Transformation of *Thielaviopsis basicola* to study host-pathogen interactions

By

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DECLARATION

I declare that the substance of this thesis is the result of my own original work. This material has not been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify to the best of my knowledge any help received in preparing this thesis and all sources used, have been acknowledged in this thesis.

Samiya Al-Jaaidi

DEDICATION

I dedicate my effort and determination to complete this study to my beloved and precious mother who passed away during the course of this study. I also dedicate this study to the rest of my family members; my father, my sisters, Salma, Aysha and Suad, and my brothers, Omar and Yasser. I thank you for your patience, encouragement and support during the course of this study.

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(Read! In the name of your Lord who created – Created the human from something, which clings. Read! And your Lord is most Bountiful – He who taught the use of the Pen, Taught the human that which he knew not).

(The Holy Qur'an)

ABSTRACT

Thielaviopsis basicola, a filamentous fungus, is a soil-borne plant pathogen belonging to the teleomorphic genus *Ceratocystis* (perithecial ascomycete). Different strains are capable of attacking a wide range of host plants causing black root rot, a seedling disease. Control strategies based on cultural practices, biocontrol agents, chemical fungicides, and genetically determined host resistance have not yet solved the issue of the loss of yield of agricultural crops. The main aim of this project was to investigate the molecular aspects of host-pathogen interactions, generate new knowledge and make progress towards the development of new control strategies for black root rot.

For *T. basicola* to cause black root rot, it germinates in soil forming a germ tube that elongates to reach the plant roots, attaches to the root surface and penetrates into the root hairs or epidermal cells. It is possible that signalling mechanisms may be required at different stages of the infection process for its progress. *In vitro* pathogenicity and water agar assays were developed in order to understand and appreciate the ability of *T. basicola* to perceive signals and respond by germination and/or directed growth towards various plants and in order to analyse the susceptibility of various plants, *in vitro* pathogenicity and water agar assays were developed. The results provided evidence that exudates released by roots of host and non-host plants were responsible for hyphal directional growth towards plant roots. There was little evidence to suggest that a host-specific stimulus caused hyphal directional growth. There was also little evidence to suggest that a correlation existed between hyphal directional growth towards host plants and disease severity caused by *T. basicola* isolates. Isolates that showed a strong hyphal directional growth response towards a particular susceptible plant did not necessarily cause disease. Strains of *T. basicola* isolated from particular hosts may exhibit stronger growth direction and/or pathogenicity towards other hosts.

Novel molecular tools for *T. basicola* were developed in order to investigate the molecular interaction and understand the infection process between *T. basicola* and cotton. A random insertional mutagenesis protocol using polyethylene glycol was developed in order to identify pathogenicity genes. Understanding the control of such genes will ultimately assist future studies to identify mechanisms of resistance employed by plants and to target resistance breeding in plants.

Protoplasts of *T. basicola* were transformed with the plasmid pGpdGFP containing the bacterial hygromycin phosphotransferase gene (*hph*) conferring resistance to hygromycin B. Transformation frequencies of 2.5 hygromycin B-resistant transformants/μg of transforming DNA were obtained in treatments containing 2×10^6 protoplasts. Mitotic stability analysis revealed that 90.5% of transformants resistant to 100 μg/mL of hygromycin B were mitotically stable.

To identify mutants with altered pathogenicity towards cotton, a rapid *in vitro* dipping technique was developed whereby cotton seedlings grown in water agar plates were dipped in spores of *T. basicola* transformants. A total of 202 mitotically stable transformants were screened and five pathogenicity mutants were identified with reduced pathogenicity towards cotton. This result was further confirmed in an *in vitro* soil bioassay in which cotton seedlings were grown in soil infested with spores of *T. basicola* transformants. Further pathogenicity tests revealed that the five mutants also demonstrated reduced aggressiveness to lupin seedlings.

Phenotypic characterisation revealed that the five mutants were able to grow in liquid media (potato dextrose broth and Czapek Dox), and on solid media (nutrient and minimal media). Three of the mutants (P16, P849 and P954) showed reduced melanin production and two (P737 and P888) showed enhanced melanin production. None of the mutants showed defects in their ability to germinate and grow towards cotton roots. Endoconidia and chlamydospore production were similar or higher compared to the wild-type. Microscopy studies revealed that the chlamydospore morphology of one of the mutants (P16) differed from the wild-type. All of the mutants showed low or similar tolerance to osmotic stress to the wild-type when exposed to different concentrations of sodium chloride. Microscopic studies also revealed that the cotton root lesion caused by the five mutants after 24 h of inoculation was similar when compared to the wild-type. However, seven days post-inoculation, the mutants were unable to establish a more durable biotrophic and necrotrophic phase (expansion of the lesions) compared to the wild-type.

Southern hybridisation analysis confirmed random insertion of one or more copies of the plasmid pGpdGFP into the genome of each of the five pathogenicity mutants. Attempts to rescue the integrated plasmid derived from either *Hpa*I-, *Nhe*I- or *Nru*I-digested genomic DNA were unsuccessful. This was most likely due to the large size of the restriction fragments generated by these enzymes, which did not cut within the plasmid.

Further attempts were performed using *Xba*I that cuts within the plasmid DNA in order to rescue the genomic DNA from only one flank of the integration site. This was only attempted in mutants that were not considered to have tandem copies of the plasmid. Mutants P849 and P954 did not produce any ampicillin resistant *E. coli* colonies. This was possibly due to the disruption of the ampicillin gene resulting from the integration of the transforming plasmid DNA into the fungal genome. Mutant P737 produced ampicillin resistant *E. coli* colonies that were similar to the control uncut plasmid. Further attempts to rescue the integrated plasmid derived from *Xba*I-digested genomic DNA fragments excised from a Southern hybridisation gel were unsuccessful.

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