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

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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 × 10⁵ 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 HpaI-, NheI- or NruI-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 XbaI 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 XbaI-digested genomic DNA fragments excised from a Southern hybridisation gel were unsuccessful.
# TABLE OF CONTENTS

Chapter 1 . Literature Review ................................................................. 1

1.1. General Introduction ........................................................................ 1

1.2. *T. basicola* ......................................................................................... 2
  1.2.1. Characterisation of fungi .............................................................. 2
  1.2.2. Host range ..................................................................................... 4
  1.2.3. Host specificity ............................................................................ 4
  1.2.4. Variability in culture ................................................................. 7

1.3. Morphology of *T. basicola* ................................................................. 9

1.4. The genus *Thielaviopsis* ................................................................. 11

1.5. Isolation of *T. basicola* ................................................................. 12

1.6. Geographical distribution of *T. basicola* ......................................... 13

1.7. Symptoms of black root rot ........................................................... 13
  1.7.1. Below ground symptoms ......................................................... 14
  1.7.2. Above ground symptoms ....................................................... 15

1.8. Factors promoting and suppressing black root rot ......................... 16
  1.8.1. Abiotic factors ............................................................................ 17
  1.8.2. Biotic factors ............................................................................. 19

1.9. Fungal pathogenicity in plants ....................................................... 21
  1.9.1. Infection process by *T. basicola* on roots ............................... 22
    1.9.1.1. Germination of *T. basicola* spores ..................................... 24

1.10. Nutritional classification of phytopathogenic fungi in living plants ...... 29
  1.10.1. Necrotrophs ............................................................................ 30
  1.10.2. Biotrophs ................................................................................. 30
  1.10.3. Hemibiotrophs ....................................................................... 31
  1.10.4. *T. basicola* as a hemibiotroph .............................................. 32

1.11. Control of black root rot .............................................................. 32
  1.11.1. Cultural control ....................................................................... 32
  1.11.2. Chemical control .................................................................... 34
  1.11.3. Biocontrol ................................................................................. 35
1.12. **Fungal pathogenicity genes** ................................................................................. 38

1.12.1. Formation of infection structures ................................................................. 39

1.12.2. Cuticle and cell wall degradation ................................................................. 40

1.12.3. Responding to host environment ................................................................. 41

1.12.4. Fungal toxins ............................................................................................... 42

1.12.5. Signaling genes ........................................................................................... 43

1.12.6. Novel and unclassified pathogenicity genes .............................................. 44

1.13. General aims of this study .................................................................................. 45

Chapter 2 . Pathogenicity and directional growth of *T. basicola* isolates towards various plants ........................................................................................................... 46

2.1. Introduction ........................................................................................................ 46

2.2. Materials and Methods ..................................................................................... 48

2.2.1. Culture conditions for *Thielaviopsis* isolates ............................................. 48

2.2.2. Growth media ............................................................................................... 49

2.2.3. Inoculum production .................................................................................... 49

2.2.4. Seed sterilization and seed weight ............................................................... 50

2.2.5. Pathogenicity tests ..................................................................................... 50

2.2.6. Growth rate of *Thielaviopsis* isolates ....................................................... 51

2.2.7. Growth direction and host preference tests .............................................. 51

2.2.8. Statistical analysis ....................................................................................... 54

2.3. Results .................................................................................................................. 55

2.3.1. Pathogenicity of *T. basicola* isolates towards plants............................... 55

2.3.2. Growth rates of *Thielaviopsis isolates* on ½ PDA (2.2% agar) and 1.2% water agar .................................................................................................................. 58

2.3.3. Directional growth of *Thielaviopsis* isolates towards plants .................. 59

2.3.4. Host preference tests .................................................................................... 62

2.4. Discussion .......................................................................................................... 68

Chapter 3 . PEG-mediated transformation of *T. basicola* ................................... 73

3.1. Introduction ........................................................................................................ 73

3.2. Materials and Methods .................................................................................... 76
3.2.1. Fungal strain and maintenance .......................................................... 76
3.2.2. Plasmid and bacterial strain ............................................................. 76
3.2.3. *E. coli* DH5α transformation .......................................................... 77
3.2.4. Isolation of plasmid DNA ................................................................. 78
3.2.5. Materials used: chemicals, solutions and reagents .......................... 78
3.2.6. Growth media and culture conditions ............................................. 79
3.2.7. Protocol development for transformation of *T. basicola* .................. 80
   3.2.7.1. Preparation of fungal protoplasts ............................................. 80
   3.2.7.2. Protoplast sensitivity to hygromycin B .................................... 82
   3.2.7.3. PEG-mediated transformation of fungal protoplasts with pGpdGFP .... 82
   3.2.7.4. Protoplast regeneration after transformation .............................. 83
3.2.8. Mitotic stability of hygromycin resistant phenotypes ...................... 84
3.2.9. Confirmation of transformation by Southern hybridisation ............... 85

3.3. Results .................................................................................................... 88
   3.3.1. Preparation of fungal protoplasts ................................................. 88
   3.3.2. Optimising protoplast regeneration broth and determining the effect of PEG on protoplast regeneration .......................................................... 90
   3.3.3. Sensitivity to hygromycin B ......................................................... 92
   3.3.4. Regeneration of DNA-treated protoplasts ...................................... 93
      3.3.4.1. Regeneration solid media ..................................................... 93
      3.3.4.2. Regeneration broth .............................................................. 97
      3.3.4.3. Effect of resuspending the DNA-treated protoplast pellet in 200 μL 1× STC and regeneration broth ................................................................. 101
   3.3.5. Mitotic stability of putative transformants .................................... 103
   3.3.6. Confirmation of transformation by Southern hybridisation analysis ........ 104

3.4. Discussion ............................................................................................. 109

3.5. Summary ............................................................................................... 118

3.6. Conclusion ............................................................................................. 122

Chapter 4. Screening of *T. basicola* transformants and phenotypic and molecular characterisation of pathogenicity mutants ............... 123

4.1. Introduction ........................................................................................... 123

4.2. Materials and Methods ......................................................................... 125
4.2.1. Culture conditions for \textit{T. basicola} ................................................................. 125
4.2.2. Sterilization of cotton and lupin seeds ................................................................. 125
4.2.3. Inoculum production of \textit{T. basicola} transformants for pathogenicity and directional growth tests ................................................................. 125
  4.2.3.1. Pathogenicity test on cotton roots using the dipping technique .......... 125
  4.2.3.2. Growth of \textit{T. basicola} transformants towards cotton roots .......... 126
4.2.4. Confirmation of pathogenicity and directional growth of \textit{T. basicola} pathogenicity mutants ................................................................. 126
  4.2.4.1. Inoculum production for \textit{T. basicola} pathogenicity mutants........ 126
  4.2.4.2. Pathogenicity of \textit{T. basicola} mutants on cotton and lupin roots .... 126
  4.2.4.3. Pathogenicity of \textit{T. basicola} mutants on cotton roots using \textit{in vitro} soil bioassays ................................................................. 127
  4.2.4.4. Directional growth of \textit{T. basicola} mutants towards cotton roots ...... 128
4.2.5. Molecular analysis of \textit{T. basicola} pathogenicity mutants ......................... 128
4.2.6. Characterisation of \textit{T. basicola} pathogenicity mutants .................................. 129
4.2.7. Statistical analysis .................................................................................................. 131
4.2.8. Plasmid rescue ........................................................................................................ 131

4.3. Results ...................................................................................................................... 134
  4.3.1. Primary screening assays of \textit{T. basicola} transformants ......................... 134
  4.3.2. Pathogenicity of \textit{T. basicola} mutants on cotton roots using the dipping technique bioassay ................................................................. 134
  4.3.3. Pathogenicity of \textit{T. basicola} mutants on cotton roots using \textit{in vitro} soil bioassay ................................................................. 137
  4.3.4. Growth of \textit{T. basicola} mutants towards cotton roots ......................... 139
  4.3.5. Molecular analysis of \textit{T. basicola} mutants ................................................. 140
  4.3.6. Characterisation of \textit{T. basicola} pathogenicity mutants ....................... 144
  4.3.7. Plasmid Rescue ................................................................................................. 150

4.4. Discussion ................................................................................................................. 153

Chapter 5. General Discussion ......................................................................................... 164
  5.1. \textit{T. basicola}-host specificity ............................................................................... 164
  5.2. Development of a PEG-mediated transformation protocol for \textit{T. basicola} .... 168
  5.3. Screening of \textit{T. basicola} transformants ............................................................ 170
  5.4. Alternatives to PEG-mediated transformation ......................................................... 171
5.5. Alternatives to random insertional mutagenesis ....................................... 172
5.6. Possible genes related to pathogenicity in *T. basicola* mutants ................. 173
5.7. Future directions ......................................................................................... 177
5.8. Conclusion .................................................................................................. 181
References ......................................................................................................... 183
LIST OF TABLES

Table 2.1. *Thielaviopsis* isolates ................................................................. 48
Table 2.2. Plants exposed to *Thielaviopsis* isolates ........................................ 48
Table 2.3. Rating of necrotic lesions induced by *Thielaviopsis* isolates on roots of various plants susceptible or non-susceptible to black root rot ........................................... 51
Table 2.4. Rating of directional growth of *Thielaviopsis* isolates induced by roots of various plants susceptible or non-susceptible to black root rot ........................................... 53
Table 2.5. Preferential growth of cotton isolate ............................................... 63
Table 2.6. Preferential growth of lupin isolate ................................................. 64
Table 2.7. Preferential growth of the lettuce 1 isolate ...................................... 65
Table 2.8. Preferential growth of the lupin and lettuce 1 isolates towards seeds of equal weight ........................................................................................................ 67
Table 3.1. List of solutions, buffers and reagents .............................................. 78
Table 3.2. Transformation frequency of protoplasts regenerated on regeneration solid media .................................................................................................................. 97
Table 3.3. The effect of regeneration broth on the average number of *Thielaviopsis basicola* stable transformants ................................................................. 99
Table 3.4. Transformation frequency of *Thielaviopsis basicola* protoplasts regenerated directly in regeneration broth ................................................. 100
Table 3.5. The effect of 200 µL 1× STC treatment on the average number of *Thielaviopsis basicola* stable transformants ................................................. 102
Table 3.6. Transformation frequency of *Thielaviopsis basicola* protoplasts resuspended in 200 µL 1× STC and regenerated in regeneration broth .......... 103

LIST OF FIGURES

Figure 1.1. An endoconidium produced inside a phialide .................................. 10
Figure 1.2. Black root rot in a mature cotton plant .............................................. 15
Figure 1.3. Schematic illustration of the life cycle of *Thielaviopsis basicola* leading to black root rot ................................................................. 22
Figure 1.4. Infection process of *Thielaviopsis basicola* leading to black root rot ...... 23
Figure 1.5. Germination in *Thielaviopsis basicola* .............................................. 24
Figure 1.6. Cytoplasmic streaming ...................................................................... 28
Figure 2.1. Assessment of growth direction and host preference tests ............... 53
Figure 2.2. Assessment of pathogenicity of *Thielaviopsis* isolates on roots of seedlings... 56
Figure 2.3. Pathogenicity tests using the dipping technique ........................................... 57
Figure 2.4. Representative roots of cotton and lupin seedlings infected with endoconidia of
*Thielaviopsis* isolates showing different levels of black root rot. ................................. 58
Figure 2.5. Assessment of growth rate of *Thielaviopsis* isolates ................................. 59
Figure 2.6. Assessment of directional growth efficiency of *Thielaviopsis* isolates towards
seedlings ......................................................................................................................... 61
Figure 2.7. Growth of *Thielaviopsis basicola* (cotton isolate) towards cotton seedlings... 62
Figure 3.1. Diagram of plasmid pGpdGFP (6.93 kb) ......................................................... 77
Figure 3.2. Germination of endoconidia of *Thielaviopsis basicola* ............................... 88
Figure 3.3. *Thielaviopsis basicola* protoplasts ............................................................... 89
Figure 3.4. Colonies regenerated from *Thielaviopsis basicola* protoplasts plated on
regeneration medium ................................................................................................. 90
Figure 3.5. Flow chart showing regeneration frequency of *Thielaviopsis basicola*
protoplasts in the presence and absence of PEG ......................................................... 92
Figure 3.6. DNA-treated protoplasts plated on regeneration solid media ..................... 94
Figure 3.7. Stable and Abortive putative transformants from DNA-treated protoplasts on
regeneration solid media ............................................................................................. 94
Figure 3.8. Flow chart showing *Thielaviopsis basicola* protoplasts treated with DNA on
selective regeneration media ....................................................................................... 96
Figure 3.9. Flowchart of protoplasts treated with DNA resuspended in regeneration broth.
..................................................................................................................................... 98
Figure 3.10. *Thielaviopsis basicola* transformants in regeneration broth plated on PDA
media (1.2% agar) ........................................................................................................ 100
Figure 3.11. *Thielaviopsis basicola* abortive transformants ............................................. 101
Figure 3.12. Mitotic stability of *Thielaviopsis basicola* transformants ......................... 103
Figure 3.13. Southern hybridisation analysis of NheI-digested genomic DNA from
*Thielaviopsis basicola* transformants ........................................................................ 106
Figure 3.14. Southern hybridisation analysis of XbaI-digested and undigested genomic
DNA from *Thielaviopsis basicola* unstable transformants ....................................... 108
Figure 4.1. The effect of inoculum level on disease severity on cotton seedlings inoculated
with *Thielaviopsis basicola* pathogenicity mutants using the dipping technique ....... 135
Figure 4.2. Representative roots of cotton seedlings exposed to *Thielaviopsis basicola*
pathogenicity mutants ............................................................................................... 135
Figure 4.3. Disease severity of lupin seedlings inoculated with *Thielaviopsis basicola* pathogenicity mutants using the dipping technique ................................................................. 136

Figure 4.4. Representative roots of lupin seedlings infected with endoconidia of *Thielaviopsis basicola* pathogenicity mutants ........................................................................... 136

Figure 4.5. Disease severity on cotton seedlings in soil inoculated with *Thielaviopsis basicola* pathogenicity mutants .................................................................................... 137

Figure 4.6. Representative roots of cotton seedlings grown in soil inoculated with *Thielaviopsis basicola* pathogenicity mutants ........................................................................ 138

Figure 4.7. Growth of *Thielaviopsis basicola* pathogenicity mutants towards cotton seedlings ...................................................................................................................... 139

Figure 4.8. Southern hybridisation analysis of *HpaI-, NheI- or NruI*-digested genomic DNA of *Thielaviopsis basicola* pathogenicity mutants .................................................. 141

Figure 4.9. Southern hybridisation analysis of *XbaI*-digested genomic DNA of *Thielaviopsis basicola* pathogenicity mutants ........................................................................... 143

Figure 4.10. Chlamydospore morphology of P16 .................................................................. 144

Figure 4.11. Appearance of *Thielaviopsis basicola* wild-type and pathogenicity mutants. .............................................................................................................................. 145

Figure 4.12. Assessment of vegetative growth of *Thielaviopsis basicola* pathogenicity mutants ...................................................................................................................... 146

Figure 4.13. Assessment of spore count of *Thielaviopsis basicola* pathogenicity mutants on PDA .................................................................................................................... 147

Figure 4.14. Effect of osmotic stress on vegetative growth of *Thielaviopsis basicola* pathogenicity mutants ......................................................................................... 148

Figure 4.15. Germination of *Thielaviopsis basicola* pathogenicity mutants in liquid media ......................................................................................................................... 149

Figure 4.16. Colonisation of *Thielaviopsis basicola* pathogenicity mutants after 24 h post-inoculation ........................................................................................................... 150

Figure 4.17. Mini prep products of uncut plasmid DNA from ampicillin-resistant transformants of *E. coli* strain DH5α ................................................................. 151