than 90%.

To investigate the role of MAPK *PfHog1* on osmotic stress adaptation, cultures of both wild type *P. fijiensis* and *PfHog1* mutant strains were cultured on potato dextrose agar media supplemented with 1 M NaCl. *PfHog1* mutant strains showed significant suppressed growth. Furthermore the role of *PfHog1, PfSlt2* and *PfFus3* to virulence in *P. fijiensis* were determined by inoculating silenced strains on susceptible East African Highland Bananas "Nakitembe". The silenced *PfHog1, PfSlt2* and *PfFus3/Kss1* strains showed significant reduced virulence characterised by low necrosis. Staining infected leaf tissues with lacto

phenol cotton blue further confirmed the impaired penetration and mycelia growth of the silenced *PfHog1* strains. In the *PfSlt2* and *PfFus3/Kss1* mutant strains there was impaired infectious, invasive growth. Reduced virulence was further confirmed with low fungal biomass recovery from silenced *PfHog1*, *PfSlt2* and *PfFus3/Kss1* strains through quantification of the fungal biomass using absolute quantitative PCR.

Collectively, these findings demonstrate that *PfHog1* is critical for both osmotic stress regulation and virulence of *P. fijiensis* on its host banana. Meanwhile *PfSlt2* and *PfFus3* are important in plant infection and pathogenic growth of fungal pathogens. These results support the hypothesis of silencing MAP kinase encoding genes interfere with growth, development and virulence of *P. fijiensis*. Thus, *PfHog1 PfSlt2* and Pf*Fus3* could be interesting targets for the control of black Sigatoka disease in banana.