IDENTIFICATION AND
CHARACTERISATION OF MARKERS
LINKED TO THE LEAF RUST
RESISTANCE GENE LR37
IDENTIFICATION AND CHARACTERISATION OF MARKERS LINKED TO THE LEAF RUST RESISTANCE GENE LR37

CHRISTIAAN TROSKIE

Submitted in partial fulfillment of the degree

Magister Scientiae

Forestry and Agricultural Biotechnology Institute and Department of Genetics at the University of Pretoria, Pretoria, South Africa.

2000

Supervisor: Prof. A-M Oberholster (Botha)
Co-supervisor: Dr. F. J. Kloppers
PREFACE

The results represented in this thesis follow from the study, which was carried out at the Forestry and Agricultural Biotechnology Institute and Department of Genetics at the University of Pretoria under the supervision of Prof A-M. Oberholster and the co-supervision of Dr. F.J. Kloppers.

The results represented here are original and have not been submitted in any form to another University.

Christiaan Troskie
Acknowledgements

I would like to thank the following people, organizations and institutions for their contribution towards the successful completion of this thesis:

Prof. A-M Oberholster for excellent supervision, enthusiasm and numerous opportunities.

Dr. F.J. Kloppers for his excellent (co) supervision, continued interests and practical inputs.

Prof. Z.A. Pretorius for providing plant material from the University of the Orange Free State.

Dr. H van Niekerk for providing plant material from the Small Grain Institute Bethlem, SA.

Magriet van der Nest for assistance with AFLP and sequencing software.

Shilo Loots for assistance with SSRs in Chapter 3.

Juanita, Janine, Jackie and Lieschen for proofreading.

The FABI family for assistance, research advice and friendship.

Colleagues from the Department of Genetics for supportive discussions.

My parents and brother for continued support.

The Forestry and Agricultural Biotechnology Institute and Department of Genetics for excellent research facilities.

The NRF for financial support.
## CONTENTS

List of figures iv

List of tables vi

List of abbreviations vii

Chapter 1

Introduction

Chapter 2

Molecular markers to facilitate leaf rust resistance, breeding programs in wheat (Literature review)

2.1 Introduction 5

2.2 Wheat rusts 6

2.2.1 Wheat rust species 6

2.2.2 Asexual stages of the leaf rust pathogen in the wheat plant 7

2.3 Control of rust diseases 9

2.3.1 Methods of rust control 9

2.3.2 Genes for disease control 9

2.3.3 Gene pyramiding 16

2.3.4 Success with resistance breeding 16

2.4 Wheat 17

2.4.1 General 17

2.4.2 The wheat genome 17

2.4.3 Genetic diversity of wheat plants 18

2.4.4 Genetic distance analysis of wheat plants 19

2.4.5 Mapping of the wheat genome 20

2.5 Molecular techniques 20

2.5.1 General 20

2.5.2 RFLPs 21

2.5.3 RAPDs 22

2.5.4 AFLPs 22
2.5.5 SSRs
2.5.6 SCARs

2.6 Optimization of PCR based technologies
2.6.1 Template DNA
2.6.2 MgCl₂
2.6.3 dNTPs
2.6.4 Primers
2.6.5 Enzyme
2.6.6 Reaction volume
2.6.7 Number and duration of PCR cycles
2.6.8 Gel electrophoresis

2.7 Marker assisted selection
2.7.1 General
2.7.2 Molecular markers
2.7.3 Bulk segregant analysis to identify molecular markers
2.7.4 Near isogenic lines to identify molecular markers
2.7.5 General approaches to locate molecular markers linked to genes

Chapter 3
A comparative genetic analysis using RAPDs, AFLPs and SSRs to predict the best combinations of different genotypes, with the emphasis on gene Lr37

3.1 Introduction
3.2 Materials and Methods
3.2.1 Plant materials
3.2.2 DNA isolations
3.2.3 RAPD-PCR analysis
3.2.4 AFLP analysis
3.2.5 SSR analysis
3.2.6 Genetic distance analysis among wheat genotypes

3.3 Results
3.3.1 RAPD analysis
3.3.2 AFLP analysis
3.3.3 SSR analysis
3.3.4 Dendogram analysis
Chapter 4
Identification and characterization of DNA markers linked to leaf rust resistance gene \textit{Lr37}

4.1 Introduction 54
4.2 Materials and Methods
4.2.1 Plant material 55
4.2.2 Inoculations 55
4.2.3 DNA isolation 56
4.2.4 RAPD-PCR analysis 56
4.2.5 AFLP analysis 56
4.2.6 SSR analysis 56
4.2.7 Linkage analysis 57
4.2.8 Cloning of marker fragments 58
4.2.9 Dot blot hybridization of marker fragments 59
4.2.10 Sequencing 60
4.2.11 SCAR analysis 60

4.3 Results
4.3.1 Segregation analysis 61
4.3.2 RAPD analysis 61
4.3.3 Cloning and sequencing of OPQ7 62
4.3.4 SCAR analysis of OPQ7_{1180} 62
4.3.5 AFLP analysis 70
4.3.6 SSR analysis 74

4.4 Discussion 76

Chapter 5
Conclusions 79

Chapter 6
Summary/ Opsomming 83

References 88
Appendices 112
LIST OF FIGURES

Fig. 2.1 Uredia produced by *Puccinia recondita* f. sp. *tritici* on flag leaves of susceptible wheat plants.................................................................8

Fig. 3.1 RAPD amplification profiles obtained for the 21 genotypes with primer OPA19 after separation on 2% agarose gels.................................................................41

Fig 3.2 RAPD amplification profiles obtained for the 21 genotypes with primer OPB13 after separation on 2% agarose gels.................................................................42

Fig 3.3 RAPD amplification profiles obtained for the 21 genotypes with primer OPG13 after separation on 2% agarose gels.................................................................43

Fig 3.4 A silver stained, 6% polyacrylamide gel showing SSR amplification products of different genotypes, with primer pair XGWM122..................................................45

Fig 3.5 A dendrogram based on pairwise genetic distances (Nei & Li, 1979), among the different genotypes used in the study.................................................................48

Fig 3.6 A cladogram of the different genotypes used in the study based on RAPDs, AFLPs and SSRs.................................................................49

Fig 4.1 A 2% agarose gel stained with Ethidium Bromide, showing RAPD amplification products of primer OPQ7 when exposed to UV light.................................................................64

Fig 4.2 *E. coli* cells after transformation with a pGEM®-T vector.................................................................65

Fig 4.3 An Ethidium Bromide stained, 2% agarose gel showing colony PCR amplification products when exposed to UV light.................................................................66

Fig 4.4 A 2% agarose gel showing the result of an *EcoR*I digest of plasmid pGEM®-T containing the inserted RAPD amplification product OPQ7 when exposed to UV light.................................................................67
Fig 4.5 Sequence information obtained after sequencing marker fragment OPQ7,160 with primers T7 and SP6 (Promega) from both ends .......................................................68

Fig 4.6 A 2% agarose gel showing the result of a combination of primers OPQ7L1 and OPQ7R when using genomic wheat DNA of the cultivar Karee and line RL6081 as template. The amplification products were also digested with various enzymes.................................70

Fig 4.7 A 6% non-denaturing acrylamide gel showing the results visualized with silver staining after an AFLP selective amplification.......................................................71

Fig 4.8 An Ethidium bromide stained, 2% agarose gel showing colony PCR amplification products when exposed to UV light.................................................................72

Fig 4.9 Hybridization results obtained after 32P [dATP] labeling of six randomly chosen clones (A-F) of AFLP fragment EcoACA-MseCTG470....................................................73

Fig 4.10 A 6% non-denaturing polyacrylamide gel showing the results visualized with silver staining after genomic wheat DNA is amplified with SSR marker XGWM359..............75
**LIST OF TABLES**

**Table 2.1** Classification of different infection types of wheat infected with *Puccinia recondita* f. sp. *tritici* (Stakman *et al.*, 1962; Roelfs, 1988) ...............................................................10

**Table 2.2** Designated leaf rust resistance genes, genome locations, linkage of the genes among each other, original gene sources, tester lines and molecular markers available with references and gene references. ..................................................................................12

**Table 3.1** The 21 different genotypes used to conduct a molecular genetic analysis in the study ........................................................................................................................................................................36

**Table 3.2** Pairwise genetic distances converted to a percentage among the 21 genotypes, calculated from the binary data set according to Nei & Li (1979) ........................................................................47

**Table 4.1** Segregation analysis of 35 BC$_2$F$_2$ plants from a cross between a leaf rust susceptible South African cultivar Karee and a leaf rust resistant line RL6081 .................................................................................61

**Table 4.2** Putative markers linked to leaf rust resistance gene *Lr37* obtained after screening resistant and susceptible plants with RAPDs, AFLPs and SSRs ........................................................................................................63
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphism</td>
</tr>
<tr>
<td>2AS</td>
<td>Chromosome 2A, short arm</td>
</tr>
<tr>
<td>BSA</td>
<td>Bulk segregant analysis</td>
</tr>
<tr>
<td>c</td>
<td>Recombination fraction</td>
</tr>
<tr>
<td>cn</td>
<td>Chlorotic/ Necrotic</td>
</tr>
<tr>
<td>eM</td>
<td>Centimorgan</td>
</tr>
<tr>
<td>D</td>
<td>Genetic distance between any two individuals</td>
</tr>
<tr>
<td>Dn</td>
<td>Russian wheat aphid resistance gene</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxyribonucleotides</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithioerythritol</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>et al.</td>
<td>Et alii (and others)</td>
</tr>
<tr>
<td>F</td>
<td>Index of genetic similarity</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropylthio-β-D-galactoside</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobases</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Bertani</td>
</tr>
<tr>
<td>Lr</td>
<td>Leaf rust resistance gene</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>m/v</td>
<td>Mass/volume</td>
</tr>
<tr>
<td>MAS</td>
<td>Marker-assisted selection</td>
</tr>
<tr>
<td>Mb</td>
<td>Megabases</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NILs</td>
<td>Near isogenic lines</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>Pm</td>
<td>Powdery mildew resistance gene</td>
</tr>
<tr>
<td>pM</td>
<td>Picomolar</td>
</tr>
<tr>
<td>PPi</td>
<td>Difosfate</td>
</tr>
<tr>
<td>RAPD</td>
<td>Random amplified polymorphic DNA</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
</tr>
<tr>
<td>Rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>s</td>
<td>Similarity coefficient</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SCAR</td>
<td>Sequence characterized amplified region</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>Sr</td>
<td>Stem rust resistance gene</td>
</tr>
<tr>
<td>SSRs</td>
<td>Small sequence repeats</td>
</tr>
<tr>
<td>STS</td>
<td>Sequence-tagged site</td>
</tr>
<tr>
<td>Taq</td>
<td><em>Termes aquaticus</em></td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-borate/EDTA</td>
</tr>
<tr>
<td>TEN</td>
<td>Tris-EDTA-sodium chloride</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris (hydroxymethyl)- aminomethane</td>
</tr>
<tr>
<td>U</td>
<td>One unit of enzyme</td>
</tr>
<tr>
<td>UHQ</td>
<td>Distilled and UV treated water</td>
</tr>
<tr>
<td>UPGMA</td>
<td>Unweighted pair-group mean arithmetic</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>Volts</td>
</tr>
<tr>
<td>X</td>
<td>Distance of marker to the gene</td>
</tr>
<tr>
<td>X-Gal</td>
<td>5-bromo-4-chloro-3-indolyl-β-D-galactoside</td>
</tr>
<tr>
<td>Yr</td>
<td>Yellow rust resistance gene</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μL</td>
<td>Microliter</td>
</tr>
</tbody>
</table>