

## **Acknowledgements**

I would like to thank the following individuals for their contribution to this study:

- My supervisor, Dr E Venter, for his continuous support, guidance, motivation and financial support.
- Prof A-M Oberholster for her theoretical and practical input and supervision.
- The National Research Foundation (NRF) for financial support.
- Botany Department at UJ for the use of their laboratories.
- My family and friends for their understanding and support.
- For Gustav for his support, motivation and guidance.



## List of Figures

<b>Figure 2.1:</b> An illustration of barley-----	5
<b>Figure 2.2:</b> The disease cycle of <i>Fusarium graminearum</i> -----	9
<b>Figure 2.3:</b> The interaction between plants and pathogens -----	11
<b>Figure 2.4:</b> A representation of some of the responses in signal transduction in the host during infection -----	13
<b>Figure 2.5:</b> A model representing the activation of calcium channels through the binding of G-proteins to a receptor in the plasma membrane. -----	20
<b>Figure 2.6:</b> The different hormonal pathways in a disease response -----	23
<b>Figure 2.7:</b> The interaction between <i>Blumeria graminis</i> and barley. -----	28
<b>Figure 4.1:</b> Total RNA extraction with a 1 kb ladder (lane 1), driver sample (Puma15) in lane 2 and tester sample (Puma15) in lane 3. -----	55
<b>Figure 4.2:</b> Ligation efficiency test of tester cDNA. -----	56
<b>Figure 4.3:</b> PCR products obtained after two rounds of PCR amplification with Nested PCR primer 1 and Nested PCR primer 2R.-----	57
<b>Figure 4.4:</b> The determination of SSH efficiency through Southern Blot analysis, showing the hybridisation of Puma 15. -----	58
<b>Figure 4.5:</b> Amino acid and nucleotide sequence alignment of SSH10 to its BLASTn and BLASTx best hits.-----	61
<b>Figure 4.6:</b> Amino acid and nucleotide sequence alignment of SSH11 to its BLASTn and BLASTx best hits.-----	62
<b>Figure 4.7:</b> Amino acid and nucleotide sequence alignment of SSH12 to its BLASTn and BLASTx best hits.-----	63
<b>Figure 4.8:</b> Amino acid and nucleotide sequence alignment of SSH13 to its BLASTn and BLASTx best hits.-----	64
<b>Figure 4.9:</b> Amino acid and nucleotide sequence alignment of SSH15 to its BLASTn and BLASTx best hits.-----	65
<b>Figure 4.10:</b> Amino acid and nucleotide sequence alignment of SSH16 to its BLASTn and BLASTx best hits.-----	66

**Figure 4.11:** Pathogenicity results of barley infected with *F. graminearum*.-----67

**Figure 4.12:** Expression analyses of selected TDFs. ----- 68



## List of Tables

<b>Table 2.1:</b> The characteristics and description of diseases that occur in barley plants -----	7
<b>Table 2.2:</b> A quadrant that shows the resistance or susceptibility between plants and pathogens -----	12
<b>Table 2.3:</b> Pathogenesis-Related (PR) Proteins-----	25
<b>Table 2.4:</b> Barley resistance genes from the MI group that shows resistance towards <i>B. graminis</i> -----	29
<b>Table 2.5:</b> QTL functions on different chromosomes of wheat and barley -----	33
<b>Table 4.1:</b> Putative identities of the SSH fragments attained using the BLASTx algorithm -----	59
<b>Table 4.2:</b> Putative identities of the SSH fragments attained using the BLASTn algorithm -----	60



## Abbreviations

ABA	Abscisic acid
AGT	Appressorial germ tubes
AMV	Avian Myeloblastosis Virus
Avr	Avirulence
BLAST	Basic local alignment search tool
Bp	Base pairs
°C	Degree centigrade
cDNA	Complementary DNA (refers to DNA synthesised from mRNA)
Ca <sup>2+</sup>	Calcium
CC	Coiled-coil
CLA	Carnation leaf agar
DIG	Digoxygenin-11-dUTP
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
DON	Deoxynivalenol
DTT	Dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylene diaminetetraacetic acid
<i>f.sp.</i>	<i>Forma specialis</i>
g	Gram
GRP	Glycine-rich proteins
HCl	Hydrochloric acid
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HO <sub>2</sub> / O <sub>2</sub> <sup>-</sup>	Super oxide
hpi	hours post inoculation
HR	Hypersensitive response
HRGP	Hydroxyproline-rich glycoproteins

hrs	Hours
IPTG	Isopropylthio $\beta$ -D galactoside
kb	Kilobasepairs (1kb = $10^3$ basepairs)
KD	Kernel discolouration
K <sup>+</sup>	Potassium
LRR	Leucine rich repeats
M	Molar
min	Minutes
ml	Milliliter
mM	Millimolar
mRNA	Messenger RNA
NaCl	Sodium chloride
NBS	Nucleotide binding site
NCBI	National Centre for Biotechnology Information
ng	Nanogram
P-loop	Phosphate-binding loop
PAL	Phenylalanine ammonia-lyase
PCD	Programmed cell death
PCR	Polymerase chain reaction
PGT	Primary germ tubes
PR proteins	Pathogen-related proteins
QTL	Quantitative trait loci
qRT-PCR	quantitative Reverse Transcriptase PCR
R genes	Resistance genes
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
SA	Salicylic acid
SAR	Systemic acquired resistance
sec	Seconds
SDS	Sodium dodecyl sulphate
SIR	Systemic induced resistance

SSC	Sodium citrate buffer
SSH	Suppression subtractive hybridisation
TAE	Tris-acetate-EDTA buffer
TDF	Transcript derived fragment
TIR	Toll/Interleuken-1 like receptor
Tris	2-Amino-2 (hydroxymethyl)-1,3-propanediol
7-TM	7 transmembrane
μ	Micro
μl	Microliter
μM	Micromolar
U	Units
UV	Ultraviolet
V	Volt
var	Variety
X-Gal	5-Bromo-4-chloro-3-indolyl-β-D-Galactopyranoside

