Combining wheat rust and Fusarium head blight resistance genes and QTL using marker-assisted selection

By

KATLEHO JOYCE SENOKO

Dissertation submitted in fulfilment of the requirements for the degree

Magister Scientiae Agriculturae

Department of Plant Sciences (Plant breeding)

Faculty of Natural and Agricultural Sciences

University of the Free State

Bloemfontein, South Africa

January 2014

Supervisor: **Prof. Liezel Herselman**

Co-supervisor: **Prof. ZA Pretorius**

Declaration

I, Katleho Joyce Senoko, do hereby declare that the dissertation hereby submitted by me for the degree Magister Scientiae Agriculturae in Plant Breeding at the University of the Free State represents my own original, independent work and that I have not previously submitted the same work for a qualification at another university.

I further cede copyright of the dissertation in favour of the University of the Free State

……………………….. ……………………..

Katleho Joyce Senoko **Date**

Acknowledgements

With special thanks to:

- **Professor Liezel Herselman**. Thank you for being such a dedicated supervisor, supportive, always encouraging when I thought I lost the track and for your love. Your knowledge and support in this aspect of the study gave me courage to work beyond my expectations. Thank you for your professionalism that made this research possible.

- **Professor ZA Pretorius**. Thank you for being my co-promoter and for your expertise in this study especially in plant pathology. Thank you for your assistance.

- **Scott Sydenham** and **Doctor Adre Minaar-Ontong**. Thank you for your assistance, especially with the laboratory and greenhouse work but most of all for your friendship and jokes that made me laugh for all the years I spent with you. You made me smile in the laboratory even when things were not working well. Thank you.

- **Sadie Geldenhuys.** Thank you so much for putting a smile on my face every day. Your love, support and motivation always came when I needed it the most.

- **Mafoafoa Charles and Sebofane Lehakoe Charlotte Lephoto**. Thank you for being a supportive, patient, loving husband and for never giving me any reason to doubt that I could not do this work without your support. You have given me encouragement beyond what you can ever imagine. To you my baby girl: your mere existence gives me joy and strength. I sing the praises to God for blessing me with you every single day of my life.

- My mother **'Mathabiso Senoko**. Thank you for your immeasurable love, your prayers and your wealth of knowledge of God’s Word that keep me going. You have taught me so much and you have been my inspiration and strength. I am who I am because of your love and guidance. I thank you so much.

- My amazing family in law: **Agatha, Nkhuba, 'Maagatha Lephoto**. Thank you for taking care of my daughter. Your patience and love to my daughter, thus to me, made my studies endurable. You have never made me doubt the wellbeing of my little girl while I was studying. Thank you.

- My wonderful brothers and sisters: Thank you for being who you are to me. It is a great privilege to have you in my life.

- All my colleagues and all my wonderful friends worldwide: Thank you for your daily support and encouraging words that empowered me and gave me strength to keep up even when it was really tough. Thank you.

- My sponsors: National Research Foundation (NRF) and Government of Lesotho for financial support.

Dedication

I dedicate this Masters dissertation to my late father Tšepiso Oriel Senoko. When I completed my first degree, he said to me “This is not enough” and I promised him to continue with my Masters degree but he said “I doubt if I would still be alive my girl”. Indeed he is gone.

Table of contents

Declaration ii
Acknowledgements iii
Dedication v
Table of contents vi
List of figures xi
List of tables xiii
List of abbreviations xv
CHAPTER 1 **1**
General introduction **1**
References 4
CHAPTER 2 7
Wheat rusts and Fusarium head blight: major fungal diseases of wheat 7
2.1 Introduction 7
2.2 Taxonomy and genomics of wheat 7
2.3 Wheat production in South Africa 8
2.4 Importance of wheat both worldwide and in South Africa 9
2.5 Value of plant breeding 9
2.6 Threats for wheat production 10
2.7 Wheat rust diseases important in this study 11
2.7.1 Background information on rust fungi 11
2.7.2 Stripe rust 12
2.7.2.1 Symptoms and disease development on host plants 12
2.7.2.2 Economic importance worldwide and in South Africa 13
2.7.2.3 Important genes used in breeding 13
2.7.3 Leaf rust 14
2.7.3.1 Symptoms and disease development on host plants 14
2.7.3.2 Economic importance worldwide and in South Africa 15
2.7.3.3 Genes used in breeding against leaf rust 15
2.7.4 Stem rust 15

2.7.4.1 General background 15

2.7.4.2 Symptoms and disease development on host plants 15

2.7.4.3 Economic importance worldwide and in South Africa 17

2.7.4.4 Important genes used in breeding for stem rust 17

2.8 Wheat rust resistance genes and QTL important for the current study 17

2.8.1 Leaf rust resistance genes 17

2.8.1.1 Lr19 18

2.8.1.2 Lr34/Yr18/Sr57 18

2.8.2 Stripe rust resistance genes and quantitative trait loci 19

2.8.2.1 QYrsgi-7D and QYr.i-2B.1 20

2.8.2.2 YrSp 20

2.8.3 Stem rust resistance genes 21

2.8.3.1 Sr2 21

2.8.3.2 Sr26 22

2.9 Fusarium head blight (FHB) 23

2.9.1 General information 23

2.9.2 Symptoms and disease development on host plants 23

2.9.3 Economic importance worldwide and in South Africa 24

2.9.4 Important resistance sources used in breeding for Fusarium head blight resistance in the current study 25

2.9.5 Breeding for Fusarium head blight resistance and marker-assisted selection 26

2.9.6 Fusarium head blight resistance quantitative trait loci used in the current study 26

2.9.6.1 Fhb1/Qfhs.ndsu-3BS 26

2.9.6.2 Qfhs.ifa-5A 27

2.9.6.3 Qfhs.ndsu-3AL 27

2.9.6.4 Qfhs.ndsu-6B 28

2.9.6.5 QTL on chromosome 7A 28

2.9.7 FHB resistant sources used in the study 28

2.9.7.1 CM-82036 28

2.9.7.2 Frontana 29

2.9.8 Molecular markers and plant breeding 29

2.9.8.1 Restriction fragment length polymorphism 30
2.9.8.2 Random amplified polymorphic DNA 30
2.9.8.3 Amplified fragment length polymorphism 30
2.9.8.4 Microsatellites or simple sequence repeats 31
2.9.8.5 Sequence characterised amplified region/ Sequence tagged site markers 32
2.9.8.6 Single nucleotide polymorphism 32
2.9.9 Molecular marker-assisted selection 33
2.10 Reports on combining different resistance genes in wheat using marker-assisted selection 34
2.10.1 Combining leaf rust resistance genes 34
2.10.2 Combining Fusarium head blight resistance quantitative trait loci 34
2.11 Conclusion 36
2.12 References 36
CHAPTER 3 **59**
Selection of rust resistant lines **59**
3.1 Introduction 59
3.2 Materials and methods 63
3.2.1 Plant material 63
3.2.2 Material sampling 64
3.2.3 DNA isolation 64
3.2.4 SSR analyses 65
3.2.4.1 PCR cycling conditions 66
3.2.5 Visualisation of amplified fragments 66
3.2.5.1 Agarose gel electrophoresis 66
3.2.5.2 Polyacrylamide gel electrophoresis (PAGE) 66
3.2.5.3 Silver staining 70
3.2.6 Data analyses 70
3.2.7 Phenotypic screening 70
3.2.7.1 Materials and methods 70
3.3 Results 71
3.3.1 Genotypic screening of self-pollinated segregating populations 71

3.3.1.1 Comparison and selection of best individuals of family S16 74

3.3.1.2 Comparison and selection of best individuals of family S178 76

3.3.1.3 Comparison and selection of best individuals of family S726 76

*3.3.1.4 Comparison and selection of best individuals of family S79*1 78

3.3.1.5 Marker distribution in individuals of all four tested families 79

3.3.2 Phenotypic screening 81

3.3.2.1 *Screening of individual plants of families S16(7.3) and S726(3.2): verification of molecular markers present in selected lines* 81

Leaf rust resistance evaluation 82

Stem rust resistance evaluation 83

Stripe rust resistance evaluation 84

3.3.2.2 Phenotypic evaluation of original parental lines 85

3.4 Discussion 86

3.5 Conclusion 89

3.6 References 89

CHAPTER 4 **94**

Combining wheat rust and Fusarium head blight resistance genes and quantitative trait loci using marker-assisted selection **94**

4.1 Introduction 94

4.2 Materials and methods 97

4.2.1 Plant material 97

4.2.2 Sampling of leaf material and DNA isolation 98

4.2.3 SSR analyses 98

4.2.3.1 Markers linked to rust resistance genes/QTL 98

4.2.3.2 FHB marker screening of parental lines, F_1 progeny of each cross and the double cross population 99

4.2.3.3 PCR cycling conditions 102

4.2.4 Visualisation of amplified fragments 102

4.2.4.1 Agarose gel electrophoresis 102

4.2.4.2 Polyacrylamide gel electrophoresis (PAGE) 102

4.2.4.3 Gel Scan 3000 103

4.2.5 Data analyses 103
4.3 Results 104
4.3.1 Screening of parental lines using markers linked to rust resistance 104
4.3.2 Screening of parental lines using markers linked to FHB resistance 106
4.3.3 F_1 cross identification 106
4.3.4 Genotypic screening of double cross population 109
4.3.5 Segregation patterns for screened markers 112
4.4 Discussion 114
4.5 Conclusion 118
4.6 References 119
CHAPTER 5 124
General conclusions and recommendations 124
Summary 128
Opsomming 130

List of figures

Figure 2.1	Symptoms of wheat plants infected by stripe rust	13
Figure 2.2	Symptoms of wheat plants infected by leaf rust	14
Figure 2.3	Symptoms of wheat plants infected by stem rust	16
Figure 2.4	*Fusarium graminearum* infection in wheat: note the bleached heads	24
Figure 3.1	Experimental plan followed by Sydenham (2007) to obtain the double cross lines used in the current study	62
Figure 3.2	An agarose gel for marker csLV34 used to detect homozygous and heterozygous individuals in families S16, S178 and S726 for *Lr34* resistance	72
Figure 3.3	Segregation patterns for individuals of family S16 on a silver stained polyacrylamide gel for marker Gwm148 (*QYr.sgi-2B.1*) showing the different allele sizes of the four parental lines	74
Figure 3.4	Total number of markers observed for each individual of family S16	75
Figure 3.5	Total number of markers observed for each individual of family S178	77
Figure 3.6	Total number of markers observed for each individual of family S726	77
Figure 3.7	Total number of markers observed for each individual of family S791	79
Figure 3.8	Total number of observed markers in individual lines across all screened families	80
Figure 3.9	Leaf rust resistance evaluation on selected progeny and parental lines using race UVPt20	83
Figure 3.10	Stem rust resistance evaluation on selected progeny and parental lines using race UVPtg60	84
Figure 3.11	Stripe rust resistance evaluation on selected progeny and parental lines using race 6E22A+	85

Figure 4.1	Crossing scheme to combine rust and FHB resistance genes and/or QTL into a single wheat genotype	96
Figure 4.2	A silver stained polyacrylamide gel of S725(3.2)/Frontana F_1 individuals screened using the DuPw227 marker	108
Figure 4.3	Comparison of cross success percentages for cross 1 and cross 2 within individual plantings as confirmed by SSR markers	109
Figure 4.4	Frequency distribution showing the number of double cross F_1 individuals containing a specific number of molecular markers	110

List of tables

Table 2.1	Examples of successful combining of major genes and/or QTL into single wheat genotypes using marker-assisted breeding (modified table from Gupta et al., 2010)	35
Table 3.1	Characteristics of four families selected from the double cross population screened by Sydenham (2007)	63
Table 3.2	SSR-PCR reaction volumes and mixtures (in µl) for the different primer combinations used in the study	67
Table 3.3	SSR markers, primer pair sequences, targeted genes/QTL, parental cultivar sources and references for primers used in the study	68
Table 3.4	PCR cycling conditions and specific cycling programmes used in the study as standardised by Sydenham (2007)	69
Table 3.5	Expected allele sizes of parental lines (Sydenham, 2007)	73
Table 3.6	Chi square test for segregation patterns of the S16 population for all markers tested	76
Table 3.7	Chi square test for segregation patterns of the S726 double cross population for all markers tested	78
Table 3.8	Merits of the selected lines S16(7.3) and S726(3.2) and their parental lines S16 and S726	81
Table 3.9	Disease score results for selected rust resistant lines and parental lines	82
Table 3.10	Summary of genotypes' performance to different rust isolates screened and genes present in each tested line or cultivar based on genotypic and phenotypic data	86
Table 4.1	PCR cycling conditions and specific programmes used for primers linked to FHB resistance as well as the primer linked to the rust resistance gene *Lr34/Yr18/Sr57*	100
Table 4.2	SSR markers, primer pair sequences, targeted genes/QTL and parental cultivar sources used for markers linked to FHB resistance including the new marker used for the rust resistance gene *Lr34/Yr18/Sr57*	101

Table 4.3	Allele sizes of fragments amplified in the parental lines using markers linked to rust resistance genes and QTL	105
Table 4.4	Allele sizes of fragments amplified in the parental lines using markers linked to FHB resistance QTL	107
Table 4.5	Best performing individuals of the double cross population	111
Table 4.6	Segregation ratios of 10 molecular markers tested on 954 individuals of the double cross population using Chi square analysis	113

List of abbreviations

AFLP	Amplified fragment length polymorphism
APR	Adult plant resistance
APS	Ammonium persulfate
BGRI	Borlaug Global Rust Initiative
bp	Base pairs
°C	Degrees Celsius
CAPS	Cleaved amplified polymorphic sites
cDNA	Complimentary DNA
cm	Centimetre(s)
cM	Centimorgan(s)
CTAB	Hexadecyltrimethylammmonium bromide
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
dNTPs	2’-deoxynucleoside 5’-triphosphate
DON	Deoxynivalenol
dpi	Days post-inoculation
E	Expected
EDTA	Ethylene-diaminetetraacetate
F_1	First generation
F_2	Second generation
FHB	Fusarium head blight
g	Gram(s)
g	Gravitational force
gDNA	Genomic DNA
h	Hour(s)

ha	Hectare
HTISC	High temperature induced seedling chlorosis
indel	Insertion/deletion
L	Litre(s)
Lr	Leaf rust
M	Molar
MAS	Marker-assisted selection
Mbp	Mega base pairs
$MgCl_2$	Magnesium chloride
min	Minute(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
NaCl	Sodium chloride
ng	Nanogram(s)
nm	Nanometre(s)
O	Observed
PAGE	Polyacrylamide gel electrophoresis
PBC	Pseudo-black chaff
PCR	Polymerase chain reaction
Pgt	Stem rust
pH	Power of hydrogen
pmol	Picomole(s)
Pst	Stripe rust
Pt	Leaf rust
QTL	Quantitative trait loci
r/s	Revolutions per second

RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SA	South Africa
SCAR	Sequence characterised amplified region
s	Second(s)
SNP	Single nucleotide polymorphism
Sr	Stem rust
SSR	Simple sequence repeat
STS	Sequence tagged site
Taq	*Thermus aquaticus*
TBE	Tris-borate/EDTA
TE	Tris-Cl/EDTA
TEMED	Tetramethylethylenediamine
Tris-HCl	Tris(hydroxymethyl) aminomethane
U	Unit(s)
USA	United States of America
UV	Ultraviolet
V	Volt(s)
v/v	Volume per volume
W	Watt(s)
WL	Wavelength
w/v	Weight per volume
Yr	Yellow rust
µg	Microgram(s)
µl	Microlitre(s)
µM	Micromolar(s)

CHAPTER 1

General introduction

Wheat (*Triticum aestivum* L.) is counted among the most commonly cultivated cereal crops with over 600 million tons harvested each year (Priyamvada et al., 2011). Wheat was already cultivated about 10 000 years ago as part of the Neolithic revolution that was distinguished as a period of transition from hunting and gathering food to one of settlement and agriculture (Gupta et al., 2006; Shewry, 2009). The most primitive cultivated wheat varieties were landraces selected by farmers from wild species because of their good agronomic traits. However, that selection process was not considered scientific from a plant breeding perspective. Nevertheless, wheat's domestication was associated with selection of genetic traits that separated landraces from wild relatives (Shewry, 2009). About 95% of wheat grown worldwide is hexaploid bread wheat with the remaining 5% being tetraploid durum wheat and small amounts of other wheat species (einkorn, emmer and spelt) (Curtis, 2010). In the past, significant growth in wheat production was achieved through conventional breeding (Gupta et al., 2010).

Wheat is used for consumption by both humans and animals (Han et al., 2005). From direct use, wheat provides more than 35% of dietary calories in the developing world and 74% in the developed countries (Shiferaw et al., 2013). In South Africa (SA), the total production of wheat is estimated at 1.8 million tons but the production is not sufficient for domestic use hence SA imports wheat (Smit et al., 2010). Wheat is predicted to remain an important crop with about 68% of the produce earmarked for direct consumption by the year 2020 and its worldwide consumption is projected to be about 746 million tons in 2020 (Bureau for Food and Agricultural Policy, 2011).

Bread wheat has ample genetic diversity which has led to the development of over 25 000 varieties adapted to a wide range of temperate environments (Shewry, 2009). Though wheat is adapted and can be produced under different climatic conditions (Bushuk, 1998), improved

production can be achieved by expanding the wheat area, improving yield per unit area planted, and by minimising pre- and post-harvest losses (Curtis, 2010).

The most important constraints affecting wheat production include drought, diseases, insects and weeds. Major diseases that affect wheat yield negatively are leaf, stripe and stem rust, septoria tritici blotch, powdery mildew, common and dwarf bunts, loose smut and tan and head blight (Shewry, 2009). These diseases cause great losses to the quality and quantity of the crop worldwide (Priyamvada et al., 2011). In sub-Saharan African countries, stem rust and stripe rust are the most important rust diseases compared to leaf rust (Shiferaw et al., 2013). In SA, infectious (fungal, bacterial and viral) and non-infectious diseases affect wheat yield negatively but most research has been directed towards wheat rusts (Smit et al., 2010). Todorovska et al. (2009) added that wheat rusts have been among the most important diseases around the world because they occur everywhere. Emerging new diseases and new pathogen genotypes pose threats to crop production and provide challenges to breeders because they have to develop varieties that can perform better under unpredictable environments (Brown, 2008).

The three mentioned rust diseases are common foliar fungal diseases of wheat (Priyamvada et al., 2011). Leaf rust is more frequent and common in many areas than stem or stripe rust (Todorovska et al., 2009). Stem rust is the most important disease of wheat worldwide and reduces yield by about 50% to 100% when coupled with root diseases under favourable conditions (Shiferaw et al., 2013). The stripe rust pathogen is able to spread rapidly between widely separated wheat production areas and has become common in SA (Pretorius et al., 2007).

In addition to rust diseases, Fusarium head blight (FHB or scab) is another common and damaging fungal disease of cereals that causes losses in grain yield and quality and contaminates harvested grain with mycotoxins (Buerstmayr et al., 2003). According to Smit et al. (2010) wheat production in SA, under irrigation, has been affected by FHB since its first detection.

Yield losses due to biotic stresses can be prevented by the use of resistance genes (Lupton, 1987). A major challenge for breeders is to minimise disease outbreaks by developing new cultivars with durable resistance (Tiwari et al., 2008). Kaur et al. (2008) stated that wheat cultivars become susceptible to diseases due to the lack of a genetic base for resistance and the rapid rate of evolution of pathogens, making it necessary to search for new sources of resistance. For wheat there are numerous sources of resistance to diseases available, although not all are of equal value (Johnson and Jellies, 1992).

Considerable efforts have been made towards wheat improvement to the extent that improved cultivars and agricultural techniques have been under development by plant breeders and agronomists for several years (Shewry, 2009). Although progress has been achieved towards wheat improvement, further research is still necessary to maintain stability of wheat production under unfavourable environments. In addition, climate change is expected to change the actions of crop diseases and make the performance of varieties difficult. Hence, collaboration between plant pathologists and breeders is important to develop crop varieties with durable disease resistance (Brown, 2008).

Research to improve wheat yields includes combining germplasm through crossing, application of biotechnology techniques, hybrid wheat development and basic studies on the physiology of the wheat plant (Curtis, 2010). With application of conventional crossing and the use of new molecular techniques, new varieties can be developed within a short period of time. Resistance genes can easily be identified for use in breeding programmes using marker-assisted selection (MAS) rather than using only phenotype trait selection (Todorovska et al., 2009).

Several studies have shown that application of both MAS and conventional plant breeding produces better results by shortening the plant breeding cycle. The best plants can be selected from large segregating populations using genotypic rather than phenotypic selection only.

In a previous study by Sydenham (2007), rust resistance genes/quantitative trait loci (QTL) from Kariega (*Lr34/Yr18/Sr57* and *QYr.sgi-2B.1*), Avocet*YrSp* (*YrSp* and *Sr26*), Blade (*Sr2*

and *Sr26*) and CS*Lr19*-149-229 (*Lr19* and *Lr34/Yr18/Sr57*) were combined for durable rust resistance and selection was done using MAS. Based on marker data, the best two lines [S16(7.3) and S726(3.2)] with the highest number of markers linked to the different rust resistance genes in a homozygous state were selected. The S16(7.3) line tested positive for five homozygous and one heterozygous marker while two markers were absent. The S726(3.2) line tested positive for four homozygous and two heterozygous markers with two markers absent. Combination of these two lines should yield a line containing five potential rust resistance genes in total and if crossed with FHB resistant cultivars CM-82036 (type II resistance) and Frontana (type I resistance), will result in a wheat line containing five rust (stem, leaf and yellow rust) and two FHB (type I and type II) resistance genes/QTL.

The aim of the current study was therefore to combine five rust resistance genes/QTL from S16(7.3) and S726(3.2) and five FHB resistance QTL from CM-82036 and Frontana into a single wheat genotype using MAS. Combining wheat rust and FHB resistance genes/QTL using MAS should ensure higher levels of resistance to both rust and FHB, leading to durable resistance, resistance to a wider range of diseases, and a shortened breeding period.

References

Brown JKM (2008) Breeding for disease resistance as an intergral component of crop protection. Endure International Conference. Diversifying Crop Protection, 12-15 October 2008. La Grande-Motte, France, pp 1-5

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theoretical and Applied Genetics 107:503-508

Bureau for Food and Agricultural Policy (2011) The South African Baseline, pp 1-82 http://www.bfap.co.za (Cited March 2012)

Bushuk W (1998) Wheat breeding for end-product use. Euphytica 100:137-145

Curtis BC (2010) Wheat in the world. http://www.fao.org/ (Cited January 2014)

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. Molecular Breeding 26:145-161

Gupta SK, Charpe A, Prabhu K, Haque Q (2006) Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theoretical and Applied Genetics 113:1027-1036

Han FP, Fedak G, Ouellet T, Dan H, Somer DJ (2005) Mapping of genes expressed in *Fusarium graminearum* infected heads of wheat cultivar Frontana. Genome 48:88-96

Johnson R, Jellies GJ (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3-22

Kaur S, Bansal UK, Khanna R, Saini RG (2008) Genetics of leaf and stripe rust resistance in a bread wheat cultivar Tonichi. Journal of Genetics 87:191-194

Lupton FGH (1987) Wheat breeding. Chapman and Hall, London, 418 pp

Pretorius ZA, Pakendorf KW, Marais GF, Prins R, Komen JS (2007) Challenges for sustainable cereal rust control in South Africa. Australian Journal of Agricultural Research 58:593-601

Priyamvada M, Saharan S, Tiwari R (2011) Durable resistance in wheat. International Journal of Genetics and Molecular Biology 3:108-114

Shewry PR (2009) Wheat. Journal of Experimental Botany 60:1537-1553

Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5:291-317

Smit HA, Tolmay VL, Barnard A, Jordaan JP, Koekemoer FP, Otto WM, Pretorius ZA, Purchase JL, Tolmay JPC (2010) An overview of the context and scope of wheat (*Triticum aestivum*) research in South Africa from 1983 to 2008. South African Journal of Plant and Soil 27:81-96

Sydenham SL (2007) Pyramiding wheat rust resistance genes using marker-assisted selection. MSc dissertation, Department of Plant Sciences, University of the Free State, 108 pp

Tiwari R, Kumar Y, Priyamvada M, Saharan MS, Mishra B (2008) Marker-assisted approach for incorporating durable rust resistance in popular Indian wheat cultivars. In: Proceedings of the XI International Wheat Genetics Symposium, August 24-29, 2008, Brisbane Queensland, Australia, pp 852-854

Todorovska E, Christov N, Slavov S, Christova P, Vassilev D (2009) Biotic stress resistance in wheat breeding and genomic selection implications. Biotechnology and Biotechnology Equipment 23:1417-1426

CHAPTER 2

Wheat rusts and Fusarium head blight: major fungal diseases of wheat

2.1 Introduction

Wheat (*Triticum* spp.) is the world's most important crop after maize and highly significant in terms of food security. It contributes to about 41% of cereal calories from direct consumption worldwide (Shiferaw et al., 2013). It is used to make food, feed, beverages and biofuel (Lupton, 1987). Among cultivated wheat, bread wheat is one of the main staple foods in the world. Globally, its production is estimated to 680 million tons per year planted on about 225 million hectares (Sharma et al., 2013). However, the demand is expected to increase to about 813 million tons in 2030 and to more than 900 million tons in 2050 (FAO, 2006). Wheat is produced in a wide range of climates although it is most favourably adapted to cool, dry environments and least favourably adapted to warm, moist climates (Lupton, 1987).

2.2 Taxonomy and genomics of wheat

Wheat is classified amongst the group of wild grasses from the family Poaceae. The genus *Triticum* originated in the arid zones of western Asia (Scott, 1990; Cornell and Hoveling, 1998). Wheat species differ from one another based on morphology, physiology and genetics (Peterson, 1965). Cultivated wheat is classified into four groups based on polyploidy levels namely diploids (2n=2x=14) that include einkorn wheat, tetraploids (2n=4x=28) that include durum wheat, species with wild and cultivated variants (emmer, durum, rivet, Polish and Persian wheats) and hexaploids (2n=6x=42) that include spelt, bread, club and Indian short wheat (Poehlman, 1987; Bonjean and Angus, 2001). The most commonly cultivated wheat groups are bread wheat which is an allohexaploid with an AABBDD genome and durum wheat (AABB) (Lupton, 1987; Waines and Ehdaie, 2007).

The wheat genome consists of about 80% repetitive sequences and measures almost 16 000 Mega base pairs (Mbp) (Gupta et al., 2006). The wheat genome (16 x 10^9 bp) is larger than that of barley and maize (both at 5 x 10^9), followed by rice (4 x 10^8 bp) which has the smallest genome amongst the most important field crops. Bossolini et al. (2006) and Langridge et al. (2001) stated that the three related genomes of wheat (A, B and D) and the genome size and structure make it difficult to perform genetic analyses.

2.3 Wheat production in South Africa

Wheat was first introduced in SA by Europeans upon Jan van Riebeeck's arrival in the Cape in 1652. The first seed was harvested on 13 January 1653 though production was not successful at the time due to cultivars that were not adapted to the Cape region. Van Niekerk in Smit et al. (2010) said wheat is produced in three distinct areas in SA. Winter wheat is sown under dryland area in the Free State, spring wheat is grown on dryland conditions in the Western Cape and irrigated spring wheat is grown near rivers in the summer rainfall region. Eighty percent of production is under dryland conditions and 20% is under irrigation (Smit et al., 2010).

The main areas producing wheat in SA are the Western Cape, Northern Cape, Free State, parts of the Southern Cape, North-West and Mpumalanga. There are almost 16 million hectares of arable land available for crop production in SA (Hannon, 2012). In 2012, the estimated wheat production in the Western Cape was 775 200 tons, contributing 43% towards the country's production, followed by Free State with 370 500 tons (21%) and Northern Cape with 277 200 tons (16%). The area under wheat production in 2011 was about 604 700 ha with yield expectation of 2 million tons and declined to 511 200 ha in 2012 with yield expectation of 1 784 million tons which was lower than the previous year. The expected yield for 2012 was 3.49 tons/ha (http://www.sagis.org.za/Flatpages).

2.4 Importance of wheat both worldwide and in South Africa

Wheat was domesticated about 10 000 years ago and is one of the world's most important crops (Gupta et al., 2006). The major wheat producing countries are The People's Republic of China, India, United States of America (USA), France, Russia, Canada and Australia (Oerke and Dehne, 2004; Panozzo and Eagles, 1998; Randhawa et al., 2013). Wheat is mainly planted for human consumption and to a limited extent for feeding livestock and industrial use (Han et al., 2005). Seventy percent of wheat is used for human consumption, 20% for animal feed and the remaining quantity for industrial processing (Shiferaw et al., 2013). Wheat and related grasses such as barley and rye have always been important and its use as food goes back to the Stone-Age era (Cornell and Hoveling, 1998).

Breeders and farmers divided wheat species in terms of baking qualities with preference to high protein and starch contents or by grain colour or growing season such as winter and summer wheat cultivars (Curtis et al., 2002). On the other hand, in the USA wheat was divided by varieties or texture of endosperm for grading purposes as being hard red winter wheat, hard red spring wheat, soft red winter wheat, white wheat and durum (USDA, 2013). Bread making qualities for hard wheat includes milling yield, protein quality and the strength of the dough while soft wheat quality depends on starch, pentoson and protein concentrations (Guttierin et al., 2001). Wheat quality is vital to meet the requirements of the end user which includes grain size, protein concentration, protein composition, starch composition and lipid concentration (Panozzo and Eagles, 1998).

2.5 Value of plant breeding

The main aim of plant breeding is to improve cultivars for the benefit of farmers and their livelihood (Gepts and Hancock, 2006). Improvement and domestication of crops originated from conventional breeding through selection and by combining or reshuffling genes within the same gene pool (Jauhar, 2006). Breeding programmes are therefore aimed at improving a single trait such as an agronomic trait or disease resistance or to improve many traits simultaneously without lowering the performance of the already accumulated traits (Johnson

and Jellies, 1992). Gepts and Hancock (2006) added that wheat breeding is aimed at developing varieties that are adapted to different environmental conditions and having heritable traits, such as multiple disease resistance, traits of interest to growers, processers and consumers.

The successful defence mechanism for minimising crop damage caused by biotic factors is mainly through breeding for disease and pest resistance (Johnson and Jellies, 1992). Hence, accumulation of different resistance genes in a single genotype confirms the success in resistance breeding (Bartos et al., 2002). However, the skill of selecting desirable plants laid the basis of plant breeding in the past and will remain an important factor in future breeding programmes (Chahal and Gosal, 2002).

2.6 Threats for wheat production

Wheat production is threatened by damage due to diseases, weeds and pests, from sowing till maturity and during storage after harvest (Cook and Veseth, 1991). The highest levels of damage on crops are caused mainly by pathogens other than pests and weeds (Oerke and Dehne, 1997). A report by Pellegrineschi et al. (2001) indicated that wheat fungal pathogens inflicted losses of up to 10% to global wheat production. Pathogens evolve fast on the host due to a lack of a good genetic base for durable resistance (Kaur et al., 2008). In SA, the introduction of new pests and diseases such as the Russian Wheat Aphid in 1978, and stripe (yellow) rust in 1996, accompanied by the emergence of new biotypes and pathotypes since the original incursions, have severely impacted wheat production (Smit et al., 2010). The introduction and local adaptation of stem rust races in the Ug99 group serve as an example of a recent threat to wheat production in SA (Visser et al., 2011).

Outbreaks of diseases need to be controlled to maintain high yield (Lupton, 1987). However, according to Walker et al. (2002) control measures are only used when damage has already occurred, resulting in yield loss. More emphasis and efforts are therefore directed towards breeding for disease and pest resistance, as it is one of the most reliable methods of protecting

crops from losses due to biotic factors before the occurrence of damage (Johnson and Jellies, 1992). Since the focus area for this study is wheat rusts and FHB, only these wheat diseases will be discussed in this chapter.

2.7 Wheat rust diseases important in this study

2.7.1 Background information on rust fungi

Rust fungi are parasitic and obligate biotrophs that survive, develop and reproduce on living plant tissue. There are about 7 000 species of rust fungi that cause diseases on cereal crops and ornamental plants (Mohanan, 2010). These species interact with their specific hosts in a "gene for gene mode depending on the presence or absence of avirulence gene(s) in the pathogen and resistance gene(s) in the host" (Eckardt, 2006). Flor (1971) stated that incompatibility between host and pathogen occurs only if a resistance gene and its corresponding avirulence gene interacts.

Rust fungi have life cycles with up to five different spore stages. Many rusts require two separate host plants to complete their life cycle and are known to be heteroecious while others are autoecious, completing their life cycle on one host plant (Eckardt, 2006). Successful infection occurs when rust fungi develop special infection structures (haustoria) that penetrate the host cell and damage the plant by using its nutrients (Hahn and Mendgen, 2001).

Selection for rust resistance is based on seedling and/or field responses of breeding populations. However, molecular markers closely linked to the rust resistance genes provide a highly reliable option for the selection of important genes in breeding programmes and can be done in the absence of pathogens (Bariana et al., 2001). In the past, cereal rust diseases were of great importance for crop production in SA but genetic information for wheat resistance was not available. In SA, breeding for rust resistance started in the 20th century by transferring stem rust resistance from Rieti to local cultivars (Pretorius et al., 2007).

Three pathogens causing rust diseases namely *Puccinia triticina* Eriks., *P. graminis* Pers. f. sp. *tritici* Eriks. and *P. striiformis* West. f. sp. *tritici* Eriks. attack wheat. Breeding resistant cultivars is therefore important to minimise losses caused by these rust species (Vida et al., 2009). Wheat rust fungi are obligate parasites and are one of the contributing reasons for low yields in wheat and cereals such as barley and rye. These diseases are dispersed in the form of dikaryotic urediniospores, which can be transported by air movement over long distances (Roelfs, 1988).

2.7.2 Stripe rust

Stripe rust, caused by *P. striiformis.* f. sp. *tritici* is an important constraint to wheat production in cool environments and is the most damaging to grain among the three rust diseases of wheat (Singh et al., 2000). Moisture and low temperatures favour the occurrence of stripe rust and it also occurs in tropical areas of higher altitude (Boshoff et al., 2002). Despite its historical incidence in cooler climates, devastating stripe rust epidemics have now been reported from warmer regions where the disease was considered unimportant (Hovmøller et al., 2011).

2.7.2.1 Symptoms and disease development on host plants

Infection occurs anytime from the first leaf stage to just before physiological maturity. Symptoms are noticed a few days after infection and under favourable conditions, formation of urediospores starts about two weeks after infection (Chen, 2005). Typical symptoms include elongated, bright yellow to orange stripes which consist of rust pustules that run parallel to the leaf veins (Figure 2.1). The pustules consist of masses of rust spores and are called uredinia (Bowden, 2006).

The stripe rust pathogen can attack the glumes, awns and kernels of the plant (Knott, 1989). The stripe rust pathogen uses water and nutrients from the host plants thus lowering yield (Chen, 2005).

Figure 2.1 Symptoms of wheat plants infected by stripe rust (Photo by ZA Pretorius)

2.7.2.2 Economic importance worldwide and in South Africa

The primary loss due to stripe rust results from defoliation and shrivelling of kernels and losses of up to 75% have been reported (Knott, 1989). However, according to Chen (2005) yield losses can reach 100% if infection occurs at very early stages of plant growth and under favourable conditions. In SA, stripe rust was first observed in Moorreesburg, Western Cape in August 1996, emerged in the western Free State in 1997, and eventually spread to other wheat production areas in the country including Lesotho (Boshoff et al., 2002). The disease caused a widespread epidemic on spring wheat in 1996 because of cultivar susceptibility and favourable weather conditions (Ramburan et al., 2004; Moldenhauer et al., 2006). The economic impact of the stripe rust incursion in SA was discussed by Pretorius et al. (2007). Management decisions, fungicide costs, loss of susceptible germplasm, fewer varieties, additional surveys and new infrastructure were observed by the local wheat industry after the introduction.

2.7.2.3 Important genes used in breeding

The use of stripe rust resistant cultivars is the most economical, effective and environment friendly method to reduce damage and losses caused by the disease (Liu et al., 2007). About 105 resistance genes have been identified and denoted *Yr* followed by either a number or a

letter (http://www.graingenes). Resistance can be expressed at seedling stage, also called all-stage resistance, and at the adult plant stage (Chen, 2005).

2.7.3 Leaf rust

Wheat leaf rust, sometimes called brown rust, is caused by *P. triticina* (Curtis et al., 2002) and is the most common and widely distributed foliar disease of wheat (Mebrate et al., 2008). It causes great losses during warm and dry summers and multiplies fast when dew or misty conditions prevail (Bartos et al., 2002).

2.7.3.1 Symptoms and disease development on host plants

The leaf rust causing fungus is air-borne and typical symptoms are small, round, orange-red pustules (Figure 2.2). It primarily attacks the leaf blades and to a lesser extent leaf sheaths and glumes (Knott, 1989). The primary damage results from premature defoliation of the plants which results in shrivelling of kernels (Scott, 1990).

Figure 2.2 Symptoms of wheat plants infected by leaf rust (Photo by ZA Pretorius)

2.7.3.2 Economic importance worldwide and in South Africa

Leaf rust can cause 30% to 50% yield losses in wheat (McIntosh et al., 1995). However, it causes less damage compared to stem rust but can cause greater losses if it occurs more frequently (Knott, 1989). In SA, epidemics of leaf rust were severe in the Western Cape in 2009 and the disease was also detected in KwaZulu-Natal, Eastern and Northern Cape. The most frequent occurring race of leaf rust is 3SA133 and it has been found in SA during the past 20 years (Terefe and Pretorius, 2010). Previously a yield gain of 56% was reported when wheat was chemically protected from leaf rust infection (Pretorius et al., 2007).

2.7.3.3 Genes used in breeding against leaf rust

Leaf rust can be economically controlled by the use of resistant cultivars. However, resistance against the leaf rust pathogen is based on the presence of effective leaf rust (*Lr*) resistance genes (Šliková et al., 2004). There are about 108 *Lr* genes and 17 *Lr* QTL known (http://www.graingenes). Success in breeding for resistance worldwide was observed when partial or adult plant resistance (APR) was exploited (Bartos et al., 2002).

2.7.4 Stem rust

2.7.4.1 General background

Stem rust or black rust is caused by *P. graminis*. f. sp. *tritici* (Bartos et al., 2002). The stem rust pathogen is classified into races according to the reactions of resistance (*Sr*) genes in wheat differential lines (Singh et al., 2002). It occurs in most places where wheat is grown and infection takes place when dew and/or misty wet conditions are accompanied by temperatures of 15°C to 30°C. Due to its preference for higher temperatures, stem rust usually appears later in the season when the wheat plant is already in the grain filling stage (Roelfs, 1988).

2.7.4.2 Symptoms and disease development on host plants

The fungus causing stem rust of wheat requires two distinct hosts to complete its full life cycle. The primary hosts for *P. graminis* are wheat, barley and triticale and some closely related species. One of the secondary hosts is *Berberis vulgaris* L. (Singh and Rajaram,

2002). The fungus has five types of spores i.e. pycniospores (spermatia), aeciospores, urediniospores (urediospores), teliospores and basidiospores. The disease cycle starts with the exposure of the new wheat crop to stem rust inoculum. Symptoms of the disease are visualised as erumpent uredinial pustules mainly on the stems and leaf sheaths about 7 to 10 days after infection (Leonard and Szabo, 2005).

The stem rust pathogen is air-borne and noted by the appearance of large, elevated reddish brown rust pustules on the leaves, leaf veins, ears, awns and stems of susceptible cultivars. It can attack all of the above ground parts of the plant. Stem rust produces dark brown-red and elongated pustules (Figure 2.3). On leaves the pustules can be of various sizes and shapes but on young leaves of fully susceptible plants they are often diamond shaped (Knott, 1989).

Figure 2.3 Symptoms of wheat plants infected by stem rust (Photo by ZA Pretorius)

Martin et al. (1976) stated that the rusted plants transpire at a greatly accelerated rate which reduces the expected yield of the crop. The extent of loss is aggravated by a loss of

photosynthetic area and mechanical destruction of plant tissue. Under favourable conditions for the development of stem rust, 100% yield loss can occur.

2.7.4.3 Economic importance worldwide and in South Africa

Yield losses caused by stem rust in the middle of the 20th century were about 20% to 30% in Eastern and Central Europe and countries such as Australia, China and India (Leonard and Szabo, 2005). Admassu et al. (2009) stated that the new stem rust race Ug99 or TTKS, isolated for the first time in Uganda in 1999, is evolving and becoming virulent infecting many wheat varieties. Pathotypes within the Ug99 race group have been detected in Kenya, Ethiopia, Yemen, Sudan and Iran (Sharma et al., 2013). According to Singh et al. (2011) Ug99 races pose a threat to wheat production and food security. In SA stem rust races have been identified in the Ug99 lineage. TTKS was first reported in the year 2000 being virulent to *Sr21*. Currently there are four Ug99 races, TTKSF, TTKSP, PTKST and TTKSF+ that were detected in SA (Pretorius et al., 2012a; 2012b; Visser et al., 2011). Throughout the course of wheat production in SA stem rust has been a major constraint and challenge to farmers and breeders (Pretorius et al., 2007).

2.7.4.4 Important genes used in breeding for stem rust

The risk of rust infection can be minimised by the use of resistant cultivars. Several wheat cultivars worldwide show stem rust resistance due to the presence of resistance genes. There are 90 reported resistance genes against the stem rust pathogen (http://www.graingenes). Many genes have shown race specificity as opposed the APR gene *Sr2* which provides resistance against all races (Singh et al., 2006; Tsilo et al., 2008).

2.8 Wheat rust resistance genes and QTL important for the current study

2.8.1 Leaf rust resistance genes

Leaf rust resistance in wheat is conveyed by major genes that condition resistance at all growth stages and minor genes that become more effective during adult stages. Alien *Lr* genes have been transferred into hexaploid bread wheat through inter-genomic transfer from

wild relatives (Tyryshkin et al., 2006). There are molecular markers such as sequence tagged sites (STS), simple sequence repeats (SSR), sequence characterised amplified region (SCAR), cleaved amplified polymorphic sites (CAPS) and single nucleotide polymorphism (SNP) available for leaf rust resistance genes (Todorovska et al., 2009).

2.8.1.1 Lr19

The leaf rust resistance gene *Lr19* is located on chromosome 7DL of wheat and is derived from *Thinopyrum ponticum* (Host) D.R. Dewey (Gupta et al., 2006). It conveys resistance to all leaf rust races in many countries including China and SA (Li et al., 2006). When effective *Lr19* provides a 0; infection type to leaf rust infection (McIntosh et al., 1995). *Lr19* is linked to *Sr25* (Mclntosh et al., 1976; Bariana et al., 2007). Previously *Lr19* was not utilised extensively because it was believed to have a connection with yellow flour colour (Cherukuri et al., 2003) but this linkage was broken (Marais, 1992). The white endosperm recombinant line contained the *Lr19* gene without the *Y* gene and *Lr19* was relocated to chromosome 7BL. No negative effects on yield and quality have been detected for this segment (Prins et al., 1997).

STS markers have been developed in wheat to screen for *Lr19* by Prins et al. (2001). Prins et al. (2001) developed a marker STSLr19 that amplifies a 130 bp fragment linked to *Lr19* resistance. The dominant STSLr19_{130} marker was derived from an amplified fragment length polymorphism (AFLP) marker.

2.8.1.2 Lr34/Yr18/Sr57

Lr34/Yr18/Sr57 originated from *T. aestivum* and is located on chromosome 7DS (Chelkowski and Stepien, 2001). It was first described by Dyck et al. (1966) in the wheat cultivar Frontana and named after its chromosome position had been determined (Dyck, 1987). Cultivars that contain *Lr34* and other additional genes express high levels of leaf rust resistance even under heavy infestation (Roelfs, 1988; Singh and Rajaram, 1992). It is race non-specific and effective at adult plant stages. The gene is however expressed in seedlings at low temperatures (McIntosh et al., 1995). It provides durable rust resistance (Lagudah et al.,

2006) and is often more effective when combined with other *Lr* genes (German and Kolmer, 1992). Roelfs (1988) confirmed that the combination of *Lr34* with *Lr12* and/or *Lr13* provided durable leaf rust resistance in some cultivars. According to Krattinger et al. (2009) *Lr34* is associated with the stripe rust resistance gene *Yr18*, powdery mildew gene *Pm38* and a phenotypic marker *Ltn1* for leaf tip necrosis and therefore provides multiple resistance in wheat. It has recently been shown that *Lr34* conditions stem rust resistance and the gene is thus referred to as *Lr34/Yr18/Pm38/Sr57* (Prins et al., 2011).

The STS marker csLV34 was developed by Lagudah et al. (2006) and the SSR marker Smw10 was developed by Bossolini et al. (2006). The two markers have been used in molecular screening for *Lr34* (Bariana et al., 2007). The codominant STS marker csLV34 was developed from an restriction fragment length polymorphism (RFLP) marker and has been successfully used to indicate the presence of *Lr34/Yr18* in many wheat cultivars by amplifying a 150 bp fragment. Genetic linkage between the marker csLV34 and *Lr34/Yr18* was estimated at 0.4 cM (Lagudah et al., 2006). Six new markers (cssfr1, cssfr2, cssfr3, cssfr4, cssfr5 and cssfr6) linked to *Lr34* were developed based on sequence information from resistant and susceptible lines. These markers were tested on several wheat cultivars and produced perfect diagnostic values. Marker combinations cssfr5 and cssfr6 were most valuable for MAS. Marker cssfr5 is easily detected using agarose gel electrophoresis. These markers are perfect markers because they target the *Lr34* gene directly and are furthermore codominant (Lagudah et al., 2009).

2.8.2 Stripe rust resistance genes and quantitative trait loci

Stripe rust resistance genes are designated as *Yr* followed by a unique number or letter and distinguished by different chromosomal locations and responses to different stripe rust pathogens. Advances in biotechnology promoted the use of AFLP, CAPS, STS and SSR markers to distinguish *Yr* genes providing resistance to the stripe rust pathogen (Chen, 2005).

2.8.2.1 QYrsgi-7D and QYr.i-2B.1

Two major QTL, *QYr.sgi-7D* and *QYr.sgi-2B.1* on chromosomes 7D and 2B, respectively, have been identified in the SA wheat cultivar Kariega. The combination of these two QTL with a third QTL on chromosome 4A has provided durable and effective APR in Kariega. The 2B and 7D QTL convey different forms of resistance. The *QYr.sgi-7D* QTL, confirmed by Prins et al. (2011) to be the *Lr34/Yr18/Sr57* locus for multiple disease resistance (Krattinger et al., 2009), has shown durable resistance worldwide. In lines carrying only *QYr.sgi-2B.1* resistance is expressed as a strong hypersensitive response (Prins et al., 2011).

The SSR markers Gwm295-7D and Gwm148-2B were used to screen for the QTL in Kariega. Gwm295-7D was located closest to the *QYr.sgi-7D* QTL while *QYr.sgi-2B.1* was detected on chromosome 2B with Gwm148-2B as the closest marker (Ramburan et al., 2004). However, Prins et al. (2011) used the two flanking markers Barc200 and wPt6278 for screening the *QYr.sgi-2B.*1 QTL and Barc352 and Gwm111 for the *Lr34/Yr18/Sr57* on chromosome 7DL after increasing the population used by Ramaburan et al. (2004). Since the *QYr.sgi-7D* QTL has been indicated to be the resistance gene *Lr34/Yr18/57*, the presence of the QTL is detected using molecular markers cssfr1-cssfr6 developed by Lagudah et al. (2009).

2.8.2.2 YrSp

The *YrSp* gene is derived from the cultivar Spaldings Prolific and is located on chromosome 2BS (Sui et al., 2009). It is a dominant gene that provides seedling resistance to stripe rust. Although two AFLP markers linked to the *YrSp* gene have been identified by Mathews (2005), they could not be applied in a breeding programme due to poor linkage. *YrSp* is typically expressed by an immune phenotype (0; on a 0 to 4 scale) in seedlings to SA stripe rust pathotypes. The gene has also been transferred to the Australian wheat cultivar AvocetS as part of a near-isogenic set of lines, each containing a different *Yr* gene (Mathews, 2005).

2.8.3 Stem rust resistance genes

Breeding for stem rust resistance was encouraged by the major threats from the disease in North America but the disease lost its importance in Europe due to successful resistance breeding (Bartos et al., 2002). Among characterised and identified stem rust resistance genes, the *Sr2* gene is the only non-race specific gene that provides durable resistance to stem rust (Admassu et al., 2009).

Stem rust race TTKSK, commonly known as Ug99, is virulent to many *Sr* resistance genes. Ug99 was detected in Uganda in 1998 and characterised in 1999 (Pretorius et al., 2000). The strain posed a threat to wheat production globally and has led to the foundation of the Borlaug Global Rust Initiative (BGRI) (Sharma et al., 2013). Virulence of race Ug99 is known for the resistance genes *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e, Sr9g*, *Sr10*, *Sr11*, *Sr12*, *Sr16*, *Sr17*, *Sr18*, *Sr19*, *Sr20*, *Sr23*, *Sr30*, *Sr31*, *Sr34*, *Sr38,* *SrMcN* and *SrWld-1* (Pretorius et al., 2000; Jin et al., 2008; Sharma et al., 2013). Virulence to these genes is significant because of their current use in agriculture (Jin et al., 2009). Variants of Ug99 with virulence for *Sr24*, *Sr36* and the *Sr* gene in cv. Matlabas have also been reported (Singh et al., 2011, Pretorius et al., 2012b).

2.8.3.1 Sr2

Sr2 is a recessive and slow rusting resistance gene that provides partial resistance with variable levels of disease in adult plants (Singh et al., 2006). It is derived from *T. dicoccum*, situated on the short arm of chromosome 3B, and has conferred durable rust resistance against all races of *P. graminis* including to race Ug99 and its derivatives worldwide for more than 60 years. The gene was originally transferred from the tetraploid emmer wheat (Yaroslav) to the cultivars Hope and H44-24 (Sharp et al., 2001).

Sr2 plays an important role in wheat breeding programmes (Bartos et al., 2002). *Sr2* is phenotypically difficult to select for but pseudo-black chaff (PBC), a dark pigmentation around the stem internodes and glumes, is closely associated with *Sr2* and has been used as a

morphological marker to select for the gene (Hayden et al., 2004). According to Brown (1997) high temperature induced seedling chlorosis (HTISC) is also used as a morphological marker that confirms the presence of *Sr*2. The *Sr2* gene is linked to the leaf rust resistance gene *Lr27* (Sharp et al., 2001) and *Yr7* (Bariana et al., 2001).

Single major genes provide insufficient and short lived resistance due to genetic shifts or the appearance of new virulence (Kaur et al., 2008). *Sr2* in combination with other resistance genes showed effective protection against Ug99 (Todorovska et al., 2009). The gene did not provide sufficient resistance to Ug99 when used alone but showed high levels of resistance when combined with genes such as *Sr25* and other unknown *Sr* genes (Singh et al., 2007). DNA markers closely linked to *Sr2* include a codominant SSR markers Gwm533, codominant STS markers stm598tcac and stm559tgag and a CAPS marker csSr2 (Hayden et al. 2004; Mago et al., 2005; Bariana et al., 2007; Pretorius et al., 2012b). However, Spielmeyer et al. (2003) reported that marker Gwm533, amplifying a fragment of 120 bp, gave false results, hence it is seen as not being reliable in screening for *Sr2*.

2.8.3.2 Sr26

Sr26 is a translocation from *Agropyron elongatum* to chromosome 6AL of wheat (Knott, 1961; 1968). *Sr26* has not been widely deployed in commercial wheat varieties due to negative effects on yield (The et al., 1988). However, Mago et al. (2005) reported that wheat lines that contain reduced segments of the *Sr26* translocation are available. The presence of the *A. elongatum* segment that contains the stem rust resistance had small but additive effects in reducing leaf and yellow rust severities (Singh et al., 2005). *Sr26* is closely linked to the dominant STS DNA marker Sr26#43 that amplifies a 207 bp fragment (Mago et al., 2005). Eagle was the first Australian wheat variety carrying *Sr26* (Martin, 1971). *Sr26* is considered one of the effective genes against race Ug99 and its derivatives and thus plays an important role in breeding for effective stem rust resistance (Joshi et al., 2008). The typical low infection type range for *Sr26* in seedlings is 0; to 2- (McIntosh et al., 1995).

2.9 Fusarium head blight (FHB)

2.9.1 General information

FHB is mainly caused by *Fusarium graminearum* Schwabe [anamorph: *Gibberella zeae* Schwein (Petch)] and is one of the most important fungal wheat diseases worldwide (Yang et al., 2005). The disease negatively affects yield and grain quality by damaging wheat kernels and contaminating the grain with mycotoxins (Gilbert and Tekauz, 2000). It mainly favours warm and moist weather conditions (Rubella and Kistler, 2004). Optimal conditions for infection and spreading of the disease are warm temperatures between 22°C and 26°C accompanied by high humidity (Teich, 1989).

2.9.2 Symptoms and disease development on host plants

The fungus survives and reproduces in crop residues that remain on the soil surface and is dispersed by wind or rain to wheat crops. Crops are susceptible to infection during the flowering period but infection can still progress during kernel development (McMullen et al., 2008). The first symptoms on infected plants vary from purple to black necrotic lesions on the base of florets and on glumes. As the infection progresses, the diseased spikelets become light tan or bleached in appearance (Rubella and Kistler, 2004). These bleached heads are noticeable on a susceptible variety (Figure 2.4).

The fungus may continue to infect the stem (peduncle) below the head causing a brown/purplish discolouration. The infected kernels are shrivelled, light in weight and dull greyish or pinkish. These kernels sometimes are called tomb-stones because of their angular and dusty outer shell. However, if infection occurs late in kernel development, infected kernels are normal in size, but have a dull appearance (Lin et al., 2004).

Figure 2.4 *Fusarium graminearum* infection in wheat: note the bleached heads. (Photo by WM Kriel)

2.9.3 Economic importance worldwide and in South Africa

FHB can cause yield losses up to 70% under favourable conditions. Losses result from shrivelled kernels with lighter weight (Bai et al., 2000). Infected grain may also germinate poorly, resulting in seedling blight and a poor crop stand. Quality reductions may also occur if fungal toxins (mycotoxins) are produced in infected seed. The toxin reduces the grade quality at the market (Bai and Shaner, 2004). Rubella and Kistler (2004) stated that these effects cause problems in marketing and processing of infected wheat grain. Apart from yield and quality reduction, FHB also produces different trichothecene mycotoxins, such as deoxynivalenol (DON), that make wheat grain hazardous for consumption as food or animal feed (Buerstmayr et al*.*, 2002; Shi et al., 2008).

FHB was first described in 1884 in England as a threat to both wheat and barley (Muriuki, 2001). In SA the disease was first detected in 1980. The main species that cause FHB in SA are *F. graminearum*, *F. culmorum* (Wm. G.Sm) Sacc and *F. crookwellense* (Burgess, Nelson and Toussoun). The first two are associated with a warmer climate and the latter with cooler climates (Kriel and Pretorius, 2006). Epidemics of FHB result in devastating economic losses

to the wheat industry and this suggests a need for more resistance sources (McMullen et al., 1997).

2.9.4 Important resistance sources used in breeding for Fusarium head blight resistance in the current study

Five resistance mechanisms to FHB in wheat were discussed by Mesterhazy (1995) with type I representing defence to first disease attack, type II representing defence to colonisation, type III representing defence for accumulation of mycotoxins, type IV representing defence for kernel infection and type V representing tolerance. Availability of resistance genes for breeding FHB resistant cultivars can reduce losses to FHB disease (Shen et al., 2003). Buerstmayr et al. (2003) stated that type I resistance works against initial infection and is usually measured by the number of infected spikelets. Certain morphological characters appear to enable the plant to escape initial infection (Parry et al., 1995).

The most widely used defence mechanism is type II because it is easy to evaluate under monitored environments (Shi et al., 2008). Other types of resistance are known to be present and protect some wheat lines despite the presence of FHB (McMullen et al., 2008). According to Gilbert and Tekauz (2000) resistance types III, IV and V are difficult to manipulate and/or expensive to screen for and are not being used that often in breeding programmes.

FHB resistance is a quantitative trait in wheat and is affected by environmental effects such as temperature, humidity, plant development stage and abundance of inoculum (Parry et al., 1995; Ma et al., 2006). It is a difficult trait to select for because of its low heritability and the amount of resources required to correctly assess performance (Bourdoncle and Ohm, 2003). Fortunately, the genetics of FHB resistance in wheat are becoming more clear and there is a better understanding of the genome for manipulation (Somers et al., 2005).

Breeding for FHB resistance can minimise losses in yield and quality (Anderson et al., 2001). The Chinese cultivar Sumai 3, Brazilian cultivar Frontana, Romanian cultivar F201R and the

Korean line Chokwang show resistance to FHB infection (Shen et al., 2003; Yang et al., 2005). Popular sources for FHB resistance include Nobeokabouzu, Bozu, Sumai 3 and Beijing 8 but these cultivars do not have good agronomic traits (Bartos et al., 2002). Sumai 3 is most often used as a resistance resource of FHB in wheat breeding around the world (Handa et al., 2008).

2.9.5 Breeding for Fusarium head blight resistance and marker-assisted selection

Breeding for FHB resistance using conventional methods is possible but time consuming and expensive (Buerstmayr et al., 2003). Hence, DNA-based markers provide techniques that may be used to support conventional breeding, especially for traits that are difficult to select for (Somers et al., 2005). Molecular markers have been identified and linked to QTL associated with various types of FHB resistance, mainly in Sumai 3 (Anderson et al., 2001; Buerstmayr et al., 2002) and application of MAS in breeding for FHB resistance is considered a valuable tool for accelerating and increasing progress of breeding programmes (Matilda, 2006).

2.9.6 Fusarium head blight resistance quantitative trait loci used in the current study

More than 100 QTL linked to FHB resistance have been reported and reviewed in wheat and the QTL utilised most often are those on chromosomes 3BS (*Fhb1*), 5AS (*Qfhs.ifa-5A*) and 6BS (*Fhb2*) (Buerstmayr et al., 2009).

2.9.6.1 Fhb1/ Qfhs.ndsu-3BS

A major QTL, *Qfhs.ndsu-3BS,* is responsible for type II FHB resistance and has been derived from the cultivar Sumai 3 (Liu and Anderson, 2003). *Qfhs.ndsu-3BS* is located on chromosome 3BS and explained 15% to 60% of the FHB phenotypic variation in different mapping populations of Sumai 3 and Wangshuibai (Buerstmayr et al., 2002). *Qfhs.ndsu-3BS*

appeared to be associated mainly with resistance to fungal spread (Buerstmayr et al., 2003). It was originally mapped and verified using RFLP analysis. Molecular marker analysis using microsatellite markers suggested that the QTL was positioned between microsatellite markers Bar133, Gwm493 and Gwm533 (Anderson et al., 2001). Further studies were conducted to increase marker density in the chromosome region which aided the fine mapping of the resistance gene (Liu and Anderson, 2003). Liu et al. (2006) indicated that *Fhb1* mapped to a similar position as the *Qfhs.ndsu-3BS* gene and was re-designated as *Fhb1*. Buerstmayr et al. (2009) stated that the *Fhb1* gene provides durable FHB resistance and was found in mapping studies based on resistant Chinese sources. Zhou et al. (2002) reported that the QTL on chromosome 3B is associated with low levels of DON accumulation within kernels.

2.9.6.2 Qfhs.ifa-5A

The *Qfhs.ifa-5A* QTL is responsible for type I FHB resistance and is located on chromosome 5A (Buerstmayr et al., 2002). It was derived from the wheat cultivars Sumai 3 and CM-82036 (Zhou et al., 2003). *Qfhs.ifa-5A* has a small impact on FHB resistance when used alone. A combination of *Fhb1* and *Qfhs.ifa-5A* provided slightly higher resistance compared to resistance shown by *Fhb1* or *Qfhs.ifa-5A* alone (Salameh et al., 2011). Molecular marker analysis using SSRs indicated that *Qfhs.ifa-5A* was located between locus pair Gwm304 and Xgwm293 on chromosome 5A (Anonymous, 2007). *Qfhs.ifa-5A* explained 20% of the phenotypic variation for FHB severity and is associated with resistance to fungal penetration (Buerstmayr et al., 2003).

2.9.6.3 Qfhs.ndsu-3AL

The major QTL *Qfhs.ndsu-3AL* is associated with type I FHB resistance and was originally detected in Frontana on chromosome 3A (Chen et al., 2003). The QTL was mapped between the intervals of marker Gwm720 and DuPw227. It is associated mainly with reducing initial fungal infection but it is reported to have minor effect on the spread of the disease (Steiner et al., 2004).

2.9.6.4 Qfhs.ndsu-6B

QTL *Qfhs.ndsu-6B* is a major type II QTL for FHB resistance and was detected on the short arm of chromosome 6B in Sumai 3, close to the centromere (Anderson et al., 2001). This QTL is also known as *Fhb2* (Waldron et al., 1999). The QTL region is flanked by SSR markers Gwm133 and Gwm644 and the QTL on chromosome 6B was found to be closer to the marker Gwm644. *Fhb2* provides FHB resistance as a single gene in susceptible cultivars (Cuthbert et al., 2007).

2.9.6.5 QTL on chromosome 7A

The QTL on chromosome 7A is a minor type II QTL for FHB resistance and was detected on chromosome 7A in Sumai 3 and Wangshuibai (Zhou et al., 2002; Shi et al., 2008). The QTL on chromosome 7A has been reported to have significant effects of resistance to the spread of FHB and to reduce DON content within kernels and is detected using SSR markers Gwm60 and Barc49 (Zhou et al., 2002).

2.9.7 FHB resistant sources used in the study

2.9.7.1 CM-82036

The line CM-82036-1TP-10Y-OST-10Y-OM-OFC, abbreviated as CM-82036, is associated with type II FHB resistance and originated from a cross between Sumai 3 and Thornbird and was developed in a shuttle breeding programme between CIMMYT Mexico and South-America (McCartney et al., 2005). The major QTL associated with FHB resistance is located on chromosome 3B (*Qfhs.ndsu-3BS* or *Fhb1)* explaining 60% of the phenotypic variance at marker interval Gwm533-Gwm493 followed by QTL located on chromosomes 5A (*Qfhs.ifa-*5A) and 1B. CM-82036 is a donor of *Fhb1* (type II) and *Qfhs.ifa-5A* (type I). Combination of these two genes increases FHB resistance in derivatives of CM-82036 spring wheat (Salameh et al., 2011). Previous studies indicated that the QTL detected on chromosome 6B of Sumai 3 was not transferred to CM-82036 (Buerstmayr et al., 2002).

2.9.7.2 Frontana

The Brazilian cultivar Frontana originated from a cross between Fronteira and Mentana (Liu and Anderson, 2003). Frontana is a source of type I FHB resistance which is based on morphological traits though there are no published results to support this hypothesis (Buerstmayr et al., 2009). The important QTL associated with FHB resistance in Frontana was mapped to chromosome 3A explaining 16% of the phenotypic variation between the Gwm720-Dupw227 interval, followed by smaller effects on chromosomes 1B, 2A, 2B, 4B, 5A and 6B (Steiner et al., 2004).

2.9.8 Molecular markers and plant breeding

Plants have been differentiated based on morphological characteristics in traditional breeding (Khalighi et al., 2008). Traditional breeding involves the introduction of both genes of interest and undesirable characters into cultivated plants (Farooq and Azam, 2002). It is a lengthy process because it requires backcrossing to ensure transfer of desirable traits. However, the use of molecular markers has become familiar in crop improvement (Wenzel, 2006). The ever increasing demand for wheat has encouraged the use of molecular marker technologies to enhance wheat production (Landjeva et al., 2007). Molecular markers in plant breeding improve the effectiveness of conventional plant breeding by indirect selection of only the traits of interest for both simple traits and QTL (Gupta et al., 2010).

Molecular markers offer many opportunities for breeding programmes (Bartos et al., 2002). Molecular markers are based on the recognition of polymorphisms in the DNA sequence (Feuillet and Keller, 2004). In resistance breeding they are used to identify genes linked to the desirable traits in varieties whose genetic background is known or unknown and to verify the transfer of such genes into elite genotypes (Vida et al., 2009). According to Castro et al. (2003) molecular markers are useful in developing cultivars with pyramided resistance genes. Hence, the advantage of molecular marker technologies for genotypic screening is the use and selection of multiple genes in a population, characterisation of species and fingerprinting (Rafalski et al., 1996; Anderson, 2007).

Molecular markers that have been used in plant breeding include RFLP, random amplified polymorphic DNA (RAPD), CAPS, AFLP, SSR and SNP (Somers, 2004). A specific RAPD, RFLP or AFLP fragment linked to the gene of interest may be used and converted to SCAR markers for more effective detection of polymorphism (Borner et al., 2002). Two types of markers have been used; the old system that depends on hybridisation and the newer system that uses the polymerase chain reaction (PCR) to amplify DNA (Karp et al., 1997). DNA-based markers detect small differences (Somers, 2004; Khalighi et al., 2008) and have been utilised for mapping genes of interest in cereal crops (Lapitan and Jauhar, 2006).

2.9.8.1 Restriction fragment length polymorphism

RFLPs were the initial neutral molecular marker technique developed by Botstein et al. (1980) to be applied in genome mapping (Tanksley et al., 1989). RFLP is a codominant marker that is based on DNA hybridisation. Genomic DNA is digested by restriction enzymes to detect differences in DNA fragment sizes between individuals (Varshney et al., 2004). This marker type is characterised by a low level of polymorphism and is expensive and slow which resulted in few RFLP maps in self-pollinated crops (Cao et al., 2002).

2.9.8.2 Random amplified polymorphic DNA

RAPD is a dominant marker based on PCR that has been used for genome mapping and characterisation. The technique was developed by Welsh and McClelland (1980) and Williams et al. (1990). RAPD uses a single PCR primer designed at random to amplify several different regions of the genome (Gupta et al., 1999). Najimi et al. (2002) stated that RAPD technology has been useful for several crops but its use in wheat has been limited by low levels of polymorphism and a lack of reproducibility due to low annealing temperatures (Varshey et al., 2004).

2.9.8.3 Amplified fragment length polymorphism

AFLP is a DNA marker analysis system based on the use of PCR and restriction enzyme analyses. The AFLP technique is based on a combination of RFLP and RAPD analyses but discriminates a higher number of polymorphisms within a short period of time. It is

considered a robust marker technique that is efficient and reproducible (Vos et al., 1995). It has been used for mapping and gene tagging in plants with large genomes and low polymorphism rates such as wheat (Gupta et al., 1999). It can be used to generate better information in a single analysis using a small amount of DNA (Farooq and Azam, 2002). However, the technique is costly and takes a longer time than other PCR techniques (Varshney et al., 2004).

2.9.8.4 Microsatellites or simple sequence repeats

Microsatellites or SSRs are codominant DNA markers based on PCR and are present in eukaryotic genomes (Chen et al., 2003). They are short tandem repeats of about two to six bp (Gupta et al., 1999). These repeats are present throughout plant and animal genomes that show high levels of polymorphism based on the number of tandemly repeated units of a locus (Jonah et al., 2011). The tandem repeated base pair units are arranged in repeats of mono, di-, tri-, tetra, penta and hexa nucleotides with different lengths of repeat motifs (Farooq and Azam, 2002).

Variation in the number of repeat units results in highly polymorphic banding patterns (Farooq and Azam, 2002) which are detected by PCR, using locus specific flanking region primers. The difference is brought by mutation in the sequence of base pairs. Sequence polymorphisms in the loci occur by insertion or deletion of one or more repeat units (Tautz and Renz, 1984). Mutation rates are high ranging from 10^3 to 10^6 per generation. SSRs are useful in genome evolution by creating and maintaining genetic variation because of their high mutability (Weber and Wong, 1993). Studies have shown that the rate of slippage during the replication of simple DNA sequences is dependent on the nature of the repeat sequence (Schotter and Tautz, 1992). Gupta et al. (1999) found dinucleotides units to be longer and more frequent than the other sequence nucleotide motifs in their study. In contrast, Gao et al. (2003) stated that trinucleotides were the most abundant repeats in rice, wheat, maize and soybean, with rice being the highest.

SSRs have been considered a useful marker technology, with the advantages of being efficient, stable, codominant, abundant and distributed evenly throughout eukaryotic genomes and with higher levels of polymorphism than other molecular techniques (Holton, 2001).

Their nature of being heritable makes them important for monitoring gene flow, for screening larger populations and forensics. SSRs may even be used across species and genus boundaries (Farooq and Azam, 2002).

In wheat SSR markers have been used to tag several genes (Gupta et al., 2010). Molecular maps are available to advance gene tagging (Röder et al., 1998; Stephenson et al., 1998). However, SSRs are expensive to develop and not economic for developing complex maps (Karaoglu et al., 2005).

2.9.8.5 Sequence characterised amplified region/ Sequence tagged site markers

SCARs are genomic DNA bands on a distinct locus that has been defined genetically and identified by PCR amplification using a pair of specific oligonucleotide primers (McDermoth et al., 1994). SCARs are markers that are defined by the exact primer location and sequence and can be amplified by PCR (Farooq and Azam, 2002). They are distinguished by a pair of PCR primers and developed from markers identified with other techniques such as AFLP, RAPD or RFLP (Gupta et al., 1999). SCARs are either dominant or codominant. The products of SCARs are subjected to restriction enzymes if there is no variation among products being screened. Utility of the technique is limited by a need to sequence fragments prior to designing of primers. SCARs are vigorous but characterised by low levels of polymorphism (Farooq and Azam, 2002).

2.9.8.6 Single nucleotide polymorphism

SNPs are one of the newest marker technologies used in plant breeding. It is a codominant genetic marker based on the use of PCR. They are abundant and detect DNA sequence polymorphisms. The technique detects a single base difference or small insertions or deletions (indels) in a DNA sequence (Vignal et al., 2002). However, SNPs are less informative with lower levels of polymorphism than SSR markers but are highly utilised for rapid genotyping and MAS (Bernardo et al., 2012). Lai et al. (2012) used the technique to identify 38 928 SNPs between three wheat cultivars: Excalibur, RAC875 and Kukri which enabled the selection of differences within genes of interest. Matsuda et al. (2012) also used SNP in wheat for mapping of the hybrid necrosis gene *Net2*, located on chromosome 2D. The mapping population was generated from a cross between KU-2075 and KU-2025. Fifteen cDNAs were polymorphic between the two populations KU-2075 and KU-2025.

2.9.9 Molecular marker-assisted selection

Molecular MAS is the process of selecting desirable phenotypic characters in plants based on DNA fragments or information linked to molecular markers (Jonah et al., 2011). Morphological markers are available for selection of traits of interest in breeding programmes but the method alone is not reliable. Selection of plants based on DNA banding patterns is reliable and is currently an attractive tool for plant breeders. However, combining both traditional and MAS methods improves the accuracy and efficacy in selection of desirable traits (Bariana et al., 2007) by shortening the breeding process (Šliková et al., 2003).

There are several advantages of using MAS over selection using morphological characteristics only. Selection of traits can be done during the developmental stage before the plant reaches maturity (Collard et al., 2005). With the use of MAS, screening can be done for desirable genes in segregating progeny populations (Vida et al., 2009). MAS facilitates combining and selection of a number of important characters at the same time (Haussmann et al., 2001; Singh et al., 2001) and it is possible to screen for complex traits within a short period of time (Somers, 2004). Heterozygotes and homozygotes can be easily discriminated with the use of codominant marker screening (Chen et al., 2003).

MAS enables the selection of varieties at early stages and limits assumptions that crosses have been successful (FAO/IAEA, 2008). MAS involves the use of different molecular markers to detect variation in DNA banding patterns. Unfortunately there is no perfect marker technique for all studies. Therefore a choice of the ideal technique depends on the objective of study but a good marker should be closely linked to the gene of interest, reliable, polymorphic, simple to generate and interpret and allow high throughput analysis (Varshney et al., 2004).

2.10 Reports on combining different resistance genes in wheat using marker-assisted selection

2.10.1 Combining leaf rust resistance genes

A few reports on combining rust and FHB resistance genes into a single genotype are available in wheat (Singh et al., 2011). Some examples of combining resistance genes for various crops are given in Table 2.1. Šlikova et al. (2004) as well as Singh et al. (2004) combined leaf rust resistance genes *Lr19* and *Lr24* using MAS and both studies were successful in enhancing leaf rust resistance. Singh et al. (2004) used the lines Thatcher NIL and Inia66//CMH81A575 for transferring *Lr19* and the genotypes Arkan, Blue Boy II, Agent and CII7907 for the gene *Lr24*.

2.10.2 Combining Fusarium head blight resistance quantitative trait loci

Singh et al. (2011) summarised the use of MAS in FHB resistance breeding. Miedaner et al. (2006) and Wilde et al. (2007) used MAS to combine two QTL from CM-82036 located on chromosomes 3B and 5A and one QTL on chromosome 3A donated by Frontana. The aim of the studies was to improve FHB resistance and reduce DON content. Another study was done by Shi et al. (2008) for multiple FHB resistance. A pyramided FHB line WSY from a cross between Sumai 3, Wangshuibai and Nobeokabouzu was analysed for accumulated FHB resistance QTL. WSY tested positive for the QTL on chromosomes 1BL, 2AS, 2BL, 2DS, 3AS, 5AS, 6BS and 7AL. Wilde et al. (2008) and Miedaner et al. (2009) combined the QTL *Qfhs.lfl-6AL* and *Qfhs.lfl-7BS* from the cultivar Dream and QTL on chromosome 2BL from line G16-92. The double cross population generated was subjected to FHB infection to compare phenotypic and marker selection. Miedaner et al. (2009) concluded that QTL effects for FHB resistance are small hence combining many alleles using MAS is important to increase resistance.

Table 2.1 Examples of successful combining of major genes and/or QTL into single wheat genotypes using marker-assisted gene combining (modified table from Gupta et al., 2010)

Traits	**Number of genes/QTL**	**Targeted genes/QTL**	**Marker type**	**References**
Leaf rust resistance	2 genes	*Lr19, Lr24*	STS	Singh et al. (2004)
Leaf rust resistance	2 genes	*Lr19, Lr24*	*Ep-D1c*, STS	Šliková et al. (2004)
FHB resistance	3 QTL	3A, 3B, 5A	SSR	Miedaner et al. (2006)
FHB resistance and DON content	3 QTL	3A, 3B, 5A	SSR	Wilde et al. (2007)
FHB resistance	Multiple resistance	1BL, 2BL, 5AS, 7AL, 2AS, 2DS, 3AS, 6BS	SSR	Shi et al. (2008)
FHB resistance	3 QTL	*Qfhs.lfl-6AL, Qfhs.lfl-7BS,* 2BL QTL	SSR	Wilde et al. (2008)
FHB resistance	3 QTL	*Qfhs.lfl-6AL, Qfhs.lfl-7BS,* 2BL QTL	SSR	Miedaner et al. (2009)

FHB = Fusarium head blight; QTL = quantitative trait loci; DON = deoxynivalenol; STS = sequence tagged site; SSR = simple sequence repeat

2.11 Conclusion

Based on this literature review, wheat is recognised as one of the important cereal crops worldwide. It is valued mainly for consumption for both humans and animals. The current tonnage of wheat produced is not enough to meet the high demand. This implies that there should be an increase in wheat production universally. However, the three rust diseases (leaf, stem and stripe rust) threaten global wheat production. The situation is worsened by the introduction of new rust races such as stem rust race Ug99. On the other hand FHB is a disease that contributes to the loss in yield and quality of wheat. Breeding for resistance against these diseases has been done but there is still a need for developing new and improved cultivars. Combining resistance genes into a single genotype can result in durable resistance against these diseases. The traditional breeding methods plus the use of molecular techniques allow selection of new cultivars carrying desirable traits within a shorter period of time. The current study will therefore involve combining rust and FHB resistance genes and/or QTL in a single genotype. As indicated in the literature review, the simplicity of using informative SSR on large populations makes it a suitable marker for this study.

2.12 References

Admassu B, Lind V, Friedt W, Ordon F (2009) Virulence analysis of *Puccinia gramini* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathology 58:362-369

Anderson JA (2007) Marker-assisted selection for Fusarium head blight resistance in wheat. International Journal of Food Microbiology 119:51-53

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theoretical and Applied Genetics 102:1164-1168

Anonymous (2007) Evaluation of genetic diversity of Fusarium head blight resistance in European winter wheat. Institution of Agricultural and Fisheries Research. Plant Sciences, Melle, Belgium, pp 1-3

Bai GH, Shaner GE (2004) New Fusarium head blight resistance sources from Asian wheat. Annual Review of Phytopathology 42:135-161

Bai GH, Shaner G, Ohm H (2000) Inheritance of resistance to *Fusarium graminearum* in wheat. Theoretical and Applied Genetics 100:1-8

Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lu M (2007) Breeding triple rust resistance wheat cultivars for Australia using conventional and marker-assisted selection technologies. Australian Journal of Agricultural Research 58:576-587

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA (2001) Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Australian Journal of Agricultural Research 52:1247-1255

Bartos P, Sip V, Chrpova J, Vacke J, Stuchlikova E, Blazkova V, Sarova J, Hanzalova A (2002) Achievements and prospects of wheat breeding for disease resistance. Czech Journal of Genetics and Plant Breeding 38:16-28

Bernardo AN, Ma H, Zhang D, Bai G (2012) Single nucleotide polymorphism in wheat chromosome region harboring *Fhb1* for Fusarium head blight resistance. Molecular Breeding 29:477-488

Bonjean AP, Angus WJ (2001) The World Wheat Book - A History of Wheat Breeding. Lavoisier Publishing, France, pp 4-94

Borner A, Schumann E, Furste A, Coster H, Leithold B, Roder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 105:921-936

Boshoff WHP, Pretorius ZA, van Niekerk BD (2002) Establishment, distribution and pathogenicity of *Puccinia striiformis* f. sp. *tritici* in South Africa. Plant Diseases 86:485-492

Bossolini E, Krattinger S, Keller B (2006) Development of simple sequence repeat markers specific for the *Lr34* resistance region of wheat using sequence information from rice and *Aegilops taushii*. Theoretical and Applied Genetics 113:1049-1062

Botstein D, White RL, Scholnick M, Davis RW (1980) Construction of genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314-331

Bourdoncle W, Ohm HW (2003) Quantitative trait loci for resistance to Fusarium head blight in recombinant inbred wheat lines from the cross Huapei 57-2/Patterson. Euphytica 131:131-136

Bowden RL (2006) Wheat stripe rust. Department of Pathology. Kansas State University. http://www.oznet.ksu.edu/pathext/factSheets/Wheat/Wheat%20Stripe%20Rust.asp (Cited November 2008)

Brown GN (1997) The inheritance and expression of leaf chlorosis associated with gene *Sr2* for adult plant resistance to wheat stem rust. Euphytica 95:67-71

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breeding 128:1-26

Buerstmayr H, Lemmens L, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theoretical and Applied Genetics 104:84-91

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theoretical and Applied Genetics 107:503-508

Bureau for Food and Agricultural Policy (2011) The South African Baseline, pp 1-82. http://www.bfap.co.za (Cited March 2012)

Cao J, Zhao JZ, Tang DJ, Shelton MA, Earle ED (2002) Broccoli plants with pyramided *cry1Ac* and *cry1C Bt* genes control diamondback moths resistant to Cry1A and Cry1C proteins. Theoretical and Applied Genetics 105:258-264

Castro AJ, Chen X, Hayes PM, Mundt C, Johnston M (2003) Pyramiding qualitative trait locus (QTL) alleles determining resistance to barley stripe rust: Effects on seedling stage. Crop Science 43:651-659

Chahal GS, Gosal SS (2002) Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches. CRS press Narosa Publishing House India, 604 pp

Chelkowski J, Stepien L (2001) Molecular markers for leaf rust resistance genes in wheat. Journal of Applied Genetics 42:117-126

Chen XM (2005) Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Canadian Journal of Plant Pathology 27:314-337

Chen X, Soria AM, Yan G, Sun J, Dubcovysky J (2003) Development of sequence tagged and cleaved amplified polymorphic sequence markers for wheat stripe rust resistance gene *Yr5*. Crop Science of America 43:2058-2064

Cherukuri DP, Gupta SK, Charpe A, Kou S, Prabhu KV, Singh RB, Haq QMR, Chauhan SVS (2003) Identification of a molecular marker linked to an *Agropyron elongatum*-derived gene *Lr19* for leaf rust resistance in wheat. Plant Breeding 122:204-208

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169-196

Cook RJ, Veseth RJ (1991) Wheat Health Management. American Phytopathology Society, United States of America, pp 41-59

Cornell HJ, Hoveling AW (1998) Wheat Chemistry and Utilization. Technomic Publishing Company, United States of America, pp 1-13

Curtis BC, Rajaram S, Macpherson HG (2002) Bread Wheat Improvement and Production No. 30 Food and Agriculture Organisation of the United Nations, Rome Italy, pp 552-560

Cuthbert PA, Somers DJ, Brule-Babel A (2007) Mapping *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 114:429-437

Dyck PL (1987) The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467-469

Dyck PL, Samborski DJ, Anderson RG (1966) Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. Canadian Journal of Genetics and Cytology 8:665-671

Eckardt NA (2006) Identification of rust fungi avirulence elicitors. Plant Cell 18:1-3

FAO (2006). http://www. faostat.fao.org (Cited November 2011)

FAO/IAEA (2008) Plant Breeding and Genetics. http://www.iaea.org/nata/d2-cropquality.html (Cited March 2008)

Farooq S, Azam F (2002) Molecular markers in plant breeding-1: Concepts and characterization. Pakistan Journal of Biological Sciences 5:1135-1140

Feuillet C, Keller B (2004) Molecular markers for disease resistance, the example of wheat In: Lorz H, Wenzel G (Eds) Biotechnology in Agriculture and Forestry, volume 55. Molecular markers, Springer-Verlag Berlin Heidelberg, pp 290-294

Flor HH (1971) The current status of gene-for-gene concept. Annual Review of Phytopathology 9:275-296

Gao L, Tang J, Li H, Jia J (2003) Analysis of microsatellites in major crops assessed by computational and experimental approaches. Molecular Breeding 12:245-261

Gepts P, Hancock J (2006) The future of plant breeding. Crop Science 46:1630-1634

German SE, Kolmer JA (1992) Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. Theoretical and Applied Genetics 84:97-105

Gilbert J, Tekauz A (2000) Review: Recent developments in research on Fusarium head blight in Canada. Canadian Journal of Plant Pathology 22:1-8

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present and future possibilities. Molecular Breeding 26:145-161

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390

Gupta SK, Charpe A, Prabhu K, Haque Q (2006) Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theoretical and Applied Genetics 113:1027-1036

Guttierin MJ, Stark JC, O'Brien K, Souza E (2001) Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. Crop Science 41:327-335

Han FP, Fedak G, Ouellet T, Dan H, Somer DJ (2005) Mapping of genes expressed in *Fusarium graminearum* infected heads of wheat cultivar Frontana. Genome 48:88-96

Hahn M, Mendgen K (2001) Signal and nutrient exchange at biotrophic plant-fungus interfaces. Plant Biology 4:322-327

Handa H, Namiki N, Xu D, Ban T (2008) Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Molecular Breeding 22:71-84

Hannon P (2012) South Africa looks far and wide for long-term growth. African Agriculture Review Nedbank Capital September 3:7

Haussmann BIG, Hess DE, Omanya GO, Reddy BVS, Welz HG, Geiger HH (2001) Major and minor genes for stimulation of *Striga hermonthica* seed germination in sorghum, and interaction with different striga populations. Crop Science 41:1507-1512

Hayden MJ, Kuchel H, Chalmers KJ (2004) Sequence tagged microsatellites for the Xgwm533 locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 109:1641-1647

Holton TA (2001) Plant Genotyping by Analysis of Microsatellites. CAB International Australia, pp 15-18

Hovmøller MS, Sorenson CK, Walter S, Justesen, AF (2011) Diversity of *Puccinia striiformis* on cereals and grasses. Annual Review of Phytopathology 49:197-217

http://www/graingenes (Cited January 2014)

http://www.sagis.org.za/Flatpages (Cited January 2014)

Jauhar PP (2006) Modern biotechnology as an integral supplement to conventional plant breeding: The prospects and challenges. Crop Science 46:1841-1859

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R, Fetch T Jr (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis*. f. sp. *tritici*. Plant Disease 92:923-926

Jin Y, Szabo LJ, Rouse MN, Fetch T Jr, Pretorius ZA, Wanyera R, Njau P (2009) Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici.* Plant Disease 93:367-370

Johnson R, Jellies GJ (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3-22

Jonah PM, Bello LL, Lucky O, Midau A, Moruppa SM (2011) Review: The importance of molecular markers in plant breeding programmes. Global Journal of Science Frontier Research 11:4-12

Joshi AK, Mishara B, Prashar M, Tomar SMS, Singh RP (2008) Ug99 race of stem rust pathogen: challenges and current status of research to sustain wheat production in India. Indian Journal of Genetics 68:1-11

Karaoglu H, Lee CMY, Meyer W (2005) Survey of simple sequence repeats in completed fungal genomes. Molecular Biology and Evolution 22:639-649

Karp A, Kresovich S, Bhat KV, Ayand WG, Hodgkin T (1997). Molecular tools in plant genetics resources conservation: a guide to the technologies. IPGRI Technology, Bulletin No. 2

Kaur S, Bansal UK, Khanna R, Saini RG (2008) Genetics of leaf and stripe rust resistance in a bread wheat cultivar Tonichi. Journal of Genetics 87:191-194

Khalighi M, Arzani A, Poursiahbidi MA (2008) Assessment of genetic diversity in *Triticum* spp. and *Aegilops* spp. using AFLP markers. African Journal of Biotechnology 7:546-552

Knott DR (1961) The inheritance of rust resistance. VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. Canadian Journal of Plant Sciences 41:109-123

Knott DR (1968) Translocations involving *Triticum* chromosomes and *Agropyron* chromosomes carrying rust resistance. Analytical Journal of Genetic Cytology 10:695-696

Knott DR (1989) The wheat rusts: breeding for resistance. Springer, Berlin, pp 76-125

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Liselotte L, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360-1363

Kriel WM, Pretorius ZA (2006) Fusarium head blight: a summary of the South African situation. Proceedings of the 2005 National Fusarium head blight Forum, Milwaukee, Wisconsin, United States of America, pp 243-245

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theoretical and Applied Genetics 119:889-898

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene in wheat. Theoretical and Applied Genetics 114:21-30

Lai K, Duran C, Berkman PJ, Lorenc MT, Jiri S, Manoli S, Hayden M, Forrest KL, Fleury D, Baumann U, Zander M, Mason AS, Batley J, Edwards D (2012) Single nucleotide polymorphism discovery from wheat next-generation sequence data. Plant Biotechnology Journal 10:743-749

Landjeva S, Korzun V, Bomer A (2007) Molecular markers: actual and potential contributions to wheat genome characterization and breeding. Euphytica 156:271-296

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001) Trends in genetic and genome analyses in wheat: a review. Australian Journal of Agricultural Research 52:1043-1077

Lapitan N, Jauhar PP (2006) Molecular markers, genomics and genetic engineering in wheat. In: Singh RJ, Jauhar PP (Eds) Genetic Resources, Chromosome Engineering and Crop Improvement: Cereals. Taylor and Francis Group, LLC, United States of America, pp 99-109

Leonard KJ, Szabo LJ (2005) Rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6:99-111

Li X, Yang W, Li Y, Liu D, Yan H, Meng Q, Zhang T (2006) A SSR marker for leaf rust resistance gene *Lr19* in wheat. Agricultural Sciences in China 5:111-115

Lin F, Kong ZX, Zhu HL, Xue SL, Wu JZ, Tian DG, Wei JB, Zhang CQ, Ma ZQ (2004) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 × Wangshuibai population. I. Type II resistance. Theoretical and Applied Genetics 109:1504-1511

Liu F, Niu Y, Deng H, Tan G (2007) Mapping of a major stripe rust resistance gene in Chinese native wheat variety Chike using microsatellite markers. Journal of Genetics and Genomics 34:1123-1130

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microlinearity among wheat, rice and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Functional and Integrated Genomics 6:83-89

Liu S, Anderson JA (2003) Marker assisted evaluation of Fusarium head blight wheat germplasm. Crop Science 43:760-766

Lupton FGH (1987) Wheat Breeding. Chapman and Hall, London, 418 pp

Ma HX, Zhang KM, Gao L, Bai GH, Chen HG, Cai ZX, Lu WZ (2006). Quantitative trait loci for resistance to Fusarium head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. Plant Pathology 55:739-745

Mago R, Bariana HS, Dundas IS, Spielmyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse germplasm. Theoretical and Applied Genetics 111:496-504

Marais GF (1992) The identification of a common wheat *Thinopyrun distichum* translocated chromosome with a locus homeoallelic to *Lr19*. Theoretical and Applied Genetics 85:73-78

Martin JH, Leornard WH, Stamp DL (1976) Pricniples of field crop production 3rd edition. Collier Macmillan Publishers London, pp 430-484

Martin RH (1971) Eagle - a new wheat variety. Agricultural Gazette of New South Wales 825:206-207

Mathews LJ (2005) A host pathogen study of stripe rust resistance in *Triticum aestivum.* MSc dissertation, University of the Free State, South Africa, 181 pp

Matilda C (2006) Detection of QTLs linked to Fusarium head blight resistance in Romanian winter wheat. Romanian Agricultural Research Number 23

Matsuda R, Lehisa JCM, Takumi S (2012) Application of real-time PCR-based SNP detection for mapping of *Net2*, a causal D-genome gene for hybrid necrosis in interspecific crosses between tetraploid wheat and *Aegilops tauschii*. Genes and Genetics Systems 87:137-143

McCartney CA, Somers DJ, McCallum BD, Thomas J, Humphreys DG, Menzies JG, Brown PD (2005) Microsatellite tagging of the leaf rust resistance gene *Lr16* on wheat chromosome 2BSc. Molecular Breeding 15:329-337

McDermoth JM, Brandle U, Dutly F, Haemmerli UA, Keller S, Muller KE, Wolf MS (1994) Genetic variation in powdery mildew of barley: development of RAPD, SCAR and VNTR markers. Phytopathology 84:1316-1321

McIntosh RA, Dyck PL, Green GJ (1976) Inheritance of leaf rust and stem rust resistances in wheat cultivars Agent and Agatha. Australian Journal of Agricultural Research 28:37-45

McIntosh RA, Wellings CR, Park RF (1995) Wheat Rusts: An Atlas of Resistance Genes. CSIRO Australia, Kluwer Academic Publishers, Dordrecht, Netherlands, 200 pp

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81:1340-1348

McMullen M, Zhong S, Neate S (2008) Fusarium head blight (Scab) of small grains. Plant diseases management, North Dakota State University Extension Service, pp 804

Mebrate SA, Oerke EC, Dehne HW, Pillen K (2008) Mapping of the leaf rust resistance gene *Lr38* on wheat chromosome arm 6DL using SSR markers. Euphytica 162:457-466

Mesterhazy A (1995) Types and components of resistance to Fusarium head blight of wheat. Plant Breeding 114:377-386

Miedaner T, Wilde F, Korzun V, Ebmeyer E, Shmolke M, Hartl L, Schön CC (2009) Marker selection for Fusarium head blight resistance based on quantitative trait loci (QTL) from two European sources compared to phenotypic selection in winter wheat. Euphytica 166:219-227

Miedaner T, Wilde F, Steiner B, Buerstamayr H, Korzun V, Ebmeyer E (2006) Stacking quantitative trait loci (QTL) for Fusarium head blight resistance from no-adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. Theoretical and Applied Genetics 112:562-569

Mohanan C (2010) Rust fungi of Kerata. Kerata, India: Kerata Forest Research Institute. ISBN 81-85041-72-5, 148 pp

Moldenhauer J, Moerschbacher BM, van der Westhuizen AJ (2006) Histological investigation of stripe rust (*Puccinia striiformis* f. sp. *tritici*) development in resistant and susceptible wheat cultivars. Plant Pathology 55:460-474

Muriuki JG (2001) Deoxynivalenol and nivalenol in pathogenesis of Fusarium head blight in wheat. Thesis, University of Minnesota. 134 pp

Najimi B, Boukhatem N, El Jaafari S, Jlibene M, Paul R, Jacquemin JM (2002) Amplified fragment length polymorphism (AFLP) analysis of markers associated with *H5* and *H22* Hessian fly resistance genes in bread wheat. Biotechnology of Agronomy, Sociology and Environment 6:79-85

Oerke EC, Dehne HW (1997) Global crop production and efficacy of crop protection - current and future trends. European Journal of Plant Pathology 103:203-215

Oerke EC, Dehne HW (2004) Safeguarding production losses in major crops and the role of crop protection. Crop Protection 23:275-285

Panozzo JF, Eagles HA (1998) Cultivar and environmental effects on quality characteristics in wheat. I. Starch. Australian Journal of Agricultural Research 49:755-766

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals - a review. Plant Patholology 44:207-238

Pellegrineschi A, Mclean S, Salgado M, Velazquez L, Hernandez R, Brito RM, Noguera M, Medhurst A, Hoisington D (2001) Transgenic wheat plants: a powerful breeding source. Euphytica 119:133-136

Peterson RF (1965) Wheat - Botany, cultivation and utilization. Grampian Press Ltd, London, pp 5-21

Poehlman JM (1987) Breeding field crops, 3rd Edition. AVI Publishing Company Westport, USA, pp 187-210

Pretorius ZA, Jin Y, Bender CM, Herselman L, Prins R (2012a) Seedling resistance to stem rust race Ug99 and marker analysis for *Sr2*, *Sr24* and *Sr31* in South African wheat cultivars and lines. Euphytica 186:15-23

Pretorius ZA, Pakendof KW, Marais R, Prins R, Komen JS (2007) Challenges for sustainable cereal rust control in South Africa. Australian Journal of Agricultural Research, 58:593-601

Pretoruis ZA, Singh RP, Wagoire WW, Payne TA (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Disease 84:203

Pretorius ZA, Szabo LJ, Boshoff WHP, Herselman L, Visser B (2012b) First report of a new TTKSF race of wheat stem rust (*Puccinia graminis*. f. sp. *tritici*) in South Africa and Zimbabwe. Plant Disease 96:590

Prins R, Marais GF, Pretorius ZA, Janse BJH, Marais AS (1997) A study of modified forms of the *Lr19* translocation of common wheat. Theoretical and Applied Genetics 95:424-430

Prins R, Groenewald JZ, Marais GF, Snape JW, Koebner RMD (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theoretical and Applied Genetics 103:618-624

Prins R, Pretorius ZA, Bender CM, Lehmensiek A (2011) QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega x Avocet S doubled haploid wheat population. Molecular Breeding 27:259-270

Rafalski JA, Vogel JM, Morgante M, Powell MW, Andre C, Tingey SV (1996) Generating and using DNA markers in plants. In: Biren B, Lai E (Eds) Non-mammalian Genomic Analysis: A Practical Guide Academic Press, London, pp 75-134

Ramburan VP, Pretorius ZA, Louw JH, Boyd LA, Smith PH, Boshoff WHP, Prins R (2004) A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar Kariega. Theoretical and Applied Genetics 108:1426-1433

Randhawa HS, Asif M, Pzniak C, Clarke JM, Graf RJ, Fox SL, Humphreys DG, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Hucl P, Spaner D (2013) Application of molecular markers to wheat breeding in Canada. Plant Breeding 132:458-471

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023

Roelfs AP (1988) Resistance to leaf rust in wheat. In: Simmonds NW, Rajaram S (Eds) Breeding Strategies for Resistance to the Rusts of Wheat. CIMMYT: Mexico. Federal District Mexico, pp 10-22

Rubella SG, Kistler CH (2004) Pathogen profile heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5:515-525

Salameh A, Buerstmayr M, Steiner B, Neumayer A, Lemmens M, Buerstmayr H (2011) Effects of introgression of two QTL for Fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on Fusarium head blight resistance, yield and quality traits. Molecular Breeding 28:485-494

Schotter C, Tautz D (1992) Slippage synthesis of simple sequence DNA. Nucleic Acids Research 20:211-215

Scott DB (1990) Wheat diseases in South Africa. Department of Agricultural Development, Republic of South Africa, pp 1-13

Sharma RK, Singh PK, Vinod, Joshi AK, Bhardwaj SC, Bainis NS, Singh S (2013) Protecting South Asian wheat production from stem rust (Ug99) epidemic. Journal of Phytopathology 161:299-307

Sharp PJ, Johnston S, Brown G, McIntosh RA, Pallota M, Carter M, Bariana HS, Khatkar S, Lagudah ES, Singh PR, Khairallan M, Potter R, Jones MG (2001) Validation of molecular markers for wheat breeding. Australian Journal of Agricultural Research 52:1357-1366

Shen X, Ittu M, Ohm HW (2003) Quantitative trait loci conditioning resistance to Fusarium head blight in wheat line F201R. Crop Science 43:850-857

Shi JR, Xu DH, Yang HY, Lu XQ, Ban T (2008) DNA marker analysis for pyramided Fusarium head blight (FHB) resistance QTLs from different germplasm. Genetica 133:77-84

Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5:291-317

Singh RP, Datta D, Priyamvada M, Singh S, Tiwari R (2004) Marker-assisted selection for leaf rust resistance genes *Lr19* and *Lr24* in wheat (*Triticum aestivum* L.). Journal of Applied Genetics 45:399-403

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, Govindan V (2011) The emergence of Ug99 races of the stem rust fungi is a threat to world wheat production. Annual Review of Phytopathology 49:465-481

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua M, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspective in Agriculture, Veterinary Science and Natural Resources 54:1-13

Singh RP, Huerta-Espino J, Roelfs AP (2002) The wheat rusts. FAO Plant Production and Protection Series 30: Bread Wheat: Improvement and Production. http://www.wheat/FAOdocumentrepositoryfile/y411eog.html (Cited September 2012)

Singh RP, Huerta-Espino J, William HM (2005) Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. Turkey Journal of Agriculture 29:121-127

Singh RP, Kinyua MG, Wanyera R, Njau P, Jin Y, Huerta-Espino J (2007) Spread of a highly virulent race of *Puccinia graminis tritici* in eastern Africa: Challenges and opportunities 12:51-57

Singh RP, Nelson JC, Sorrells ME (2000) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Science 40:1148-1155

Singh RP, Rajaram S (1992) Genetics of adult-plant resistance to leaf rust in Frontana and three CIMMYT wheats. Genome 35:24-31

Singh RP, Rajaram S (2002) Breeding for disease resistance in wheat. In: Curtis BC, Rajaram S, Gomez Macpherson H (Eds) Bread Wheat: Improvement and Production. Food and Agricultural Organisation of the United Nations, Rome, Italy, pp 317-330

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal GS (2001) Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *xa21*) using marker-assisted selection into *Indica* rice cultivar PR106. Theoretical and Applied Genetics 102:1011-1015

Šliková S, Gregová E, Bartoš P, Hanzalová A, Hudcovicová M, Kraic J (2004) Development of wheat genotypes possessing a combination of leaf rust resistance genes *Lr19* and *Lr24*. Plant Soil Environment 50:434-438

Šliková S, Gregová E, Bartoš P, Kraic J (2003) Marker-assisted selection for leaf rust resistance in wheat by transfer of gene *Lr19.* Plant Protection Science 39:13-17

Smit HA, Tolmay VL, Barnard A, Jordaan JP, Koekemoer FP, Otto WM, Pretorius ZA, Purchase JL, Tolmay JPC (2010) An overview of the context and scope of wheat (*Triticum aestivum*) research in South Africa from 1983 to 2008. South African Journal of Plant and Soil 27:81-96

Somers DJ (2004) Molecular marker systems and their evaluation for cereal genetics. In: Gupta PK, Varshney RK (Eds) Cereal Genomics. Kluwer Academic Publishers Netherlands, pp 19-30

Somers DJ, Thomas J, DePauw R, Fox S, Humphreys G, Fedak G (2005) Assembling complex genotypes to resist Fusarium in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 111:1623-1631

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Science 43:333-336

Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theoretical and Applied Genetics 109:215-224

Stephenson P, Bryan G, Kirby J, Collins A, Devos KM, Busso C, Gale MD (1998) Fifty new microsatellite loci for the wheat genetic map. Theoretical and Applied Genetics 97:946-949

Sui XX, Wang MN, Chen XM (2009) Molecular mapping of a stripe rust resistance gene in spring wheat cultivar Zak. Phytopathology 99:1209-1215

Tanksley SD, Young ND, Patterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding new - tools for an old science. Biotechnology 7:257-264

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12:4127-4138

Teich AH (1989) Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp. Topics in Secondary Metabolism 2:269-282

Terefe T, Pretorius Z (2010) Update on wheat rust. SA Grain, pp 96-98

The TT, Latter BDH, McIntosh RA, Ellison FW, Brennan PS, Fisher J, Hollamby GJ, Rathjen AJ, Wilson RE (1988). Grain yields of near isogenic lines with added genes for stem rust resistance. In: Miller TE, Koebner RMD (Eds) Proceedings of the 7th Wheat Genetics Symposium, Bath Press, UK, pp 901-909

Todorovska E, Christov N, Slavov S, Christova P, Vassilev D (2009) Biotic stress resistance in wheat breeding and genomic selection implications. Biotechnology and Biotechonology Equipment 23:1417-1426

Tsilo TJ, Jin Y, Anderson JA (2008) Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Science 48:253-261

Tyryshkin LG, Gul'tyaeva EI, Alpat'eva NV, Kramer I (2006) Identification of effective leaf-rust resistance genes in wheat (*Triticum aestivum*) using STS markers. Russian Journal of Genetics 42:662-666

USDA (2013) http://www.ers.usda.gov/topics/crops/wheat/background.aspx (Cited January 2014)

Varshney A, Mohaptra T, Sharma RP (2004) Molecular mapping and marker-assisted selection of traits for crop improvement. In: Srivastava PS, Narula A, Srivastava S (Eds) Plant Biotechnology and Molecular Markers. Anamaya publishers, New Delhi, India, pp 289-313

Vida G, Gal M, Andrea Uhrin A, Veisz O, Syed NH, Flavell AJ, Wang Z, Bedo Z (2009) Molecular markers for the identification of resistance genes and marker-assisted selection in breeding wheat for leaf rust resistance. Euphytica 170:67-76

Vignal A, Milan D, Sancristobal M, Eggen A (2002) A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution 34:275-305

Visser B, Herselman L, Park RF, Karaoglu H, Bender CM, Pretorius ZA (2011) Characterisation of two new *Puccinia graminis* f. sp *tritici* races within the Ug99 lineage in South Africa. Euphytica 179:119-127

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23:4407-4414

Waines JG, Ehdaie B (2007) Domestication and crop physiology: Roots of green revolution of wheat. Annals of Botany 100:991-998

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP of QTL for Fusarium head blight resistance in wheat. Crop Science 39:805-811

Walker D, Boerma RH, All J, Parrot W (2002) Combining *cry1Ac* with QTL alleles from PI 229358 to improve soybean resistance to lepidopteran pests. Molecular Breeding 9:43-51.

Weber JL, Wong C (1993) Mutation of human short tandem repeats. Human Molecular Genetics 2:1123-1128

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18:7213-7218

Wenzel G (2006) Molecular plant breeding: achievements in green biotechnology and future perspectives. Applied Microbiology and Biotechnology 70:642-650

Wilde F, Schön CC, Korzun V, Ebmeyer E, Geiger HH, Miedaner T (2007) Comparison of phenotypic and marker-based selection for Fusarium head blight resistance and DON content in spring wheat. Molecular Breeding 19:357-370

Wilde F, Schön CC, Korzun V, Ebmeyer E, Schmolke M, Hartl L, Miedaner T (2008) Marker-based introduction of three quantitative trait loci (QTL) conferring resistance to Fusarium head blight into an independent elite winter wheat breeding population. Theoretical and Applied Genetics 117:29-35

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535

Yang Z, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled haploid spring wheat population. Genome 48:187-196

Zhou WC, Kolb FL, Bai GH, Domier LL, Boze LK, Smith NJ (2003) Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. Plant Breeding 122:40-46

Zhou WC, Kolb FL, Bai GH, Domier LL, Yao JB (2002) Effect of individual Sumai 3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation within kernels in wheat. Hereditas 137:81-89

CHAPTER 3

Selection of rust resistant lines

3.1 Introduction

The three rust pathogens (leaf, stem and stripe rust) cause losses to cereal production although damage caused by these diseases varies from one another (Vida et al., 2009). Stem rust develops well under hot and humid conditions, stripe rust under cool climates while leaf rust develops under a range of different conditions which makes it the most widespread of the three diseases (Bariana et al., 2007).

The most successful, efficient and economical approach of controlling rust diseases in cereals is through the use of resistant cultivars (Castro et al., 2002; Li et al., 2006; Tyryshkin et al., 2006). Sun et al. (1997) stated that breeding resistant cultivars eliminates the use of fungicides and minimises crop losses. Breeding of new varieties using conventional breeding has been reported to be time consuming, taking 12 to 15 years (Wenzel, 2006) and is affected by genotype and environment interactions (Langridge et al., 2001). Jian-Kang and Pfeifferz (2007) added that successful conventional breeding depends on phenotypic selection and the breeder's experience, hence selection is not precise, leading to low efficiency. Breeders are interested in new technologies that could improve traditional methods by shortening the breeding programme and making it more cost effective. The new approach of applying molecular techniques has been reported to improve breeding and selection (Langridge et al., 2001).

DNA markers are therefore important for creating genetic maps that are useful to locate genes or QTL of important traits and to introduce them into cultivars using MAS (Song et al., 2005). A range of molecular markers are available in cereals, including wheat, hence can be used in gene introgression studies and wheat breeding. On the other hand, the application of molecular techniques in marker-assisted breeding was not well deployed in the past

regardless of the availability of molecular markers (Gupta et al., 1999). However, this situation is changing. A large number of molecular markers are available for use in wheat breeding to identify parental lines accurately and in combining multiple traits into a single genotype (Randhawa et al., 2013).

Microsatellites or SSRs are one of the molecular techniques that has been extensively used for identification of resistance genes in wheat (Huang et al., 2003). Langridge et al. (2001) said that SSRs are more informative in hexaploid wheat than other classes of markers and that the technique appears to be promising in breeding disease resistant cultivars. SSRs are good genetic markers for detecting differences between and within species (Farooq and Azam, 2002).

Li et al. (2006) stated that the availability of molecular markers associated with resistance genes promotes combination of important genes into a complex cultivar. Combining major insecticidal and disease resistance genes result in durable and broad-spectrum resistance against targeted organisms (Wenzel, 1985). MAS is currently used in breeding programmes for combining resistance genes of leaf, stem and stripe rust and other diseases including FHB, and has been recently used to combine stem rust resistance genes against Ug99 (Randhawa et al., 2013).

In the current study SSR analysis using known and previously mapped molecular markers was utilised to follow the introgression of leaf, stem and stripe rust resistance genes and QTL into bread wheat lines that were a result of a cross between four parental lines: Avocet*YrSp*, Blade, CS*Lr19*-149-229 and Kariega. The current study is a continuation of a research project entitled "Pyramiding wheat rust resistance genes using marker-assisted selection" that was carried out in the Department of Plant Sciences (Plant Breeding) at the University of the Free State, South Africa from 2006-2008 by Sydenham (2007). According to Ishii and Yonezawa (2007) a gene pyramiding programme involves two steps. The first step involves combining all markers into a single genome. The second step involves selection of lines from the first step that have all markers in a homozygous state. The previous study by Sydenham (2007)

addressed the first step namely combining all genes and QTL from four parental lines into a single genotype. The current study will try to address the second step.

The aim of Sydenham's study was to develop a pyramided rust resistant line. He made crosses between four parental lines containing different rust resistance genes and QTL. These lines and genes/QTL were Kariega (*Lr34/Yr18/Sr57*, Q*Yr.sgi-7D* and Q*Yr.sgi-2B.1*), Avocet*YrSp* (*YrSp* and *Sr26*), Blade (*Sr2* and *Sr26*) and CS*Lr19*-149-229 (*Lr19* and *Lr34/Yr18/Sr57*). During this study of Sydenham it was not yet known that the Q*Yr.sgi-7D* QTL in Kariega was indeed the *Lr34/Yr18/Sr57* gene. Sydenham (2007) made a series of crosses using the four parental lines as indicated in Figure 3.1 to develop the Cross 1 F_1 and Cross 2 F_1 generations. The two F_1 populations were crossed with each other to develop a double cross population of about 900 individuals. The aim was to obtain a line containing seven possible pyramided rust resistance genes/QTL. Screening of the 900 individuals of the double cross population was performed using MAS. Four lines (S16, S178, S726 and S791) each containing seven markers linked to rust resistance genes/QTL based on marker data, as shown in Table 3.1, were selected from the double cross population generated by Sydenham (2007). Unfortunately lines that tested positive for all eight markers did not produce any seed.

Since the four selected lines were still segregating, they were planted and left for self-pollination to perform molecular screening for identifying markers in a homozygous state. Self-pollinated lines were used to select (using MAS) the best rust resistant lines that contained the highest number of markers in a homozygous state for combining with FHB resistant cultivars that will be discussed in the next chapter.

The aim of the study was to select the best lines produced during the first phase of the project, containing most markers in either a homozygous or heterozygous state. This was done using MAS applying SSR analysis on individuals obtained after self-pollination of the four best double cross populations (S16, S178, S726 and S791). The best lines selected in this chapter will be used in further pre-breeding programmes that will be discussed in the next chapter.

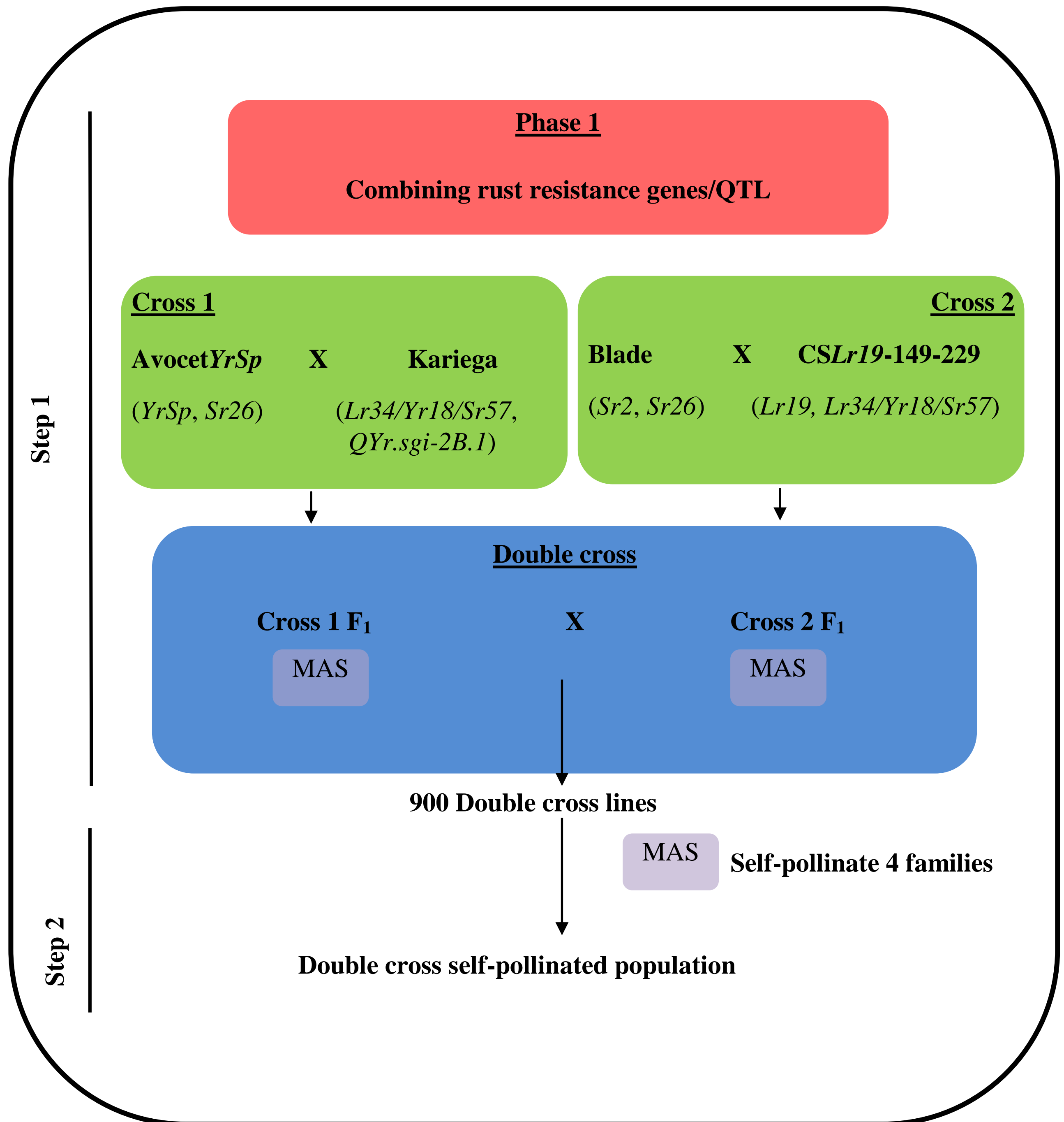


Figure 3.1 Experimental plan followed by Sydenham (2007) to obtain the double cross lines used in the current study

Table 3.1 Characteristics of four families selected from the double cross population screened by Sydenham (2007)

Population name	Homozygous markers[1]	Heterozygous markers[1]	Homozygous or heterozygous markers[1,2]	Absent[1]
S16	Gwm295 (*QYr.sgi-7D*) csLV34 (*Lr34/Yr18/Sr57*)	Gwm148 (*QYr.sgi-2B.1*) Gwm501 (*QYr.sgi-2B.1*) Gwm111 (*QYr.sgi-7D*)	$STSLr19_{130}$ (*Lr19*) Stm559gtag (*Sr2*)	Sr26#43 (*Sr26*)
S178	None	Gwm148 (*QYr.sgi-2B.1*) Gwm501 (*QYr.sgi-2B.1*) Gwm111 (*QYr.sgi-7D*) Gwm295 (*QYr.sgi-7D*) csLV34 (*Lr34/Yr18/Sr57*)	Stm559gtag (*Sr2*) Sr26#43 (*Sr26*)	$STSLr19_{130}$ (*Lr19)*
S726	None	Gwm111 (*QYr.sgi-7D*) Gwm295 (*QYr.sgi-7D*) csLV34 (*Lr34/Yr18/Sr57*) Gwm501 (*QYr.sgi-2B.1*)	*Lr19* *Sr26* Stm559gtag (*Sr2*)	Gwm148 (*QYr.sgi-2B.1*)
S791	None	Gwm148 (*QYr.sgi-2B.1*) Gwm501 (*QYr.sgi-2B.1*) Gwm111 (*QYr.sgi-7D*) Gwm295 (*QYr.sgi-7D*) csLV34 (*Lr34/Yr18/Sr57*)	$STSLr19_{130}$ (*Lr19)* Stm559gtag (*Sr2*)	Sr26#43 (*Sr26*)

1 = Gene/QTL targeted by marker is given in brackets; 2 = Due to being a dominant marker, homozygous individuals could not be discriminated from heterozygous individuals

3.2 Materials and methods

3.2.1 Plant material

The work was conducted at the Department of Plant Sciences (Plant Breeding division) of the University of the Free State, South Africa. Seed used in the study was sourced from four selected double cross families: 21 seeds of family S16 (*QYr.sgi-7D, Lr34/Yr18/Sr57, QYr.sgi-2B.1, Lr19* and *Sr2*), 21 seeds of family S178 (*QYr.sgi-7D, Lr34/Yr18/Sr57,*

QYr.sgi-2B.1, Sr26 and *Sr2,*), 21 seeds of family S726 (*QYr.sgi-7D, Lr34/Yr18/Sr57, QYr.sgi-2B.1, Sr26, Sr2* and *Lr19*) and 15 seeds of family S791 (*QYr.sgi-7D, Lr34/Yr18/Sr57, QYr.sgi-2B.1, Lr19* and *Sr2*) obtained from crosses between the original donor sources of the resistance genes/QTL used in the study; Avocet*YrSp*, Blade, CS*Lr19*-149-229 and Kariega.

Three seeds were planted in soil per 3 L pot. Cultivation was done in the greenhouse under controlled conditions of temperatures ranging from 10°C (minimum) to 25°C (maximum). Watering of plants was done on a daily basis. Starting two weeks after germination, 3:2:1(28) fertiliser was applied at a rate of 10 g/l in water with each pot receiving 50 ml. In addition, a solution of micro-nutrients was periodically applied at the same rate mentioned above. The four selected families were left to self-pollinate and all heads were covered to prevent cross-pollination.

3.2.2 Material sampling

Five to seven young leaves, 5 cm in length, were sampled from each eight week old plant using clean and sterile equipment between each cutting. Leaves were placed in sampling tubes on ice. Samples were freeze-dried (Freeze Mobile II, New York, NY, USA) for three to five days and stored at -70°C. Three to five pieces of ±2 cm freeze-dried leaves from each selected individual were cut into a 2 ml microcentrifuge tube. Two 5 mm steel ball bearings per tube were added to the leaf material for grinding to a fine powder using Qiagen's TissueLyser (Haan, Germany) at a speed of 30 r/s for 30 s.

3.2.3 DNA isolation

Total genomic DNA was isolated using a modified CTAB (hexadecyltrimethylammonium bromide) extraction method (Saghai-Maroof et al., 1984). A volume of 750 µl CTAB buffer [100 mM Tris-HCl (tris(hydroxymethyl) aminomethane) pH 8.0; 20 mM EDTA (ethylene-diaminetetraacetate) pH 8.0; 1.4 M NaCl; 2% (w/v) CTAB; 0.2% (v/v) β-mercaptho-

ethanol] was added to ±250 µl of fine leaf powder in 2 ml microcentrifuge tubes and incubated at 65°C for one hour. The suspension was extracted with 500 µl chloroform:isoamylalcohol [24:1 (v/v)] and centrifuged at 12 000 *g* for 5 min. DNA was precipitated from the aqueous phase with 500 µl (0.66 volumes) isopropanol and incubated at room temperature for 20 min followed by centrifugation at 12 000 *g* for 5 min at 5°C. The supernatant was discarded and tubes drained upside down. The precipitate was washed twice at room temperature by adding 500 µl ice-cold 70% (v/v) ethanol, incubated at room temperature for 20 min, centrifuged at 12 000 *g* for 5 min and the supernatant discarded. The DNA pellets were air dried for one hour at room temperature. Dry pellets were resuspended in 200 µl TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) and incubated at 37°C for one hour followed by addition of RNaseA to a final concentration of 200 µg/ml and incubated at 37°C for one to two hours. DNA quality and quantity were determined using electrophoresis and a spectrophotometer, respectively. Electrophoresis was carried out in 0.8% (w/v) agarose gels at 80 V for one hour in 1x UNTAN (40 mM Tris-HCl; 2 mM EDTA, pH adjusted to pH 7.4 with acetic acid) buffer and DNA visualised with ethidium bromide under ultraviolet (UV) light. The spectrophotometer was set to measure at two wavelengths (WL1 = 260 nm and WL2 = 280 nm). WL1 (260 nm) was used to measure the DNA concentration as well as the possible RNA concentration. WL2 (280 nm) was used to measure the protein contamination. DNA was diluted to a working concentration of 200 ng/µl using 0.1x TE buffer, pH 8.0.

3.2.4 SSR analyses

The following SSR and STS markers were used to screen for fragments associated with the resistance genes/QTL: Gwm111 and Gwm295 (*QYr.sgi-7D)*, Gwm501 and Gwm148 (Q*Yr.sgi-2B.1)*, Sr26#43 (*Sr26*), stm559gtag (*Sr2*), csLV34 (*Lr34/Yr18/Sr57*) and STSLr19$_{130}$ (*Lr19*). At this stage of the study it was not known that the *QYr.sgi-7D* QTL was actually the *Lr34/Yr18/Sr57* gene hence markers Gwm111 and Gwm295 were used. Furthermore, the new markers csSr2 for *Sr2* and cssfr5 for *Lr34/Yr18/Sr57* were not available at the time of screening.

3.2.4.1 PCR cycling conditions

SSR-PCR reactions were set up in volumes of either 10 µl or 20 µl (Table 3.2). All marker reaction mixes contained 4 µl genomic DNA (gDNA), double distilled water (ddH_2O), Promega *Taq* buffer (Promega, Madisen, Wl, USA), $MgCl_2$, 2'-deoxynucleoside 5'-triphosphate (dNTPs), forward and reverse primer and Promega *Taq* polymerase (Tables 3.2 and 3.3). All reagents and reaction mixes were stored on ice until use. PCR cycling conditions were used as standardised by Sydenham (2007) (Table 3.4).

3.2.5 Visualisation of amplified fragments

3.2.5.1 Agarose gel electrophoresis

SSR-PCR products for the primer pair csLV34 were mixed with 5 µl non-denaturing loading dye [15% (w/v) Ficoll; 0.24% (w/v) Bromophenol blue]. PCR products were separated on a 1.5% (w/v) agarose gel and visualised using ethidium bromide staining. Electrophoresis was performed at 80 V for one to two hours in 1x UNTAN buffer.

3.2.5.2 Polyacrylamide gel electrophoresis (PAGE)

Amplified fragments for Gwm111, Gwm295, Gwm148, Gwm501, Sr26#43, stm559gtag and $STSLr19_{130}$ were separated using 5% (w/v) denaturing polyacrylamide gel electrophoresis (PAGE) [19:1 acrylamide:bis-acrylamide; 7 M urea; 1x TBE buffer (89 mM Tris-Borate; 2 mM EDTA)]. SSR products were mixed with equal volumes of formamide loading buffer [98% (v/v) de-ionized formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cyanol]. Samples were denatured for 5 min at 95°C and immediately placed on ice before loading. Five µl of each sample was loaded. Electrophoresis was performed at a constant power of 80 W for 1-2 hours.

Table 3.2 SSR-PCR reaction volumes and mixtures (in µl) for the different primer combinations used in the study

Reagent	Gwm111 (*QYr.sgi-7D*)	Gwm295 (*QYr.sgi-7D*)	Gwm501 (Q*Yr.sgi-2B.1*)	Gwm148 (Q*Yr.sgi-2B.1*)	Sr26#43 *(Sr26)*	Stm559gtag *(Sr2)*	csLV34 *(Lr34)*	STSLr19$_{130}$ *(Lr19)*
DNA (200 ng/µl)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
ddH_2O	1.05	1.75	6.30	1.75	1.75	1.45	1.75	1.75
***Taq* Buffer (5x)**	2.00	2.00	4.00	2.00	2.00	2.00	2.00	2.00
$MgCl_2$ (25 mM)	0.80	0.80	1.80	0.80	0.80	0.60	0.80	0.80
dNTPs (1.25 mM)	0.60	0.40	0.80	0.40	0.40	0.40	0.40	0.40
Forward primer (50 ng/µl)	0.75	0.50	1.50	0.50	0.50	0.75	0.50	0.50
Reverse primer (50 ng/µl)	0.75	0.50	1.50	0.50	0.50	0.75	0.50	0.50
***Taq* Pol (5 U/µl)**	0.05	0.05	0.10	0.05	0.05	0.05	0.05	0.05
Total	**10.00**	**10.00**	**20.00**	**10.00**	**10.00**	**10.00**	**10.00**	**10.00**

ddH_2O = double distilled water; *Taq* pol = *Thermus aquaticus* polymerase

Table 3.3 SSR markers, primer pair sequences, targeted genes/QTL, parental cultivar sources and references for primers used in the study

Marker name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Targeted gene/QTL	Resistance sources	Reference
$STSLr19_{130}$	CATCCTTGGGGACCTC	CCAGCTCGCATACATCCA	*Lr19*	CS*Lr19*-149-299	Prins et al., 2001
csLV34	GTTGGTTAAGACTGGTGATGG	TGCTTGCTATTGCTGAATAGT	*Lr34*	All except Blade	Lagudah et al., 2006
stm559tgag	AAGGCGAATCAAACGGAATA	TGTGTGTGTGTGTGAGAGAGAG	*Sr2*	Blade	Hayden et al., 2004
Sr26#43	AATCGTCCACATTGGCTTCT	CGCAACAAAATCATGCACTA	*Sr26*	Avocet*YrSp*, Blade	Mago et al., 2005
Gwm148	GTAGGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	*QYr.sgi-2B.1*	Kariega	Röder et al., 1998
Gwm501	GGCTATCTCTGGCGCTAAAA	TCCACAAACAAGTAGGCGCC	*QYr.sgi-2B.1*	Kariega	Röder et al., 1998
Gwm111	TCTGTAGGCTCTCTCTCCGACTG	ACCTGATCAGATCCCACTCG	*QYr.sgi-7D*	Kariega	Röder et al., 1998
Gwm295	GTGAAGCAGACCCACAACAC	GACGCGTGCGACGTAGAG	*QYr.sgi-7D*	Kariega	Röder et al., 1998

QTL = quantitative trait loci

Table 3.4 PCR cycling conditions and specific cycling programmes used in the study as standardised by Sydenham (2007)

Marker	PCR cycling conditions
STSLr19$_{130}$	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min, 1 cycle
csLV34	94°C 5 min, 1 cycle; 94°C 1 min, 55°C 1 min, 72°C 2 min, 4 cycles; 94°C 30 s, 55°C 30 s, 72°C 30 s, 29 cycles; 94°C 30 s, 55°C 30 s, 72°C 5 min, 1 cycle
stm559tgag	95°C 2 min, 1 cycle; 95°C 30 s, 64°C 30 s, 72°C 30 s, 44 cycles; 72°C 10 min, 1 cycle
Sr26#43	94°C 3 min, 1 cycle; 94°C 45 s, 55°C 45 s, 72°C 1:15 min, 44 cycles; 72°C 10 min, 1 cycle
Gwm148	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min, 1 cycle
Gwm501	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min, 1 cycle
Gwm111	95°C 2 min, 1 cycle; 95°C 1 min, 65°C 1 min, 72°C 1 min, 44 cycles; 72°C 5 min, 1 cycle
Gwm295	95°C 2 min, 1 cycle; 95°C 1 min, 64°C 1 min, 72°C 1 min, 44 cycles; 72°C 5 min, 1 cycle

3.2.5.3 Silver staining

Samples separated using PAGE were visualised using the silver staining method based on the protocol described by the Silver Sequence™ DNA Sequencing System manual supplied by Promega. Stained gels were left to air-dry overnight. Photographs were taken by exposing photographic paper (Ilford multigrade IV RC) directly under the gel to a dim white light for approximately 20 s. SSR fragment lengths were determined by comparison with a 25 bp DNA ladder (Promega).

3.2.6 Data analyses

Chi square (χ^2) tests were used for analyses of the segregating population. Fifteen to twenty one individuals of each family were examined for the presence of each marker. Fragments were scored as present (1) or absent (0) for the presence of markers linked to targeted genes/QTL using score sheets for all samples based on allele sizes of the parental lines. Heterozygotes were discriminated from homozygotes by scoring (1) for both alleles. Data for the expected ratios were created for comparison with the observed data. Analyses were performed using chi square analysis to compare observed and expected segregation ratios of marker alleles.

3.2.7 Phenotypic screening

3.2.7.1 Materials and methods

Parental lines used in the study as well as control lines were phenotypically screened for rust resistance in the greenhouse. Screenings were done using the stem rust (*Pgt)* isolate UVPgt60, leaf rust (*Pt*) isolate UVPt20 and stripe rust (*Pst*) isolate 6E22A+. The four rust resistant parental lines: Avocet*YrSp*, Blade, CS*Lr19*-149-229 and Kariega and two off-spring lines selected from the best selected double cross population lines, S16(7.3)$P_3$5.1 and S726(3.2)$P_3$6.1, were tested at seedling stage for their response to stem, leaf and stripe rust. Two FHB resistant cultivars, CM-82036 and Frontana, were also included for inoculation while Morocco was used as a susceptible control to all three tested rust isolates. The two

FHB lines were included in the second phase of the project for combining rust and FHB resistance gene/QTL and will be discussed in depth in the next chapter.

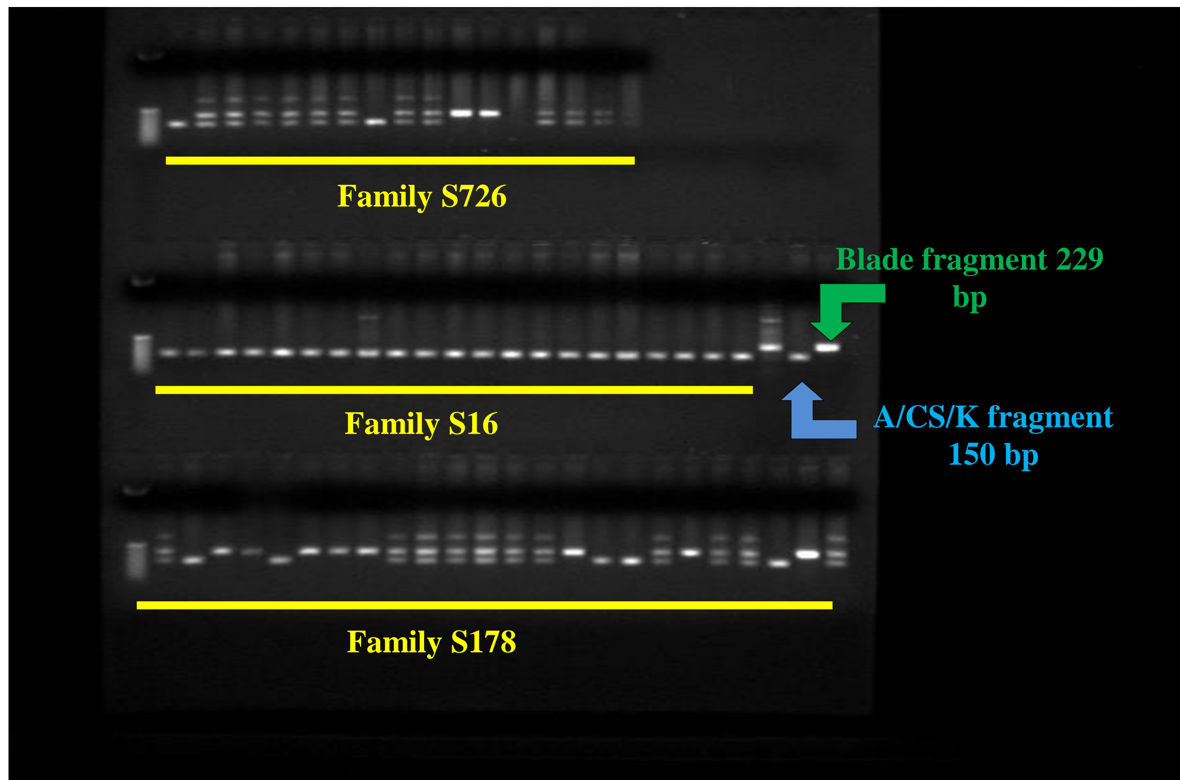
Five seeds per cultivar/line were sown per 340 ml plastic pot (10 cm diameter) in a steam-sterilised soil-peat mixture. Three sets were planted to accommodate the three different rust pathogens, using two replications. Inoculation was done on eight day-old seedlings with fresh spores of *Pgt* isolate UVPgt60, *Pt* isolate UVPt20 and *Pst* isolate 6E22A+ when the primary leaves were fully extended. Spores were suspended in light mineral oil and sprayed onto leaves in an inoculation chamber. The chamber was thoroughly rinsed between different rust inoculations. Plants were left to air-dry for about an hour before placement in a dew chamber. The stem and leaf rust inoculated plants were kept at 20-22°C in a dew chamber for 16 h. The stripe rust plants were lightly misted with sterile, distilled water and placed in a sealed container made from galvanised metal sheeting, which in turn was placed in a cold room for 48 h. The temperature in the cold room was 10°C. After plants were removed from the respective dew chambers, they were gradually dried off in a growth cabinet for 3 h.

Seedlings inoculated with stripe rust were maintained in a greenhouse cubicle at 15-20°C whereas the stem and leaf rust inoculated plants were kept at 18-25°C. Primary leaf infection types (0 to 4) scale (Stakman et al., 1962) were scored 11 days post-inoculation (dpi) for leaf rust, 14 dpi for stem rust and 17 dpi for stripe rust. Leaf material for DNA extraction was sampled before inoculation was done.

3.3 Results

3.3.1 Genotypic screening of self-pollinated segregating populations

All eight markers were tested and examined successfully on 83 individuals of four segregating self-pollinated rust resistant families (S16, S178, S726 and S951). Table 3.5 shows the expected allele sizes of the different markers and csLV34 as screened in parental lines.



A = Avocet*YrSp*; CS = CS*Lr19*-149-229; K = Kariega

Figure 3.2 An agarose gel for marker csLV34 used to detect homozygous and heterozygous individuals in families S16, S178 and S726 for *Lr34* resistance

Different segregation patterns were observed in all individuals of all four families for all tested markers. Only examples of segregation patterns of selected individual markers on gels for csLV34 (Figure 3.2) and Gwm148 (Figure 3.3) are shown.

Figure 3.2 illustrates the observed segregation patterns of individuals within and between families of S16, S178 and S726 for marker csLV34 linked to *Lr34* resistance. Marker analysis indicated that all 21 individuals of family S16 contained the resistant *Lr34* allele (150 bp) in a homozygous state. However, the *Lr34* resistance allele was segregating in families S178 and S726, being either homozygous resistant (150 bp) or heterozygous (150 bp and 229 bp) or homozygous susceptible (229 bp) for the *Lr34* resistance allele. Results

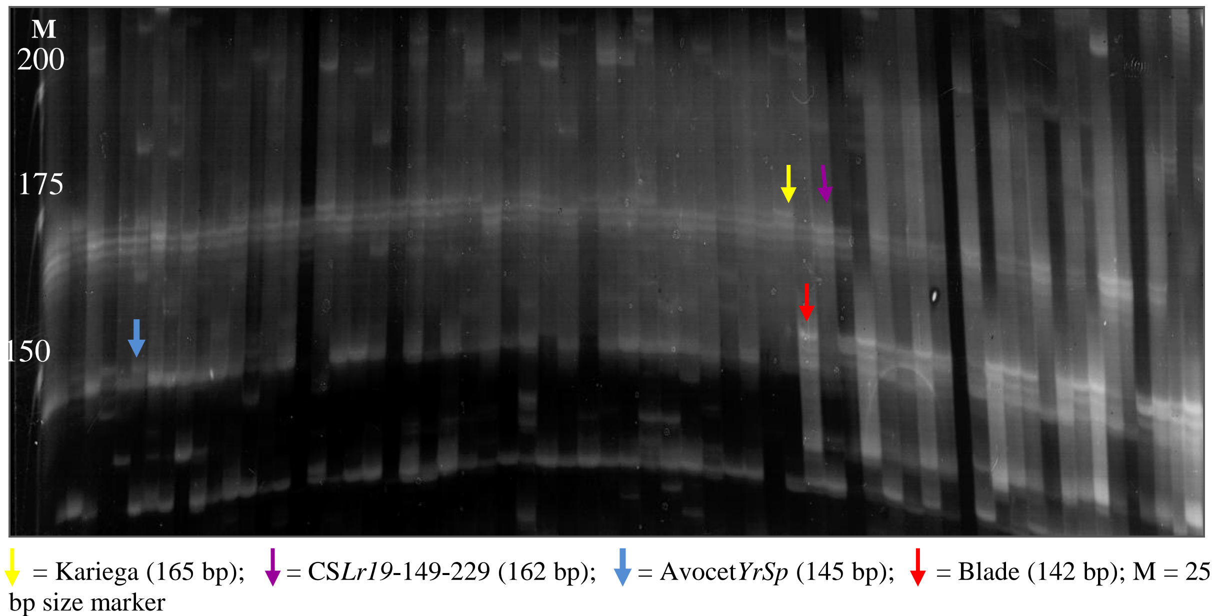
corresponded with the initial genotypic screening of the double cross family by Sydenham (2007) where family S16 was homozygous for *Lr34* while families S178 and S726 were still segregating, as shown in Table 3.1.

Table 3.5 Expected allele sizes of parental lines (Sydenham, 2007)

Marker	Targeted gene/QTL	Donor parent	Allele size in bp			
			Avocet*YrSp*	**Blade**	**CS*Lr19*-149-229**	**Kariega**
Gwm111	*QYr.sgi-7D*	Kariega	214	182	210	**204**
Gwm295	*QYr.sgi-7D*	Kariega	251	248	252	**250**
Gwm501	*QYr.sgi-2B.1*	Kariega	168	165	174	**177**
Gwm148	*QYr.sgi-2B.1*	Kariega	145	142	162	**165**
STSLr19$_{130}$	*Lr19*	CS*Lr19*-149-229	Null allele	Null allele	**100**	Null allele
Sr26#43	*Sr26*	Avocet*YrSp*, Blade	**190**	**190**	Null allele	Null allele
stm559gtag	*Sr2*	Blade	249	**237**	252	Null allele
csLV34	*Lr34*	All, except Blade	**150**	229	**150**	**150**

Allele sizes indicated in bold are linked to rust resistance; bp = base pairs

Figure 3.3 illustrates the observed segregation patterns of individuals within and between all families for marker Gwm148 linked to the *QYr.sgi-2B.1* QTL. PCR fragments were separated and visualised by silver stained PAGE. According to scores for marker Gwm148, family S16 individuals were still segregating. The Kariega allele (165 bp) that is linked to resistance, was scored as present (1) while the other fragment sizes, 142 bp for Blade, 162 bp for CS*Lr19*-149-229 and 145 bp for Avocet*YrSp,* were scored absent (0). Homozygous individuals were scored as 1,1 while heterozygous individuals were scored 1,0. All markers were scored to produce a data matrix used for further analysis.



= Kariega (165 bp); = CS*Lr19*-149-229 (162 bp); = Avocet*YrSp* (145 bp); = Blade (142 bp); M = 25 bp size marker

Figure 3.3 Segregation patterns for individuals of family S16 on a silver stained polyacrylamide gel for marker Gwm148 (*QYr.sgi-2B.1*) showing the different allele sizes of the four parental lines

3.3.1.1 Comparison and selection of best individuals of family S16

This family consisted of 21 individuals. Genotypic screening analyses indicated that the five best lines were S16(2.2), S16(3.1), S16(3.3) and S16(6.2) with a total of five and S16(7.3) with a total of six out of eight markers tested. Most lines had four markers. Lines S16(4.2) and S16(4.3) were exceptions with three markers (Figure 3.4). Lines S16(1.1) and S16(1.2) with four and S16(7.3) with five had the highest number of markers in a homozygous state. Plant S16(7.3) had the highest total number of markers, namely six markers (Gwm111, Gwm295, Gwm148, Gwm501, STSLr19$_{130}$ and csLV34), of which five were in a homozygous state.

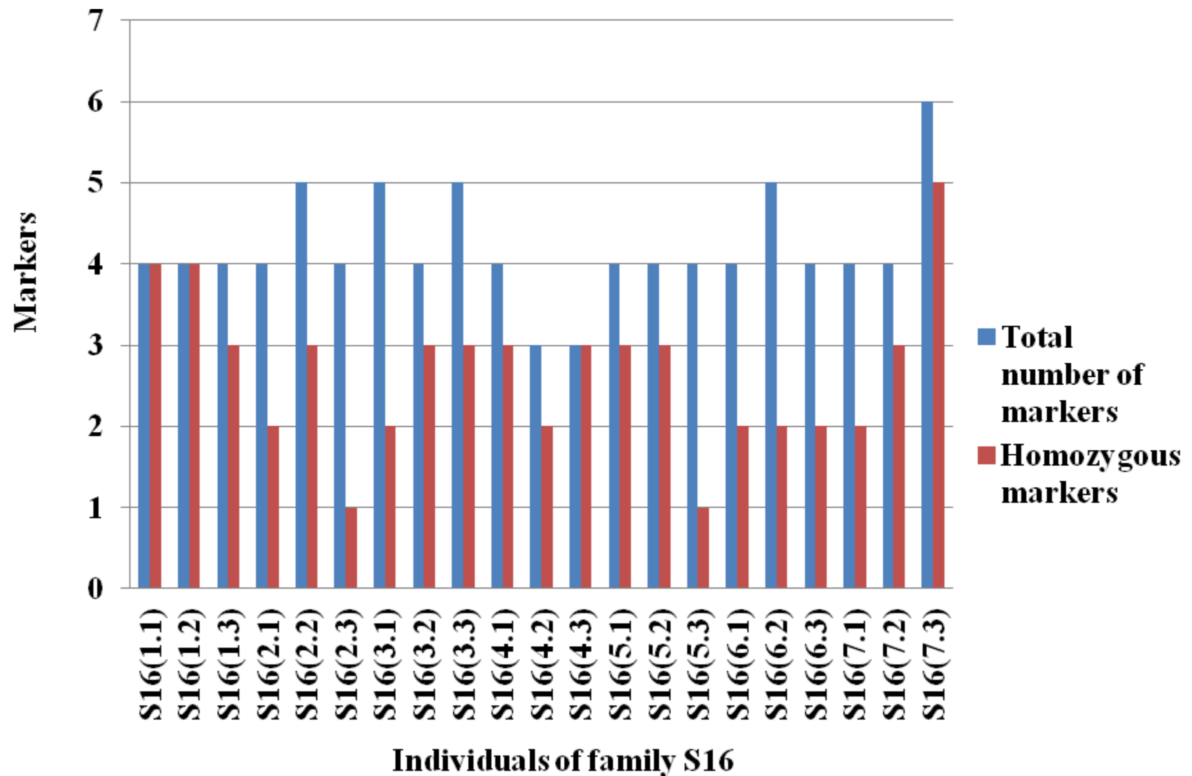


Figure 3.4 Total number of markers observed for each individual of family S16

Chi square analyses were performed to test the observed segregation ratios for molecular markers' responses to theoretical expectations. Through application of chi square analyses most observed segregation patterns for all tested markers showed a poor fit with as low as P<0.1 except for the analysis for the marker linked to *Lr34*, as shown in Table 3.6, with a good fit of probability value of P<0.9. Most markers showed a poor fit which is believed to have been caused by the small sampling population size used for screening or due to the fact that these markers were difficult to score or not linked close enough to the trait of interest. As suspected, none of the individuals of this family tested positive for the marker linked to *Sr26* after selfing because S16 originally did not contain *Sr26*.

Table 3.6 Chi square test for segregation patterns of the S16 population for all markers tested

SSR marker	Observed segregation ratio			Expected ratio	Chi square test	
	A	H	B		∑	P value
csLV34	1	-	-	1	0.00	0.900
Gwm111	2	1.7	1	1:2:1	4.30	0.100
Gwm295	4	1.3	1	1:2:1	15.05	0.001
Gwm148	2.4	1	0	1:2:1	35.57	-
Gwm501	1	6	2.5	1:2:1	2.20	0.300
STSLr19$_{130}$	1	*	*	1	*	
Sr26#43	-	-	-	-	-	-
stm559gtag	1	4	5	1:2:1	9.50	0.010

A = homozygous resistant; B = homozygous susceptible; H = heterozygous; * = the marker did not work on all tested individuals and was repeated on the selected best lines only

3.3.1.2 Comparison and selection of best individuals of family S178

Twenty individuals within family S178 were screened. Line S178(7.2) had six markers of which three were homozygous (Gwm295, Gwm501 and csLV34), followed by lines S178(1.2), S178(4.3), S178(5.2), S178(7.1) and S178(7.3) with five markers each of which between one and three were homozygous. Most lines in this family (40%) had three markers (Figure 3.5). Lines S178(2.3) and S178(3.1) had no markers in a homozygous state. All chi square analyses performed indicated probability values of P<0.1 (data not shown). The population size for the study was small hence results are less informative.

3.3.1.3 Comparison and selection of best individuals of family S726

A total of 21 individuals were successfully screened in this family for the presence or absence of markers. Line S726(3.2) had six markers of which four were probably in a homozygous state (Gwm295, Sr26#43, STSLr19$_{130}$ and csLV34) followed by lines S726(1.1), S726(2.2), S726(4.1), S726(4.3), S726(5.2), S726(7.1) and S726(7.3) with five markers each. This family had the highest number of lines with five or more markers compared to other families. Most individuals within this family had three markers (Figure 3.6).

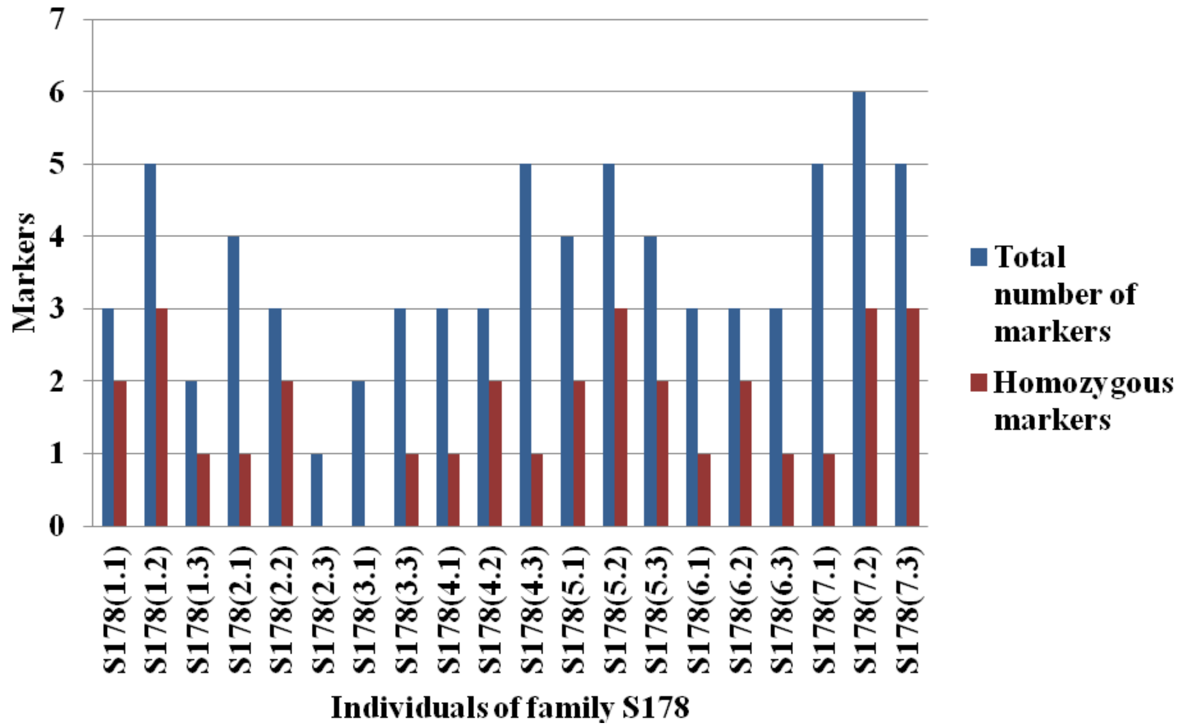


Figure 3.5 Total number of markers observed for each individual of family S178

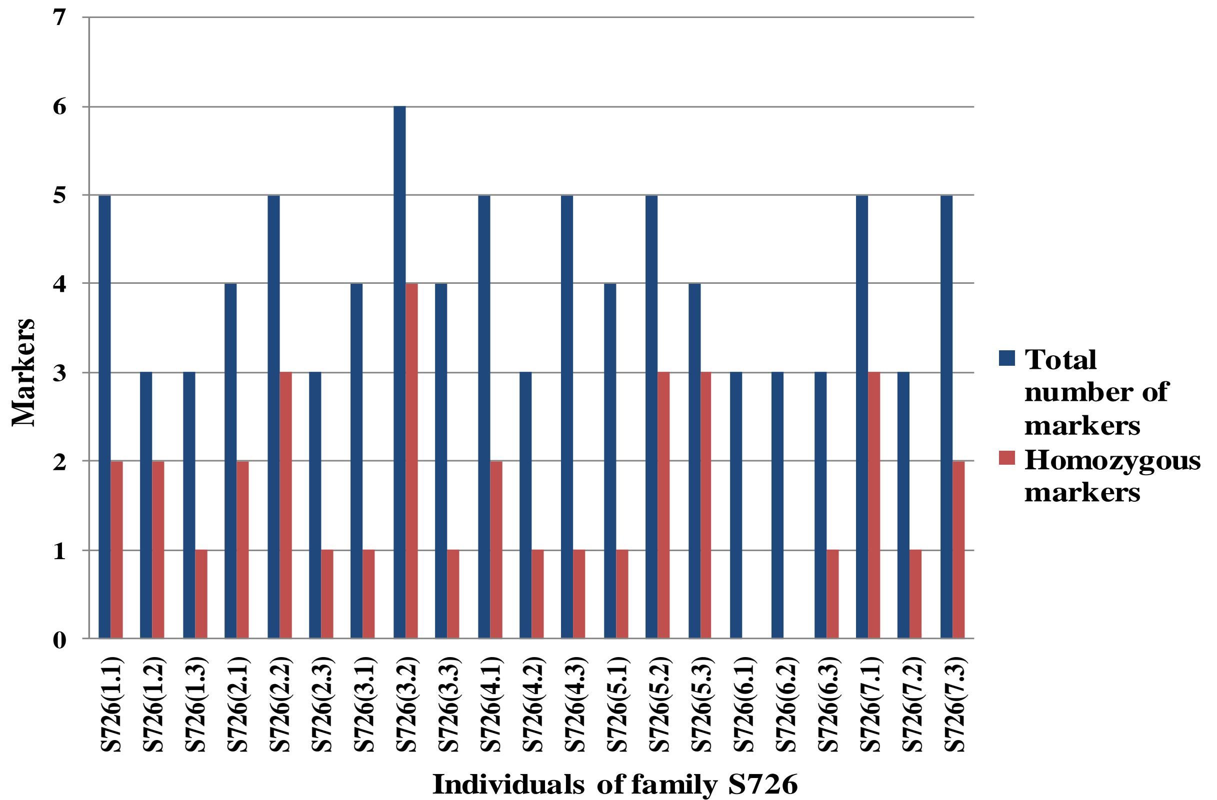


Figure 3.6 Total number of markers observed for each individual of family S726

Chi square analyses (Table 3.7) for marker Gwm148 indicated a probability value of P<0.8 for family S726.

Table 3.7 Chi square test for segregation patterns of the S726 double cross population for all markers tested

Marker	Observed segregation ratio			Expected ratio	Chi square test	
	A	H	B		∑	P value
csLV34	1	4.3	1.3	1:2:1	1.36	0.5
Gwm111	1	1	1.5	1:2:1	4.90	0.1
Gwm295	1.4	1.4	1	1:2:1	2.25	0.2
Gwm148	1	2.8	1.3	1:2:1	0.40	0.8
Gwm501	1	1	18	1:2:1	2.40	0.2
STSLr19$_{130}$	*	*	*	*	*	Missing
Sr26#43	1	0	1	1:2:1	9.2*	
stm559gtag	0	5.3	1	1:2:1	8.90*	

A = homozygous resistant; H = heterozygous; B = homozygous susceptible; * = the marker did not work on all tested individuals and was repeated on selected best lines only

3.3.1.4 Comparison and selection of best individuals of family S791

Only 15 individuals of this family were screened due to unavailability of seed. The best observed lines within this family were lines S791(2.2) and S791(4.1) with six markers each followed by lines S791(1.3), S791(4.3) and S791(5.3) with five markers each (Figure 3.7).

Line S791(2.2) had the highest number of markers in a homozygous state, namely Gwm295 and csLV34 while Sr26#43 could be either homozygous or heterozygous in this family. Most individuals of family S791 contained two homozygous markers out of the eight tested. For all tested markers in this family Chi square probabilities were P<0.1 implying that the tested hypotheses showed a poor fit, obviously resulting from the smallest population size of all tested families.

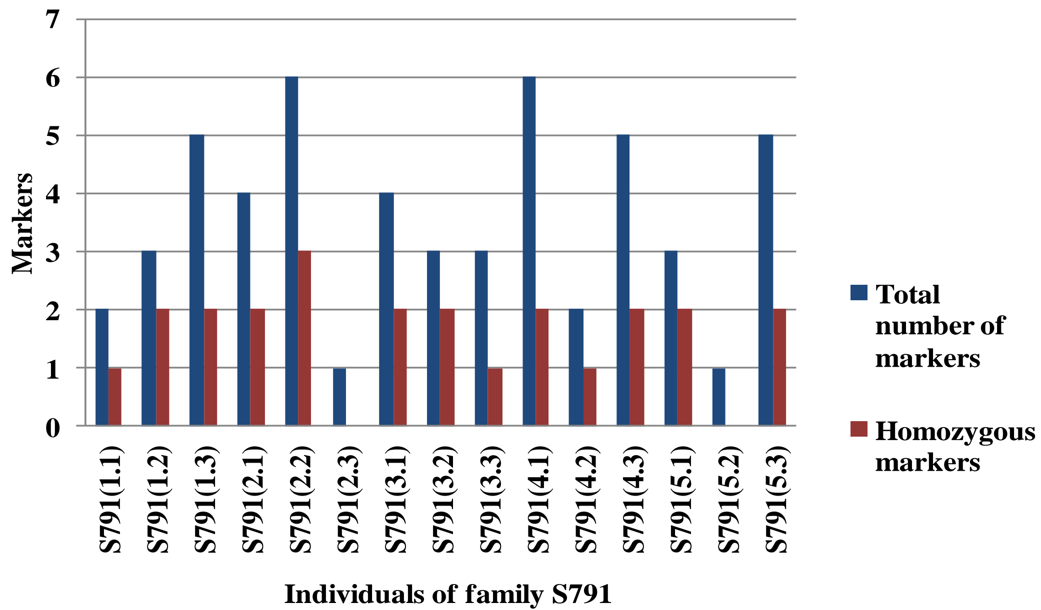


Figure 3.7 Total number of markers observed for each individual of family S791

3.3.1.5 Marker distribution in individuals of all four tested families

In a population of 77 individuals screened using eight molecular markers, most lines had either three, four or five markers present. Twenty-five lines (32%) had four markers followed by 24 (31%) and 20 (26%) lines with three and five markers, respectively (Figure 3.8). Three lines (4%) contained only one marker each while four lines (5%) tested positive for six markers, the highest number of markers detected. No lines were detected that contained all eight markers.

Based on genotypic data, all eight markers were present in the combination of two lines, namely S16(7.3) and S726(3.2), as shown in Table 3.8. Marker data indicated that S16(7.3) should have the following genes/QTL linked to the tested markers: *Lr34/Yr18/Sr57, QYr.sgi-2B.1, QYr.sgi-7D* and *Lr19* while S726(3.2) should contain the genes/QTL: *Sr26, Lr34/Yr18/Sr57, Lr19, QYr.sgi-7D* and *Sr2* (Table 3.8). These two lines were selected for crosses with FHB resistant cultivars CM-82036 and Frontana (see Chapter 4).

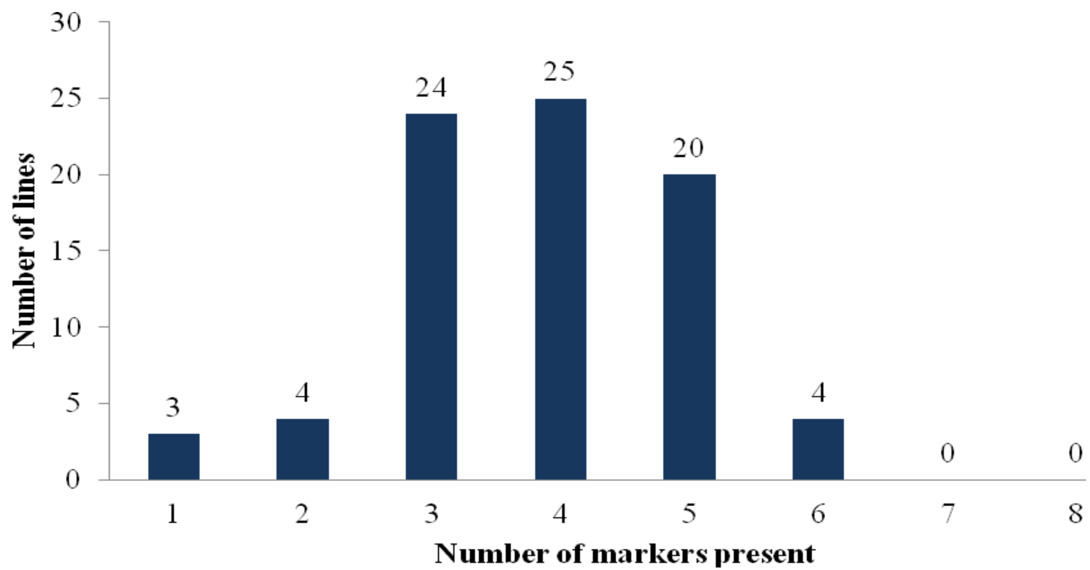


Figure 3.8 Total number of observed markers in individual lines across all screened families

Lines S16(7.3) and S726(3.2) contained more markers in a homozygous state compared to the original double cross lines S16 and S726 (Table 3.8). Line S16 contained two homozygous markers but its progeny, S16(7.3), contained four homozygous markers. A similar case was observed in line S726 that did not have any markers in a homozygous state whereas the progeny line S726(3.2) contained two homozygous markers. The presence of the *YrSp* gene was determined phenotypically in the family S726(3.2) since no marker linked to this trait was available.

Table 3.8 Merits of the selected lines S16(7.3) and S726(3.2) and their parental lines S16 and S726

Population	Homozygous markers	Heterozygous markers	Either homo- or heterozygous markers	Absent markers
S16	Gwm295 csLV34	Gwm148 Gwm111 Gwm501	$STSLr19_{130}$	Sr26#43 Stm559gtag
S726	None	Gwm111 Gwm295 csLV34 Gwm501	$STSLr19_{130}$ Sr26#43 stm559gtag	Gwm148
S16(7.3)	Gwm295 Gwm148 csLV34 Gwm111	Gwm501	$STSLr19_{130}$	Sr26#43 stm559gtag
S726(3.2)	Gwm295 csLV34	Gwm111	$STSLr19_{130}$ Sr26#43 stm559gtag *YrSp* (based on phenotypic data)	Gwm148 Gwm501

3.3.2 Phenotypic screening

3.3.2.1 Screening of individual plants of families S16(7.3) and S726(3.2): verification of molecular markers present in selected lines

Phenotypic screening was performed for selected progenies of the best selected lines S16(7.3) and S726(3.2), together with the parental lines, to verify the genotypic results. Individuals were screened and scored for leaf, stem and stripe rust resistance according to Table 3.9, using a scale of 0-4 where 0 represents a highly resistant reaction and 4 a highly susceptible reaction.

Table 3.9 Disease score results for selected rust resistant lines and parental lines

Lines screened	Screening infection type		
	Stripe rust race 6E22A+	**Leaf rust race UVPt20**	**Stem rust race UVPgt60**
S16(7.3)P3-5.1	3	0;	3
S726(3.2)P3-6.1	0;	0;	3
Avocet*YrSp*	0;	3	1
Kariega	3	3	3
Blade	missing	3	0
CS*Lr19*-149-229	3	;	3
CM-82036	;	0;	4
Frontana	3	4	3
Morocco (control)	4	4	4

Leaf rust resistance evaluation

Phenotypic screening was done at seedling stage. This implies that only resistance genes expressed at seedling stage will be detected. The selected lines S16(7.3)P3-5.1 and S726(3.2)P3-6.1 showed resistance to leaf rust with disease scores of 0;. This confirmed the genotypic data that indicated the presence of the *Lr19* gene, as indicated in Table 3.8. However, the presence of *Lr34/Yr18/Sr57* could not be detected since it is only expressed at adult plant stage and will be masked by *Lr19* if the latter is present. Figure 3.9 indicates the performance of lines inoculated with leaf rust isolate UVPt20.

Kariega was susceptible at seedling stage because it only possesses *Lr34/Yr18/Sr57*. Avocet*YrSp*, Blade and Morocco showed susceptible reactions to leaf rust with disease scores of 3 or 4 (Table 3.9). As expected, CS*Lr19*-149-229 showed a resistant reaction due to the presence of the *Lr19* gene.

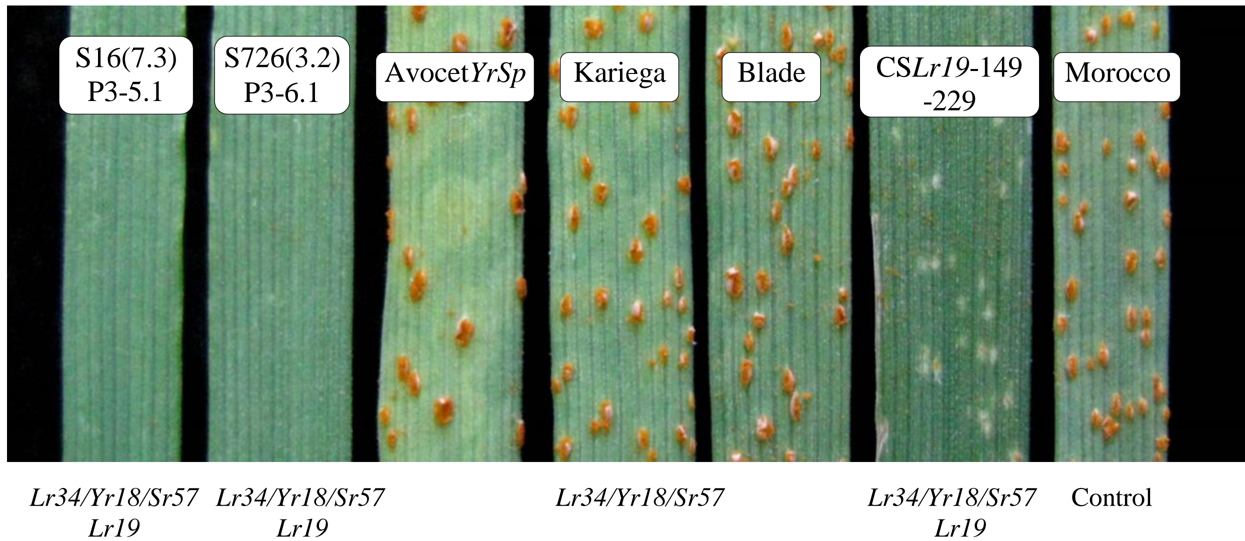


Figure 3.9 Leaf rust resistance evaluation on selected progeny and parental lines using race UVPt20. Leaf rust resistance genes indicated at the bottom of the photo are expected to be present based on marker data.

Stem rust resistance evaluation

The original parental line Avocet*YrSp* contains *Sr26* whereas Blade contains both *Sr26* and *Sr2*. The presence of *Sr26* was confirmed by resistance against stem rust race UVPtg60 tested at seedling stage (Figure 3.10). According to genotypic data S726(3.2) contained *Sr2* and *Sr26* in either homozygous or heterozygous states. However, results from phenotypic screening showed the absence of the *Sr26* gene in the tested progeny of line S726(3.2), namely S726(3.2)P3-6.1, that was screened phenotypically. Since genes in the family were still segregating this could be expected. The presence of *Sr2* could not be detected at seedling stage because it is only expressed at adult plant stage. Lines S16(7.3)P3 5.1, Kariega, CS*Lr19*-149-229 and Morocco were susceptible to the stem rust pathogen.

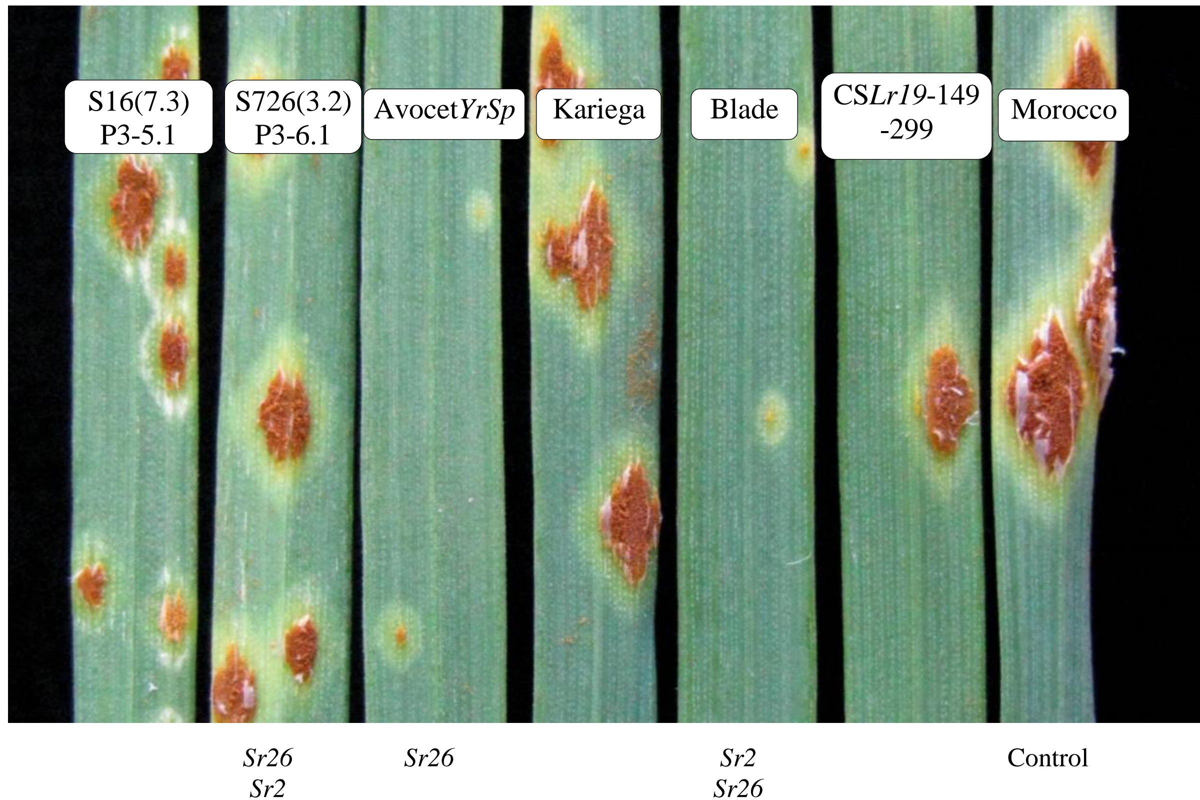


Figure 3.10 Stem rust resistance evaluation on selected progeny and parental lines using race UVPtg60. Stem rust resistance genes indicated at the bottom of the photo are expected to be present based on marker data.

Stripe rust resistance evaluation

Avocet*YrSp,* according to breeding data and genotypic data, was expected to show resistance to stripe rust race 6E22A+ while all other lines were expected to be susceptible to the pathotype. Line S726(3.2)P3-6.1 also showed resistance to stripe rust with a disease score of 0 (Table 3.9 and Figure 3.11). This was recorded as an added merit to the selected line because *YrSp* could not be selected for at molecular level due to unavailability of markers linked to the gene. Molecular data indicated that lines S16(7.3)P3-5.1, Kariega and CS*Lr19*-149-299 contained *QYr.sgi-7D*/*Lr34/Yr18/Sr57* and Q*Yr.sgi-2B.1* but these genes could not be detected at seedling stage.

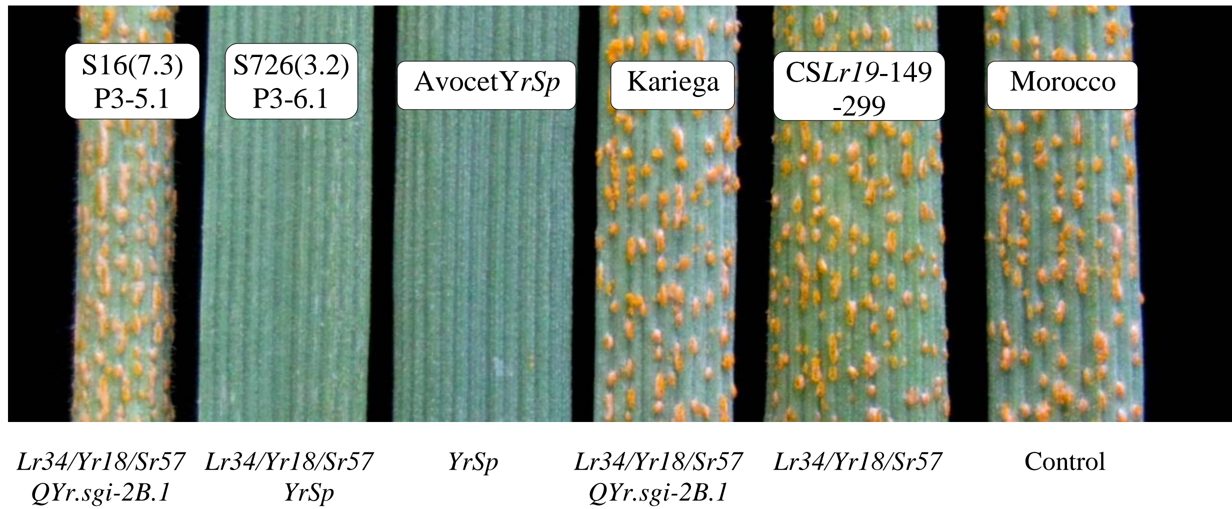


Figure 3.11 Stripe rust resistance evaluation on selected progeny and parental lines using race 6E22A+. Stripe rust resistance genes indicated at the bottom of the photo are expected to be present based on marker data.

3.3.2.2 Phenotypic evaluation of original parental lines

FHB lines that are to be used in the next phase of the study (Chapter 4), namely CM-82036 and Frontana, were also included in the phenotypic screening (Table 3.10). Screening results indicated that CM-82036 possesses resistance against leaf and stripe rust. Frontana seedlings were phenotypically susceptible to all three rusts at seedling stage.

Table 3.10 Summary of genotypes' performance to different rust isolates screened and genes present in each tested line or cultivar based on genotypic and phenotypic data

Genotype	Leaf rust isolate: UVPt20	Stem rust isolate: UVPgt60	Stripe rust isolate: 6E22A+	Confirmed genes/QTL
S16(7.3)P3 5.1	Resistant	Susceptible	Susceptible	Q*Yr.sgi-2B.1*, *Lr34/Yr18/Sr57*, ***Lr19***
S726(3.2)P3 6.1	Resistant	Susceptible	Resistant	*Lr34/Yr18/Sr57*, ***Lr19***, *Sr2*, ***YrSp***
Avocet*YrSp*	Susceptible	Resistant	Resistant	***YrSp*, *Sr26***
Kariega	Susceptible	Susceptible	Susceptible	*Lr34/Yr18/Sr57,* Q*Yr.sgi-2B.1*
Blade	Susceptible	Resistant	*	*Sr2*, ***Sr26***
CS*Lr19*-149-229	Resistant	Susceptible	Susceptible	***Lr19***, *Lr34/Yr18/Sr57*
CM-82036	Resistant	Susceptible	Resistant	*Lr34/Yr18/Sr57*
Frontana	Susceptible	Susceptible	Susceptible	*Lr34/Yr18/Sr57*
Morocco	Susceptible	Susceptible	Susceptible	

* = did not germinate; **Bold** = resistance genes effective at seedling stage

3.4 Discussion

The objective of this study was to select the best lines from the self-pollinated double cross population that contained the highest number of genes and/or QTL and to additionally select lines in which these genes/QTL were in a homozygous state. MAS in progeny of four double cross families S16, S178, S726 and S791 was performed. All chi square tests on expected segregating ratios of 1:1 and 1:2:1 versus observed ratios showed low probability values and a poor fit as a result of a small sample size. The small sample sizes of each of the four families were due to a limited number of seeds being available for screening. Across all families, markers Gwm148 and csLV34 showed a good fit for all tested individuals. The *Lr34/Yr18/Sr57* gene was present in three of the four original parental lines used to create the double cross population. It was already homozygous in line S16, before self-pollination, explaining the 100% correlation between the observed and expected segregation ratios.

The best lines between all four families screened tested positive for six of the eight markers. Of these lines, line S16(7.3) had the most markers in a homozygous state, namely four. The other four lines [S178(7.2), S726(3.2), S791(2.2) and S791(4.1)] had a fewer number of homozygous markers compared to line S16(7.3). Lines S178(2.3) and S791(2.3 and 5.2) had one marker each which was the lowest number of observed markers (Gwm501, Gwm501 and csLV34, respectively). Based on molecular marker data, the two most promising lines were S16(7.3) and S726(3.2) with six markers each. Line S16(7.3) tested positive for markers Gwm111 (*QYr.sgi-7D*), Gwm295 (*QYr.sgi-7D*), Gwm148 (*QYr.sgi-2B.1*), Gwm501 (*QYr.sgi-2B.1*), csLV34 (*Lr34*) and STSLr19$_{130}$ (*Lr19*) while S726(3.2) tested positive for markers Sr26#43 (*Sr26*), Gwm295 (*QYr.sgi-7D*), csLV34 (*Lr34*), STSLr19$_{130}$ (*Lr19*), Gwm111 (*QYr.sgi-7D*) and stm559gtag (*Sr2*). These two lines were selected for use in further breeding programmes and were selected because, based on genotypic data, all markers were present in a combination between these two selected lines. Prins et al. (2011) confirmed that the *QYr.sgi-7D* QTL is actually the *Lr34/Yr18/Sr57* gene complex, therefore screening in the next part of the study did not include markers Gwm111 and Gwm295 linked to the *QYr.sgi-7D* QTL. The use of molecular markers combined with conventional breeding helps with selection of genes of interest (Farooq and Azam, 2002). The use of molecular markers in the current study facilitated selection of individuals with genes and/or QTL of interest for the next part of the study.

The molecular marker data were confirmed using rust infection type tests. Line S16(7.3)P3-5.1 showed leaf rust resistance, confirming the presence of the seedling resistance gene *Lr19*. Line S726(3.2)P3-6.1 showed resistance to both leaf and stripe rust due to the presence of the seedling resistance genes *Lr19* and *YrSp*. However, it was susceptible to stem rust. Based on the presence of the *Sr26* gene in line S726(3.2), it was expected that plant S726(3.2)P3-6.1 would have stem rust resistance. Phenotypic screening indicated the absence of the *Sr26* gene in this line. The Sr26#43 marker is a dominant marker, thus homozygous resistant plants cannot be distinguished from heterozygous resistant plants. Phenotypic data thus confirmed that the *Sr26* gene was heterozygous in line S726(3.2). Progeny from line S726(3.2) will thus segregate for *Sr26* resistance, explaining the absence of the *Sr26* gene in plant S726(3.2)P3-6.1. Phenotypic screening furthermore indicated the presence of the *YrSp* gene in line

S726(3.2) which was an added advantage because due to a lack of molecular markers linked to the *YrSp* gene, no active selection for *YrSp* occurred throughout the breeding process.

Parental lines used to add FHB resistance to the rust resistant lines (Chapter 4) were included in the phenotypic screening. Although the FHB resistance donor line Frontana was susceptible to all three rusts at seedling stage, Roelfs (1988) reported that this cultivar is a good source of durable adult plant leaf rust resistance due to the presence of *Lr34/Yr18/Sr57*. As an additional bonus, phenotypic screening also indicated that the other FHB donor line, CM-82036, tested positive for leaf and stripe rust resistance.

Phenotypic data confirmed that combining of genes of multiple resistances into a single line provides durable resistance to a broad range of diseases. In this study phenotypic data confirmed genotypic data even though APR resistance was not be detected. Since results of phenotypic screening validated results of genotypic screening, it implies that MAS can facilitate selection of plants with multiple resistances without phenotyping (Hittalmani et al., 2000).

MAS was successfully applied to select the best two lines to be used in the next phase of the breeding scheme. As expected, the selected lines all showed higher levels of homozygosity compared to their parental lines although some genes were absent after self-pollination. The individual plant of family S178 identified by Sydenham (2007) tested positive for seven heterozygous markers. After selfing, one of the progenies of this line, S178(7.2), tested positive for six markers of which three were in a homozygous state. All individuals developed from selfing of the double cross population contained homozygous markers.

Although none of the lines tested positive for the presence of all eight combined markers, MAS helped to speed up the process of combining genes that would have taken a period of eight to twelve years if done using conventional breeding only (Langridge et al., 2001). Confirming the presence of all six accumulated genes would have been time consuming if only traditional breeding was used. Selection of plants with multiple resistance from a larger population, based on morphological markers, is difficult and laborious (Hittalmani et al.,

2000). Molecular markers are furthermore not affected by environmental conditions and can be detected at different plant growth stages (Miflin, 2000). This was advantageous since marker screening could be done at seedling stage although some of the traits selected for are APR genes/traits (*QYr.sgi-2B.1, Lr34/Yr18/Sr57* and *Sr2).* Most screenings for rust resistance in the greenhouse are also furthermore done at seedling stage. Using greenhouse rust inoculations on selected lines confirmed the presence of genotypically identified resistance genes. Thus, the presence of the *QYr.sgi-2B.1* QTL, *Lr34/Yr18/Sr57* and *Sr2* genes that could not be confirmed phenotypically demonstrated that MAS could be used to identify lines that possibly contained these traits.

Even though marker evaluation is more expensive compared to conventional breeding, combining of many genes into a single line provides durable and multiple resistance (Datta et al., 2002). Incorporation of both modern and traditional breeding methods is highly recommended because both methods are equally important to breeders (Jauhar, 2006).

3.5 Conclusion

Results generated in this study indicated that MAS was useful in selection of genes of interest and combining resistance genes into a single genotype within a shortened period of time. The developed lines from this study contain both seedling and APR genes and QTL hence are valuable donors of resistance for future breeding programmes.

3.6 References

Bariana HS, Brown GN, Bansal UK, Mian H, Stander GE, Lu M (2007) Breeding triple resistant wheat cultivars for Australia using conventional and marker selection technologies. Australian Journal of Agricultural Research 58:576-587

Castro AJ, Chen X, Hayes PM, Knapps SJ, Line RF, Toojinda T, Vivar H (2002) Coincident QTL which determine seedling and adult plant resistance to stripe rust in barley. Crop Science 42:1701-1708

Datta K, Baisakh N, Thet M, Tu J, Datta SK (2002) Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. Theoretical and Applied Genetics 106:1-8

Farooq S, Azam F (2002) Molecular markers in plant breeding: 1 Concepts and characterization. Pakistan Journal of Biological Sciences 5:1135-1140

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390

Hayden MJ, Kuchel H, Chalmers KJ (2004) Sequence tagged microsatellites for the Xgwm533 locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 109:1641-1647

Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theoretical and Applied Genetics 100:1121-1128

Huang XQ, Wang LX, Xu MX, Röder MS (2003) Microsatellite mapping of powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 106:858-865

Ishii T, Yonezawa K (2007) Optimization of marker-based procedures for pyramiding genes from multiple donor lines: I. Strategies for selecting the objective homozygous plant. Crop Science 47:1878-1886

Jauhar PP (2006) Modern biotechnology as an integral supplement to conventional plant breeding: The prospects and challenges. Crop Science 46:1841-1859

Jian-Kang W, Pfeifferz WH (2007) Simulation modeling in plant breeding: Principles and applications. Agricultural Sciences in China 6:908-921

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene in wheat. Theoretical and Applied Genetics 114:21-30

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001) Trends in genetic and genome analyses in wheat: a review. Australian Journal of Agricultural Research 52:1043-1077

Li X, Yang W, Li Y, Liu D, Yan H, Meng Q, Zhang T (2006) A SSR marker for leaf rust resistance gene *Lr19* in wheat. Agricultural Sciences in China 5:111-115

Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse germplasm. Theoretical and Applied Genetics 111:496-504

Miflin B (2000) Crop improvement in the 21st century. Journal of Experimental Botany 51:1-8

Prins R, Groenewald JZ, Marais GF, Snape JW, Koebner RMD (2001) AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. Theoretical and Applied Genetics 103:618-624

Prins R, Pretorius ZA, Bender CM, Lehmensiek A (2011) QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega x Avocet S doubled haploid wheat population. Molecular Breeding 27:259-270

Randhawa HS, Asif M, Pozniak C, Clarke JM, Graf RJ, Fox SL, Humphreys G, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Hucl P, Spaner D (2013) Review: Application of molecular markers to wheat breeding in Canada. Plant Breeding 132:458-471

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023

Roelfs AP (1988) Resistance to leaf rust in wheat. In: Simmonds NW, Rajaram S (Eds) Breeding Strategies for Resistance to the Rusts of Wheat. CIMMYT: Mexico. Federal District Mexico, pp 10-22

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of the National Academy of Sciences USA 81:8014-8018

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellites (SSR) markers in wheat. Theoretical and Applied Genetics 110:550-560

Stakman EC, Stewart DM, Loegering W (1962) Identification of Physiologic Races of *Puccinia graminis* var *tritici*. Department of Agricultural Research Service, United States of America, 53 pp

Sun GL, Fahina T, Korol AB, Turpeinen T, Grama A, Ronin YI, Nevo E (1997) Identification of molecular markers linked to the *Yr15* stripe rust resistance genes of wheat originated in wild emmer wheat *Triticum diccoides.* Theoretical and Applied Genetics 95:622-628

Sydenham SL (2007) Pyramiding wheat rust resistance genes using marker-assisted selection. MSc dissertation, Department of Plant Sciences, University of the Free State, 108 pp

Tyryshkin LG, Gult'tyaeva EI, Alpat'eva NV, Kramer I (2006) Identification of effective leaf rust resistance genes in wheat using STS markers. Russian Journal of Genetics 42:662-666

Vida G, Gal M, Uhrin A, Veisz O, Syed NH, Flavell AJ, Wang Z, Bedo Z (2009) Molecular markers for the identification of resistance genes and marker-assisted selection in breeding wheat for leaf rust resistance. Euphytica 170:67-76

Wenzel G (1985) Strategies in unconventional breeding for disease resistance. Annual Review of Phytopathology 23:149-156

Wenzel G (2006) Molecular plant breeding: achievements in green biotechnology and future perspectives. Applied Microbiology and Biotechnology 70:642-650

CHAPTER 4

Combining wheat rust and Fusarium head blight resistance genes and quantitative trait loci using marker-assisted selection

4.1 Introduction

Stripe rust and leaf rust are important diseases of wheat worldwide. This is mainly due to the pathogens' fast multiplication rate and ability to spread by air-borne dispersal from one area to another. However, historically, stem rust has been the most damaging disease of wheat. Epidemics of stem rust under highly favourable environmental conditions result in yield losses (Leonard and Szabo, 2005). In addition to wheat rusts, FHB causes yield losses in moist areas (Parry et al., 1995; McMullen et al., 1997) with the associated mycotoxin contamination of cereal food and feed (Buerstmayr et al., 2003). Grain contaminated by FHB disease has low test weight, poor baking quality and a low germination rate when used as seed (Bai and Shaner, 2004). The fungus produces DON that is harmful to animal and human health (Snijders, 1990; Bai et al., 2001). Cultivation of resistant varieties adds value to control of this fungal disease (Buerstmayr et al., 2003).

Breeding for disease and pest resistance is an effective means of protecting crops from harm caused by biotic factors. Nevertheless, breeding depends on the ability to select the best individuals from crosses and this is difficult for some diseases, especially when resistance to more than one gene or disease is combined into a single genotype. Selection for foliar resistance is usually simple, through phenotypic evaluation of areas affected and the type of lesion produced. Resistance to many diseases, including foliar diseases, have been identified and used in breeding, although there are some examples where breeding for resistance was unsuccessful due to the failure to find resistant sources (Johnson and Jellies, 1992).

A large number of rust resistance genes (for all three rust types) have been identified in wheat. Genetic variation for resistance is available in the primary gene pool of wheat and can be used in crossing and selection programmes (Salameh et al., 2011).

Breeding for rust and FHB resistance using conventional selection is feasible, but expensive (Buerstmayr et al., 2003). More than one resistance gene may be combined into agronomically adapted wheat genotypes using conventional breeding but it is labour intensive and identifying desirable plants in segregating populations is sometimes difficult (Kelly, 1995). Additionally, notable results in gene pyramiding are delayed when using only conventional breeding, due to phenotypic evaluations with different rust races during selection. Hence, application of MAS during resistance breeding in wheat enhances gene pyramiding and expedites timely release of resistant cultivars (Miedaner et al., 2009). Molecular mapping and MAS are modern tools that have been successfully used to help in the exploitation and pyramiding of different resistance genes over a short period of time (Anderson et al., 2001; Buerstmayr et al., 2002; Anderson, 2007).

Molecular markers tightly linked to desired traits are available and are useful for discovery of new plant genotypes in segregating populations (Liu et al., 2000). However, the success of this effort relies on the use of the appropriate technique (Bariana et al., 2007). Codominant SSR markers closely linked to genes of interest are useful for selection and gene pyramiding in wheat breeding programmes (Huang et al., 2003). The use of SSR markers has gained popularity because they are highly informative and polymorphic (Karaoglu et al., 2005). Resistance based on a single major gene is not durable and generating lines with a combination of resistance genes is recommended as an approach to offer long lasting resistance (Roelfs, 1988).

The aim of this study was to combine wheat rust (*Sr2*, *Sr26*, *Lr19*, *Lr34/Yr18/Sr57*, *QYr.sgi-2B.1* and *YrSp*) and FHB (type I resistance QTL on chromosome 3A and 5A and type II resistance QTL on chromosomes 3B, 6B and 7A) resistance genes/QTL into a single wheat genotype using MAS. The best rust resistance lines developed in the previous chapter were crossed with two FHB resistant donor lines, namely CM-82036 (type II resistance) and Frontana (type I resistance). The parallel crosses used during the different phases of the project are indicated in Figure 4.1.

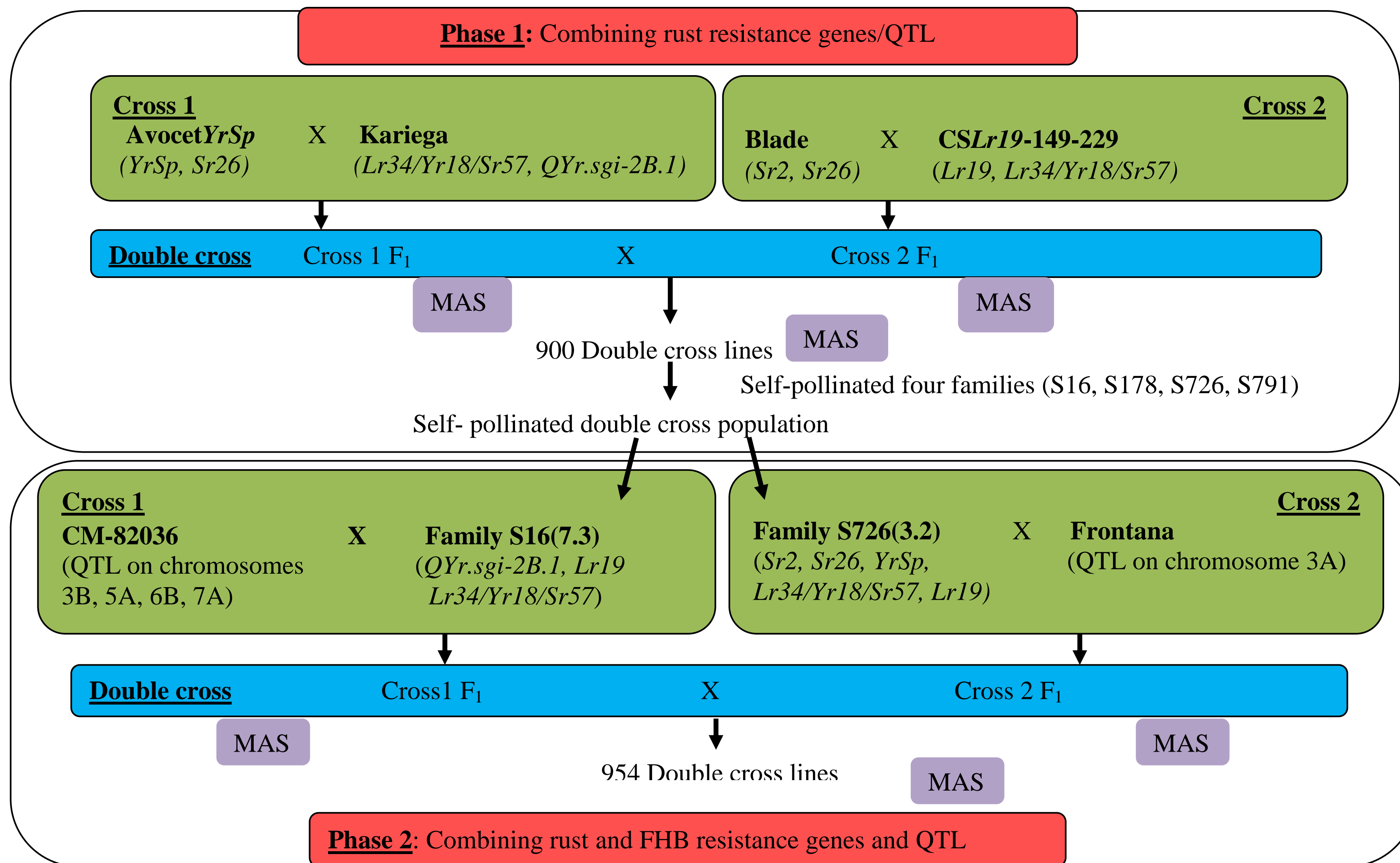


Figure 4.1 Crossing scheme to combine rust and FHB resistance genes and/or QTL into a single wheat genotype

4.2 Materials and methods

4.2.1 Plant material

Seed of the rust resistant donor lines was sourced from the double cross families selected in phase one of the study, as discussed in the previous chapter. The two selected rust resistant lines, namely S16(7.3) containing resistance genes/QTL *Lr34/Yr18/Sr57, QYr.sgi-2B.1* and *Lr19* and S726(3.2) containing *Lr34/Yr18/Sr57, QYr.sgi-2B.1, Sr26, Sr2* and *Lr19* were obtained from crosses between the original donor sources of the resistance genes used in the study, namely Avocet*YrSp*, Blade, CS*Lr19*-149-229 and Kariega. FHB resistance donor parental lines used in the second phase of the study were CM-82036 containing QTL on chromosomes 3B, 5A, 6B and 7A and Frontana containing QTL on chromosome 3A. Seed multiplication, parental screening and crosses were conducted in the greenhouse of the University of the Free State under controlled growth conditions to help minimise external environmental variation.

The FHB donor lines were obtained from Dr. H. Buerstmayr (University of Natural Resources and Life Sciences, Vienna, Australia). CM-82036 is a FHB resistant line from CIMMYT, developed from a cross between Sumai 3 and Thornbird-S (Buerstmayr et al., 2002; 2003). Frontana is a wheat cultivar from Brazil (Steiner et al., 2004).

Three seeds were planted per 3 L pot containing soil. Cultivation was done as described in section 3.2.1. A total of 96 seeds were planted in the greenhouse for generation of cross 1 [(S16(7.3)/CM-82036] (Figure 4.1). Rust resistant line S16(7.3) was used as female in cross 1 and 40 seeds of this line were planted. FHB resistant line CM-82036 was used as male and 56 seeds were planted to provide enough pollen for the crosses. Parental lines were planted at two week intervals to ensure synchronisation of flowering. A total of 46 spikes were pollinated to obtain cross 1 F_1 and 689 seeds were harvested from the crosses for future use.

A total of 96 seeds were planted in the greenhouse for generation of cross 2 [(S726(3.2)/Frontana]. Rust resistant line S726(3.2) was used as female in cross 2 and 40

seeds of this line were cultivated. FHB resistant line Frontana was used as male and 56 seeds were planted to provide enough pollen for the crosses. The same planting regime was followed as for cross 1. In the same manner, a total number of 46 spikes were pollinated to obtain progeny of cross 2 F_1 and a total number of 449 seeds were harvested for future use in the breeding programme.

A total of 600 seeds were planted in the greenhouse for generation of cross 3 (cross 1 F_1/cross 2 F_1). Progeny of cross 1 [S16(7.3)/CM-82036] was used as female in cross 3 and 240 seeds were planted. Progeny of cross 2 [S726(3.2)/Frontana] was used as male in cross 3 and 360 seeds were planted as pollinator. A total number of 215 spikes were pollinated to obtain progeny of cross 3 and 1 302 seeds of the double cross population were harvested. About 600 seeds from cross 3 were planted in the greenhouse for molecular analysis and the remaining seed was stored in the germplasm bank.

4.2.2 Sampling of leaf material and DNA isolation

Leaf material of the double cross population was sampled and DNA isolated as described in sections 3.2.2 and 3.2.3 respectively.

4.2.3 SSR analyses

4.2.3.1 Markers linked to rust resistance genes/QTL

The double cross progeny were screened for the presence of the expected markers linked to rust resistance genes using SSR analysis. A total of six markers linked to rust resistance were used, namely Gwm148 and Gwm501 linked to the *QYr.sgi-2B.1* QTL, Sr26#43 (*Sr26*), stm559gtag (*Sr2*), cssfr5 (*Lr34/Yr18/Sr57)* and STS marker $Lr19_{130}$ (*Lr19*). Since Prins et al. (2011) indicated that the *QYr.sgi-7D* QTL was actually the *Lr34/Yr18/Sr57* complex, markers Gwm111 and Gwm295 were not included during this part of the study. SSR analyses for these markers were performed as described in section 3.2.4 except for *Lr34/Yr18/Sr57*. PCR reaction conditions for these markers are given in Table 3.2, primer sequences in Table 3.3 and PCR cycling conditions in Table 3.4. Marker cssfr5, developed by Lagudah et al. (2009),

was used instead of the previously used marker csLV34, linked to the *Lr34/Yr18/Sr57* locus. Marker pair cssfr5 is a perfect marker located inside the *Lr34/Yr18/Sr57* gene region and is a codominant marker that can indicate the presence or absence the *Lr34* gene with 100% accuracy. PCR cycling conditions and primer sequences for marker cssfr5 are given in Tables 4.1 and 4.2.

4.2.3.2 FHB marker screening of parental lines, F_1 progeny of each cross and the double cross population

Previously published SSR and STS markers linked to FHB resistance QTL on chromosomes 3A, 3B, 5A, 6B and 7A were tested on the parental lines to find the most reliable and scorable markers. More than ten markers were tested to identify polymorphisms and different banding patterns were observed. Marker DuPw227 linked to the QTL on chromosome 3A was tested and produced reliable and scorable results. Markers Gwm533, Gwm493 and Barc133 linked to the QTL chromosome on 3B were tested and the marker that produced the most reliable, consistent and scorable results was Barc133 and was thus used throughout for screening the double cross population. Markers Gwm156, Gwm304, Gwm293 and Barc197.2 were tested for the QTL on chromosome 5A and Gwm156 was initially selected based on the production of scorable results. However, due to inconsistency of the Gwm156 marker when tested in the segregating double cross population, Gwm293 was used to screen for the same QTL to obtain more reliable results. Two markers, Gwm133 and Gwm644, were tested for the presence of the QTL on chromosome 6B and the most reliable results were obtained from using Gwm133. Markers Gwm130 and Gwm233 were tested for the presence of the minor QTL on chromosome 7A and Gwm233 produced the most scorable results. Some of the rust donor lines also tested positive for alleles amplified by FHB markers and this made scoring difficult.

Table 4.1 PCR cycling conditions and specific programmes used for primers linked to FHB resistance as well as the primer linked to the rust resistance gene *Lr34/Yr18/Sr57*

Marker	PCR cycling conditions	Resistance gene/QTL
cssfr5	94°C 5 min, 1 cycle; 94°C 1 min, 58°C 1 min, 72°C 1 min, 5 cycles ; 94°C 30 s, 58°C 30 s, 72°C 50 s, 30 cycles; 94°C 30 s, 58°C 30 s, 72°C 5 min, 1 cycle	*Lr34/Yr18/Sr57*
DuPw227	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min; 1 cycle	Type I FHB, 3A QTL
Barc133	94°C 3 min, 1 cycle; 94°C 45 s, 52°C 45 s, 72°C 1:10 min, 44 cycles ; 72°C 10 min, 1 cycle	Type II FHB, 3B QTL
Gwm293	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min; 1 cycle	Type II FHB, 5A QTL
Gwm156	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min; 1 cycle	Type II FHB, 5A QTL
Gwm133	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min; 1 cycle	Type II FHB, 6B QTL
Gwm233	94°C 3 min, 1 cycle; 94°C 1 min, 60°C 1 min, 72°C 2 min, 44 cycles; 72°C 10 min; 1 cycle	Type II FHB, 7A QTL

QTL = quantitative trait loci

Table 4.2 SSR markers, primer pair sequences, targeted genes/QTL and parental cultivar sources used for markers linked to FHB resistance including new marker used for resistance gene *Lr34/Yr18/Sr57*

Marker name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Gene/QTL	Resistance sources	Reference
cssfr5	L34SPF–GGGAGCATTATTTTTTTCCATCATG L34DINT9F–TTGATGAAACCAGTTTTTTTTCTA	L34DINT13R–ACTTTCCTGAAAATAATACAAGCA L34MINUSR–TATGCCATTTAACATAATCATGAA	*Lr34/Yr18/sr57*	Avocet*YrSp*, Kariega, CS*Lr19*-149-299	Lagudah et al., 2009
Dupw227	CATGTTGGGAATTTCTGTCG	CCACGAGCCATGTATCACC	3A	Frontana	Eujayl et al., 2002
Barc133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	3B	CM-82036	Röder et al., 1998
Gwm156	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	5A	CM-82036	Röder et al., 1998
Gwm293	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	5A	CM-82036	Röder et al., 1998
Gwm133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	6B	CM-82036	Röder et al., 1998
Gwm233	TCAAAACATAAATGTTCATTGGA	TCAACCGTGTGTAATTTTGTCC	7A	CM-82036	Röder et al., 1998

QTL = quantitative trait loci

The following markers were used to screen for the different QTL linked to FHB resistance; DuPw227 linked to the QTL on chromosome 3A, Barc133 linked to the QTL on chromosome 3B, Gwm156 and Gwm293 linked to the QTL on chromosome 5A, Gwm133 linked to the QTL on chromosome 6B and Gwm233 linked to the minor QTL on chromosome 7A. FHB resistance marker Barc133 linked to the QTL on chromosome 3B was used to screen progenies of cross 1 [S16(7.3)/CM-82036] to confirm the transfer of this QTL in order to confirm that progeny resulted from a cross and not self-pollination. Since this QTL was observed only in the male parental line CM-82036, the presence or absence of the fragment could be easily examined using this marker. Confirmation of combination of genes/QTL in the progenies of cross 2 [S726(3.2)/Frontana] was done using FHB resistance marker DuPw227 linked to the QTL on chromosome 3A, that was donated by the male parent Frontana.

4.2.3.3. PCR cycling conditions

SSR-PCR reactions for primers not already described in Chapter 3 were set up in a final volume of 10 µl, containing 200 ng DNA, 2 mM $MgCl_2$, 1 x Go *Taq* Flexi Polymerase buffer (Promega), 200 µM dNTPs, 25 ng of each primer and 0.25 U Go *Taq* Flexi Polymerase (Promega). PCR cycling conditions are given in Table 4.1 and primer sequences in Table 4.2.

4.2.4 Visualisation of amplified fragments

4.2.4.1 Agarose gel electrophoresis

SSR-PCR products for the primer pair cssfr5 were visualised using 1.5% (w/v) agarose gel electrophoresis as described in section 3.2.5.1

4.2.4.2 Polyacrylamide gel electrophoresis (PAGE)

SSR-PCR products for primers Gwm148, DuPw227, Barc133, Gwm156 and Gwm133 were separated using denaturing 5% PAGE and visualised using silver staining as described in sections 3.2.5.2 and 3.2.5.3.

4.2.4.3 Gel Scan 3000

Some of the screened markers were difficult to discriminate and visualise using silver staining. The following markers were visualised using the Gel Scan 3000 real-time fragment analyser: Gwm156, Gwm233, Gwm501, STSLr19$_{130}$, Sr26#43 and Gwm293. These products were separated and visualised using the Gel Scan 3000 software version 8.00.01 (Corbett Research, Sydney, Australia). Samples were separated using 5% (w/v) non-denaturing PAGE. The non-denaturing gel consisted of 1x TBE buffer, 5% acrylamide:bis-acrylamide (19:1 w/v), 0.12% (v/v) tetramethylethylenediamine (TEMED) and 0.08% (v/v) ammonium persulfate (APS) in a total volume of 25 ml. The upper chamber of the system contained 0.5x TBE buffer. The bottom chamber also contained 0.5x TBE but mixed with 1% (v/v) ethidium bromide. Samples were mixed with formamide loading buffer before loading. Samples and runs were diluted differently depending on signal strength. One microlitre of each diluted sample was loaded on the gel and run at 1 200 V for 45-90 min at 37°C. Ethidium bromide stained gels were digitally stored on the computer and photographs printed for scoring where necessary. Scoring of presence or absence was done on score sheets for all samples based on the allele sizes of the parents.

4.2.5 Data analyses

The number of alleles and allele sizes per SSR marker set were determined during parental screening and compared to the expected sizes from previous studies. The number of markers linked to resistance genes/QTL for each individual of the double cross population was determined and statistical analysis done using Chi square analysis. Fragments were scored "1" if the allele size corresponded to the allele size of the resistant parental line and as "0" if the size was different from the resistant parent's allele. Resistance was thus represented as "1" and susceptibility by "0". Heterozygotes were discriminated from homozygotes by scoring "1" for both alleles present. Data for the expected ratios were created for comparison with the observed data.

4.3 Results

4.3.1 Screening of parental lines using markers linked to rust resistance

The four parental lines S16(7.3), S726(3.2), CM-82036 and Frontana were screened with three SSR (stm559gtag, Gwm501 and Gwm148), two STS ($STSLr19_{130}$ and Sr26#43) and one SNP (cssfr5) marker linked to rust resistance. The four parental lines, Avocet*YrSp*, Blade, CS*Lr19*-149-299 and Kariega, used in phase one of the study to create the two rust resistant lines, were screened concurrently for comparison with the four current parental lines to be used for combining rust and FHB resistance genes. This was done to evaluate individual lines and for gathering more informative molecular data on what to expect in the offspring. According to results shown in Table 4.3, marker Gwm501 amplified four allele sizes 165 bp, 168 bp, 174 bp and 177 bp in Blade, Avocet*YrSp*, CS*Lr19*-149-299 and Kariega respectively. The marker was not present in line S726(3.2), CM-82036 and Frontana. Similar results were obtained for marker Gwm148, flanking the other side of the QTL. The expected alleles linked to the presence of *QYr.sgi-2B.1* were amplified in line S16(7.3) but were absent, as expected, in line S726(3.2). None of the FHB donor lines contained the *QYr.sgi-2B.1* QTL (Table 4.3).

The third SSR marker stm559gtag linked to *Sr2* amplified the 237 bp allele size linked to resistance in the donor parent Blade as well as the rust resistant line S726(3.2), as expected. The STS marker $STSLr19_{130}$ linked to *Lr19* amplified the 100 bp allele size linked to resistance as expected in the donor line CS*Lr19*-149-299 and in the two rust resistant lines S16(7.3) and S726(3.2). However, results indicated that the FHB donor line CM-82036 tested positive for the allele linked to *Lr19* resistance. The STS marker Sr26#43 associated with *Sr26* resistance amplified the expected allele size of 190 bp in AvocetYrSp, Blade and line S726(3.2). The new SNP marker, cssfr5 linked to *Lr34/Yr18/Sr57* resistance amplified the expected 751 bp allele in all screened lines/varieties except Blade. Results thus indicated that the two FHB donor lines tested positive for the *Lr34/Yr18/Sr57* locus.

Table 4.3 Allele sizes of fragments amplified in the parental lines using markers linked to rust resistance genes and QTL

Marker	**Targeted gene/QTL**	**Parental lines and allele size (bp)**							
		Avocet*YrSp*	**Blade**	**CS*Lr19*-149-299**	**Kariega**	**S16(7.3)**	**S726(3.2)**	**CM-82036**	**Frontana**
Gwm501	*QYr.sgi-2B.1*	168	165	174	**177**	**177**	Null allele	Null allele	Null allele
Gwm148	*QYr.sgi-2B.1*	145	142	162	**165**	**165**	Null allele	145	142
$STSLr19_{130}$	*Lr19*	Null allele	Null allele	**100**	Null allele	**100**	**100**	**100**	Null allele
Sr26#43	*Sr26*	**190**	**190**	Null allele	Null allele	Null allele	**190**	Null allele	Null allele
stm559gtag	*Sr2*	249	**237**	252	Null allele	Null allele	**237**	249	242
cssfr5	*Lr34*	**751**	523	**751**	**751**	**751**	**751**	**751**	**751**

Allele sizes in bold are linked to resistance genes or quantitative trait loci (QTL); bp = base pairs

4.3.2 Screening of parental lines using markers linked to FHB resistance

Both the original parental lines (Avocet*YrSp*, Blade, CS*Lr19*-149-299 and Kariega) as well as parental lines used for combining rust and FHB resistance [S16(7.3), S726(3.2), CM-82036 and Frontana] were screened with seven markers linked to FHB resistance QTL on chromosomes 3A, 3B, 5A, 6B and 7A (Table 4.4).

Barc133 (linked to the QTL on chromosome 3B), Gwm233 (7A) and DuPw227 (3A) amplified unique allele sizes that were only present in the FHB donor lines. However, the rest of the SSR markers indicated the presence of markers linked to FHB resistance in the original parental lines. The fragment linked to FHB resistance located on chromosome 5A (Gwm293) was also present in CS*Lr19*-149-299 while the fragment amplified by marker Gwm156 (5A) was also present in Kariega and the fragment amplified by marker Gwm133 (6B) was also present in Blade. However, none of these fragments were transferred to their progeny [S16(7.3) and S726(3.2)]. Although Gwm644 amplified the expected allele size of 145 bp in CM-82036, results were not repeatable and reliable and could thus not be used for further screening (Table 4.4).

4.3.3 F_1 cross identification

Two molecular markers were selected to identify successful crosses in the F_1 population for both cross 1 [CM-82036/S16(7.3)] and cross 2 [Frontana/S726(3.2)]. Barc133 was tested on 153 F_1 segregating individuals of cross 1 based on the amplification of allele sizes of 100 bp in S16(7.3) and 125 bp in CM-82036. Results confirmed that 72.54% of the tested individuals of cross 1 were true F_1 hybrids. DuPw227 was tested on 215 F_1 segregating individuals of cross 2 based on the amplification of 185 bp in S726(3.2) and 180 bp allele sizes in Frontana (Figure 4.2). Results confirmed that 92.01% of the tested individuals of cross 2 were true F_1 hybrids. Only individual plants identified as true F_1 hybrids were used in the next round of crosses.

Table 4.4 Allele sizes of fragments amplified in the parental lines using molecular markers linked to FHB resistance QTL

Marker	Targeted QTL	Parental lines and allele size (bp)							
		Avocet*YrSp*	**Blade**	**CS*Lr19*-149-299**	**Kariega**	**S16 (7.3)**	**S726 (3.2)**	**CM-82036**	**Frontana**
DuPw227	3A	185	185	185	185	185	185	185	**180**
Barc133	3B	100	120	100	100	100	100	**125**	115
Gwm293	5A	200	200	**205**	190	200	200	**205**	200
Gwm 156	5A	309	275	309	**305**	*	*	**305**	**307**
Gwm133	6B	140	**135**	133	130	140	140	**135**	*
Gwm233	7A	Null allele	252	251	250	Null allele	Null allele	**248**	249

* = No allele sizes detected in the expected size range; FHB = Fusarium head blight; QTL = quantitative trait loci

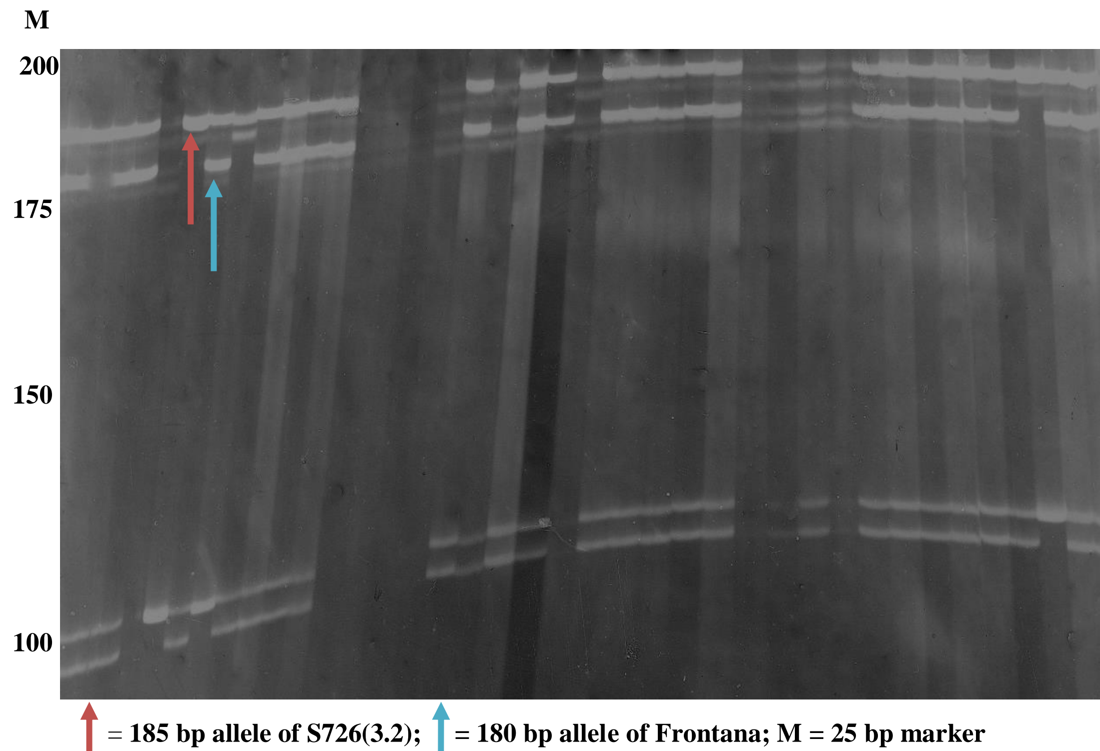


Figure 4.2 A silver stained polyacrylamide gel of S726(3.2)/Frontana F_1 individuals screened using the Dupw227 marker

Though crosses were generally successful, different planting periods seemed to have played an important role in the success rate of viable seed for both crosses. For cross 1 (Figure 4.3), among the three planting periods, the second planting was the most successful with 90% successful crosses. Successful crosses in planting one and three both showed a 80% success rate. For cross 2, the cross rate for crosses from the first planting was the lowest with a range between 60% and 65% and were not as successful as the crosses from plantings two and three that were both 80%.

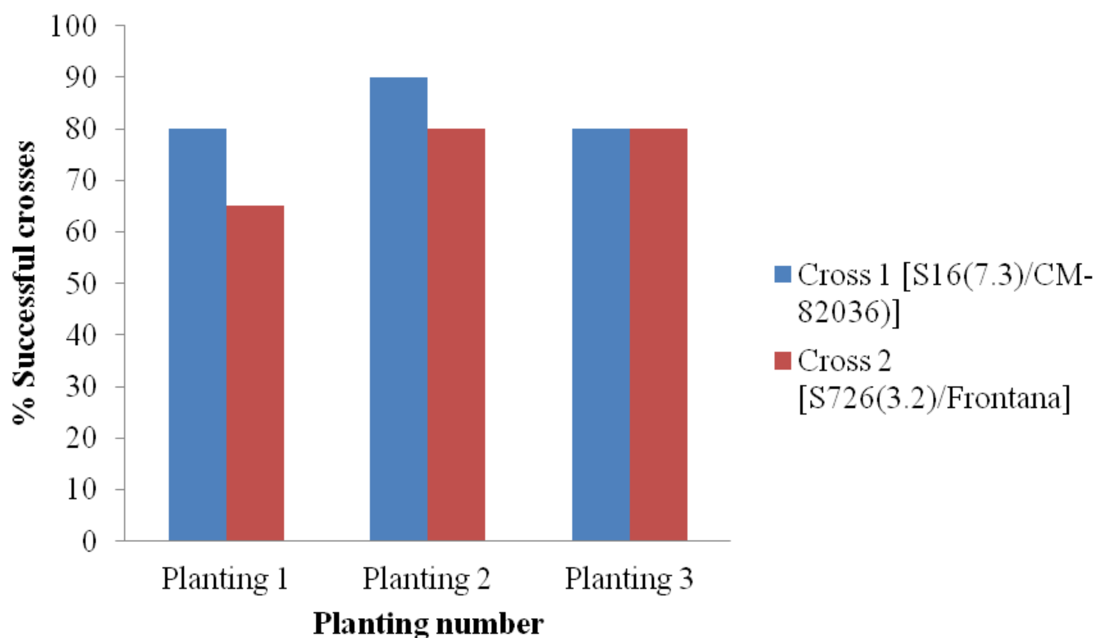


Figure 4.3 Comparison of cross success percentages for cross 1 and cross 2 within individual plantings as confirmed by SSR markers

4.3.4 Genotypic screening of double cross population

Five markers (Gwm501, Gwm148, STSLr19$_{130}$, Sr26#43 and cssfr5) linked to rust resistance (*QYr.sgi-2B.1, Lr19, Sr26* and *Lr34*) (Table 4.3) and five markers (Dupw227, Barc133, Gwm293, Gwm156, Gwm133 and Gwm233) linked to FHB resistance genes and QTL on chromosomes 3A, 3B, 5A, 6B and 7A (Table 4.4) were screened on 954 individuals of the double cross population. Although six markers linked to rust resistance (Table 4.3) were initially screened on the parental lines, results indicated that the *Sr*2 gene was absent in line S726(3.2) used in the crosses. The marker linked to *Sr2* resistance was thus not further screened in the segregating double cross population. All ten tested markers were screened and examined successfully on individuals of the double cross family. Different segregation patterns were observed for all individuals of the double cross population for all tested markers. The number of markers linked to genes/QTL of interest present in individuals of the double cross population ranged from zero to nine (Figure 4.4). Genomic data for some individuals did not give reliable results and could not be scored. Experiments could not be

repeated due to the big sample size that was screened and were thus recorded as missing data. Most of the tested individuals contained either three (233 individuals) or four markers (250 individuals). However, two individuals (plant 123.1 and plant 116.3) contained nine (90%) of the screened markers namely cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), $STSLr19_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), Sr26#43 (*Sr26*), DuPw227 (3A QTL), Barc133 (3B QTL), Gwm293 (5A QTL) and Gwm133 (6B QTL) linked to the resistance genes. Marker Gwm233 (7A QTL) was scored as missing for some individuals because it was not performing well.

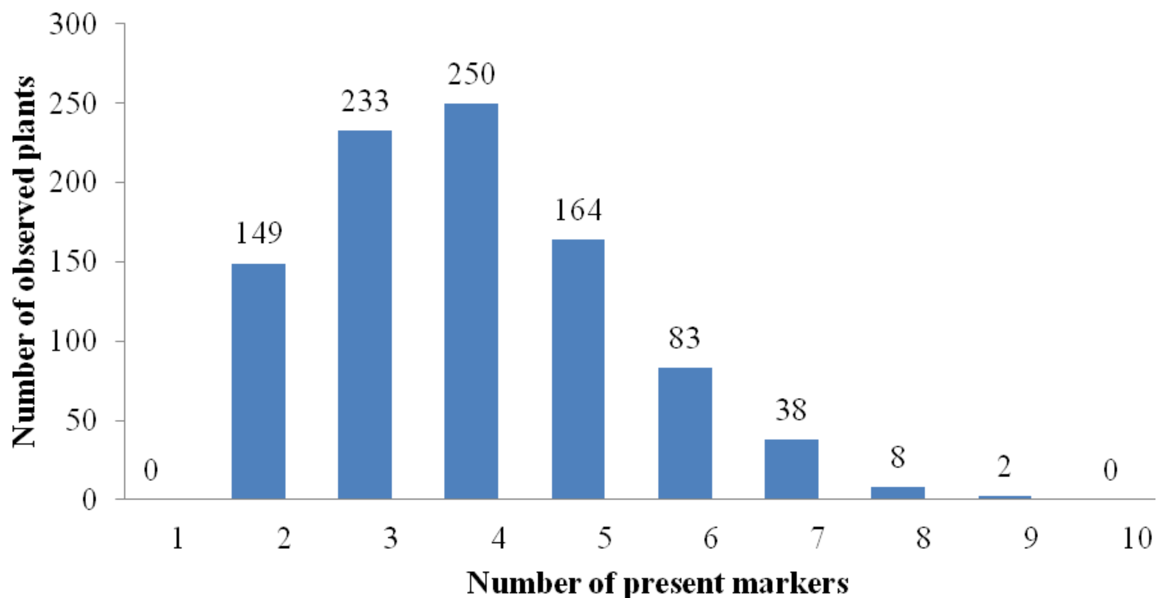


Figure 4.4 Frequency distribution showing the number of double cross F_1 individuals containing a specific number of molecular markers

A total of eight individuals (12.2, 48.1, 54.3, 88.2, 88.3, 116.1, 113.2 and 126.1) contained eight markers (Table 4.5). The marker DuPw227 was scored as missing in individuals 88.2 and 88.3. It is possible that, due to some markers that did not perform well and were thus difficult to impossible to score, some markers were scored as missing that may in fact could have been present. This could increase the number of present markers in some individuals of the population. No individuals tested positive for all ten tested markers.

Table 4.5 Best performing individuals of the double cross population

Plant number	No of markers	Present markers	Absent/missing* markers
12.2	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), DuPw227 (3A), Gwm156 (5A), Gwm133 (6B) and Gwm233 (7A)	Barc133 (3B), Sr26#43 (*Sr26*)
48.1	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), DuPw227 (3A), Barc133 (3B), Gwm156 (5A) and Gwm133 (6B)	Gwm233 (7A), Sr26#43 (*Sr26*)
54.3	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), DuPw227 (3A), Barc133 (3B), Gwm156 (5A), Gwm133 (6B) and Gwm233 (7A)	Sr26#43 (*Sr26*), Gwm501 (*QYr.sgi-2B.1*)
88.2	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Sr26#43 (*Sr26*), Barc133 (3B), Gwm293 (5A), Gwm133 (6B) and Gwm233 (7A)	Gwm501 (*QYr.sgi-2B.1*), DuPw227 (3A)
88.3	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Sr26#43 (*Sr26*), Barc133 (3B), Gwm293 (5A), Gwm133 (6B) and Gwm233 (7A)	Gwm501 (*QYr.sgi-2B.1*), DuPw227 (3A)
113.2	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), DuPw227 (3A), Barc133 (3B), Gwm293 (5A) and Gwm133 (6B)	Sr26#43 (*Sr26*), Gwm233 (7A)
116.1	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Sr26#43 (*Sr26*), DuPw227 (3A), Barc133 (3B), Gwm293 (5A) and Gwm133 (6B)	Gwm501 (*QYr.sgi-2B.1*), Gwm233 (7A)
126.1	8	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), Sr26#43 (*Sr26*), DuPw227 (3A), Barc133 (3B), Gwm293 (5A)	Gwm133 (6B), Gwm233 (7A)
116.3	9	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), Sr26#43 (*Sr26*), DuPw227 (3A), Barc133 (3B), Gwm293 (5A) and Gwm133 (6B)	Gwm233 (7A)
123.1	9	Cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1*), Sr26#43 (*Sr26*), DuPw227 (3A), Barc133 (3B), Gwm293 (5A) and Gwm133 (6B).	Gwm233 (7A)

*Underlined markers were scored as missing

4.3.5 Segregation patterns for screened markers

The expected segregation ratio of individuals was 1:1 (heterozygous resistant to homozygous susceptible) for most tested markers. All four parental lines S16(7.3), S726(3.2), CM-82036 and Frontana tested positive for *Lr34/Yr18/Sr57* (cssfr5) hence all double cross population individuals were expected to have *Lr34/Yr18/Sr57* in a homozygous state. *Lr19* was present in S16(7.3), S726(3.2) and CM-82036 (unexpected) with the expected ratio of 3:1 (homozygous/heterozygous resistant to homozygous susceptible/null allele) in the segregating population. Marker Sr26#43 is a dominant marker and can thus not distinguish between homozygous resistant and heterozygous resistant individuals for the *Sr26* allele. It was thus not known whether the gene was present in a homozygous or heterozygous state in parental line S726(3.2). If the marker was homozygous in line S726(3.2) a marker segregation of 1:1 could be expected. However, if the marker was heterozygous in line S726(3.2) a 1:3 segregation ration could be expected. Although the observed segregation ratio of 1:11 does not correspond with any of the two expected ratios, results indicated that the *Sr26* gene was probably heterozygous in the parental lines S726(3.2). Markers cssfr5 (*Lr34/Yr18/Sr57*), STSLr19$_{130}$ (*Lr19*), Gwm501 (*QYr.sgi-2B.1)* and Gwm233 (7A) segregated close to the expected ratios. All other markers did not segregate as expected because of missing values (Table 4.6).

Table 4.6 Segregation ratios of 10 molecular markers tested on 954 individuals of the double cross population using Chi square analysis

Marker	Homozygous	Heterozygous	Homozygous	E ratio	O ratio	Chi square	P value	Total	Missing
	AA	Aa	aa						
cssfr5	946			1	0.9	0	0.995	946	8
STSLr19$_{130}$		688*	234	3:1	2.9:1	0.1	0.8	922	32
Sr26#43		78*	820	1:1 or 1:3	1:10.5	127.7	0.001	898	56
Gwm148		251	595	1:1	1:2.4	9.7	0.001	846	108
Gwm501		193	539	1:3	1:2.8	0.7	0.3	732	222
Barc133		311	635	1:1	1:2.0	31.0	0.001	946	8
Dupw227		300	648	1:1	1:2.2	22.3	0.001	948	6
Gwm133		352	594	1:1	1:1.7	74.8	0.001	946	8
Gwm156		318	628	1:1	1:2	37.2	0.001	946	8
Gwm233		65	205	1:1	1:3.2	0.2	0.70	270	684

A = Resistant allele; a = Susceptible allele; E = Expected ratio; O = Observed ratio; * = Since the marker is a dominant marker, these individuals can either be Aa or AA

4.4 Discussion

Variety development takes about 12 to 15 years from initial crossing to registration (Wenzel, 2006). Conventional breeding can be shortened by the incorporation of molecular techniques. MAS is therefore a resourceful approach in conventional breeding that helps to combine many resistance genes (Šliková et al., 2003). In this study, a total of 954 segregating individuals of the double cross population derived from the cross S16(7.3)/CM-82036//S726(3.2)/Frontana were evaluated for the presence of rust and FHB resistance markers. Molecular techniques and MAS schemes were used to combine rust and FHB resistance genes and/or QTL into a single wheat genotype over a period of three years. The study was divided into two phases. Phase one was aimed at combining different rust resistance genes into one single genotype (Sydenham, 2007) and to select the best line(s) containing all rust resistance genes targeted. Phase two was aimed at combining rust and FHB resistance genes. Five molecular markers linked to rust resistance and five markers linked to FHB resistance were successfully used for screening rust and FHB resistance in parental lines and individual lines from each cross.

Genotypic screening on parental lines was conducted and results were similar to that reported by Sydenham (2007). All expected allele sizes linked to rust resistance in parental lines used in phase one were present in parental lines Avocet*YrSp*, Blade, CS*Lr19*-149-229 and Kariega. According to genotypic screening on the selected rust lines used in phase two, all markers were present between a combination of the two selected lines: S16(7.3) with Gwm501 (*QYr.sgi-2B.1*), Gwm148 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*) and cssfr5 (*Lr34/Yr18/Sr57*) and S726(3.2) with STSLr19$_{130}$ (*Lr19*), Sr26#43 (*Sr26*), cssfr5 (*Lr34/Yr18/Sr57*) and stm559gtag (*Sr2*). Results indicated the presence of *Lr34/Yr18/Sr57* in CM-82036 and Frontana. However, the gene has never been reported to be present in CM-82036. Since the cssfr5 markers is a perfect marker that targets the *Lr34* gene directly, these results should be accurate. The CM-82036 line used in this study should in future be phenotypically evaluated for the presence of the *Lr34* gene to verify the marker data. *Sr26* was absent in S16(7.3) while line S726(3.2) tested positive for *Sr26* though both phenotypic and genotypic data suggested that the gene was heterozygous and still segregating in line S726(3.2).

From results it was clear that *Sr2* was lost in progenies of line S726(3.2). This confirmed previous studies that *Sr2*, being a recessive gene, is difficult to introduce into other lines (Spielmeyer et al., 2003) and difficult to detect using phenotypic screening in segregating populations especially in the presence of other rust resistance genes (McIntosh et al., 1995). Results also indicated that the *Sr2* gene was present in a heterogeneous state in the parental line S726(3.2) and that it was not transferred to its progenies. It should further be noted that during the first phase of the project the STS marker stm559gtag was used to select for the *Sr2* gene. Although the STS marker developed by Hayden et al. (2004) gave better results than the SSR marker Gwm533 developed by Spielmeyer et al. (2003), the STS marker amplifies several alleles which makes it difficult to score accurately. Results of Sydenham (2007) also indicated that the seed source of Blade (the only donor used for the *Sr2* gene) was segregating for the *Sr2* gene. All these factors could have played a role in the fact that the *Sr2* gene was not detected in progeny of the S726(3.2) line.

Genotypic screening on parental lines for FHB resistance was successfully done. Results for markers linked to QTL on chromosome 5A indicated that CS*Lr19*-149-229 and CM-82036 contained one of the markers linked to the 5A QTL (Gwm293) while the other marker (Gwm156) for the same QTL was detected in both Kariega and CM-82036. This implies that a combination of these two sets of cultivars may result in resistance for both rust and FHB QTL located on chromosome 5A. Unfortunately none of the two selected rust donor lines [S16(7.3) and S726(3.2)] contained markers linked to the 5A QTL. As expected Frontana was the only cultivar with the QTL on chromosome 3A. Frontana was of great importance in the study for it was the only source of the QTL on chromosome 3A responsible for type I FHB resistance.

Previous studies indicated the presence of FHB resistance QTL on chromosomes 3B, 5A, 6B and 7A in Sumai 3 (Anderson et al., 2001; Buerstmayr et al., 2002; 2003; Zhou et al., 2002). A study by Sydenham et al. in 2013 (unpublished data) indicated that the markers used in this study that are linked to the FHB resistance QTL on chromosomes 3B, 5A, 6B and 7A in Sumai 3 amplified alleles of the same size in CM-82036. Thus although previous studies suggested that the QTL on chromosome 6B was not transferred from Sumai 3 to CM-82036

(Buerstmayr et al., 2002) and the QTL on chromosome 7A was not identified in CM-82036 before, markers linked to these two traits were included in the current study. Genotypic screening of the parental line CM-82036 in the current study confirmed results obtained by Sydenham et al. (unpublished data) by amplifying alleles of similar size in CM-82036 and Sumai 3.

The F_1 populations were generated from cross 1 [S16(7.3)/CM-82036] and cross 2 [S726(3.2)/Frontana] and SSR marker Barc133 was successfully employed to confirm that 83% of F_1 individuals produced in cross 1 were true hybrids. DuPw227 was successfully used to confirm a 73% success rate for cross 2. Results proved the advantages of using MAS in selection of progenies because lines that were not true crosses were eliminated. The use of markers in this case has the advantage that traits for which genetic information is required can be prioritised and investigated without a need to analyse a whole genome (Miflin, 2000). Detection of true hybrids at early stages of the breeding programme proves that conventional breeding combined with the use of MAS produce reliable results within a short period of time. It further confirms that molecular markers provide higher efficiency in parental selection by allowing a controlled combination of better genotypes and different donors in a segregating population (Wenzel, 2006).

Genotypic screening of the segregating double cross population was performed using ten markers linked to resistance genes and QTL. Results indicated that all parental lines used to combine rust and FHB resistance genes and/or QTL contained *Lr34/Yr18/Sr57* therefore all individuals of the double cross population were expected to have this locus and molecular data confirmed this. As expected, progenies of the segregating double cross population showed a 3:1 segregation ratio for the presence of the *Lr19* gene. The presence of the *Lr19* gene in progeny in a 3:1 ratio is because three of the parental lines [S16(7.3), S726(3.2) and CM-82036] tested positive for the allele size linked to *Lr19* resistance. For the other markers [Gwm148 (*QYr.sgi-2B.1*), Gwm501 (*QYr.sgi-2B.1*), Sr26#43 (*Sr26*), Barc133 (3B), Dupw227 (3A), Gwm156 (5A), Gwm133 (6B) and Gwm233 (7A)] only 50% of the population was expected to have accumulated the resistance genes in a ratio of 1:1 for each marker (heterozygous resistant to homozygous susceptible) because it was sourced from only

one parental line (donor parent). Chances of accumulating genes linked to these markers were limited due to few sources of genes used in the study. Segregation of these traits is expected in future breeding programmes, hence lines might further loose such genes that were in heterozygous state. More rounds of selfing are therefore recommended in order to obtain most markers or traits of interest in a homozygous state.

According to Chi square analyses, the p values for all markers linked to FHB resistance were <0.001 except for Gwm233 with a p value of 0.7 which is also not reliable due to a high number of missing values. The hypothesis for all markers with $p<0.001$ is thus rejected. Results suggest that there may have been external factors that caused variation while screening markers linked to the resistance genes either by chance and/or other unknown factors. These results may have been affected by missing values. Missing values were mainly due to difficulty in scoring fragments linked to resistance traits due to a combination of many genes. In future, selected lines should be screened for the major FHB QTL on chromosomes 3A, 3B and 5A. QTL on chromosome 6B and 7A are minor QTL that do not provide significant resistance against the disease. The presence of the 6B and 7A QTL in the donor line CM-82036 should furthermore be confirmed using phenotypic and genotypic screening. Chi square analyses of markers cssfr5 ($p = 0.99$), STSLr19$_{130}$ ($p = 0.8$) and Gwm501 ($p = 0.3$), showed that the observed ratios did not significantly deviate from expected ratios of 1, 3:1 and 1:3, respectively.

Results of molecular screening proved that combining single traits to obtain more complex characters is possible with the use of MAS. MAS may increase efficiency and effectiveness in plant breeding by combining many genes simultaneously (Collard et al., 2005). The following lines are recommended for further screening to obtain data for the missing values since they are the most promising lines: plants 116.3 and 123.1 that tested positive for nine markers and plants 12.2, 48.1, 54.3, 88.2, 88.3, 113.2, 116.1 and 126.1 that tested positive for eight markers linked to the resistance genes of interest. Identification of these lines would have not been easy within a period of three years of the study if conventional breeding was used alone. Selection of lines generated by conventional breeding incorporated with molecular marker techniques provides reliable results within a shortened period of time

(Todorovska et al., 2009). However, phenotypic screening is further recommended to confirm the presence of the accumulated genes detected through application of MAS. Although MAS has a potential of refining breeding programmes, its application is narrowed by the high cost of highly trained personnel (Anderson, 2007).

Phenotypic screening that was performed on the best selected progenies of S16(7.3) and S726(3.2) (see previous chapter) confirmed results of molecular screening and further indicated the presence of *YrSp* which could not be detected with the use of MAS due to absence of reliable molecular markers linked to the gene. The success of detecting this gene was assisted by infection tests that identified resistant plants.

4.5 Conclusion

In conclusion, MAS is one of the highly utilised modern techniques that is being practised to transfer or combine simple traits that are not easy to score such as disease resistance traits (Singh et al., 2004). Gupta et al. (2010) reviewed the status of application of MAS in wheat breeding. The review for the application of MAS specifically on rust and FHB resistance was discussed and indicated that MAS was successfully utilised in selection of traits of interest. However, according to the communication, since the year 2000 to 2009, few leaf rust resistance genes have been combined into a single genotype using MAS, while three QTL on different chromosomes were combined into a single genotype for FHB resistance. Only one reported study by Shi et al. (2008) involved combining eight FHB resistance QTL. No published study could be found that involved combining rust and FHB resistance genes and QTL into a single wheat genotype. Many seed companies do successfully combine disease resistance genes, but these results are usually not published or available to the scientific community. The current study is thus the first reported and publically available study where five rust resistance genes plus five FHB QTL were combined into a single genotype.

4.6 References

Anderson JA (2007) Marker-assisted selection for Fusarium head blight resistance in wheat. International Journal of Food Microbiology 119:51-53

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell-Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theoretical and Applied Genetics 102:1164-1168

Bai GH, Plattner R, Desjardins A, Kolb F (2001) Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breeding 120:1-6

Bai GH, Shaner GE (2004) Scab of wheat: prospects for control. Plant Disease 78:760-766

Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lu M (2007) Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. Australian Journal of Agricultural Research 58:576-587

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theoretical and Applied Genetics 104:84-91

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theoretical and Applied Genetics 107:503-508

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169-196

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST derived microsatellite markers for genotyping A and B genomes of wheat. Theoretical and Applied Genetics 104:399-407

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present and future possibilities. Molecular Breeding 26:145-161

Hayden MJ, Kuchel H, Chalmers KJ (2004) Sequence tagged microsatellites for the Xgwm533 locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 109:1641-1647

Huang XQ, Wang LX, Xu MX, Röder MS (2003) Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 106:858-865

Johnson R, Jellies GJ (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3-22

Karaoglu H, Lee CMY, Meyer W (2005) Survey of sequence repeats in complete fungal genome. Molecular Biology and Evolution 22:639-649

Kelly JD (1995) Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogens. Horticultural Science 30:461-465

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theoretical and Applied Genetics 119:889-898

Leonard KJ, Szabo LJ (2005) Rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6:99-111

Liu J, Liu D, Tao W, Li W, Wang S, Chen P, Cheng S, Gao D (2000) Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breeding 119:21-24

McIntosh RA, Wellings CR, Park RF (1995). Wheat Rusts: An Atlas of Resistance Genes. CSIRO Australia, Kluwer Academic Publishers, Dordrecht, Netherlands, 200 pp

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81:1340-1348

Miedaner T, Wilde F, Korzun V, Ebmeyer E, Shmolke M, Hartl L, Schön CC (2009) Marker selection for Fusarium head blight resistance based on quantitative trait loci (QTL) from two European sources compared to phenotypic selection in winter wheat. Euphytica 166:219-227

Miflin B (2000) Crop improvement in the 21st century. Journal of Experimental Botany 51:1-8

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small-grain cereals a review. Plant Pathology 44:207-238

Prins R, Pretorius ZA, Bender CM, Lehmensiek A (2011) QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega x Avocet S doubled haploid wheat population. Molecular Breeding 27:259-270

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023

Roelfs AP (1988) Resistance to leaf and stem rusts in wheat. In: Simmonds NW, Rajaram S (Eds) Breeding Strategies for Resistance to Rusts of Wheat, CIMMYT: Mexico. Federal District Mexico, pp 10-19

Salameh A, Buerstmayr M, Steiner B, Neumayer A, Lemmens M, Buerstmayr H (2011) Effects of introgression of two QTL for Fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on Fusarium head blight resistance, yield and quality traits. Molecular Breeding 28:485-494

Shi JR, Xu DH, Yang HY, Lu XQ, Ban T (2008) DNA marker analysis for pyramided Fusarium head blight (FHB) resistance QTLs from different germplasm. Genetika 133:77-84

Singh RP, Datta D, Priyamvada M, Singh S, Tiwari R (2004) Marker-assisted selection for leaf rust resistance genes *Lr19* and *Lr24* in wheat (*Triticum aestivum* L.). Journal of Applied Genetics 45:399-403

Šliková S, Gregová E, Bartoš P, Kraic J (2003) Marker-assisted selection for leaf rust resistance in wheat by transfer of gene *Lr19.* Plant Protection Science 39:13-17

Snijders CHA (1990) Fusarium head blight and mycotoxin contamination of wheat, a review. Netherland Journal of Plant Pathology 96:187-198

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Science 43:333-336

Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theoretical and Applied Genetics 109:215-224

Sydenham SL (2007) Pyramiding wheat rust resistance genes using marker-assisted selection. MSc dissertation, Department of Plant Sciences, University of the Free State, 108 pp

Todorovska E, Christov N, Slavov S, Christova P, Vassilev D (2009) Biotic stress resistance in wheat breeding and genomic selection implications. Biotechnology and Biotechnology Equilibrium 23:1417-1426

Wenzel G (2006) Molecular plant breeding: achievements in green biotechnology and future perspectives. Applied Microbiology and Biotechnology 70:642-650

Zhou W-C, Kolb FL, Bai G-H, Domier LL, Yao J-B (2002) Effect of individual Sumai 3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation within kernels in wheat. Hereditas 137:81-89

CHAPTER 5

General conclusions and recommendations

Wheat is one of the most important cereal crops that provides human food and livestock feed and has become the most abundant crop. Wheat has developed naturally from its ancient progenitors and has been exploited for the benefit of mankind by developing productive and adapted wheat varieties. The two classes of wheat that are currently cultivated in many countries are common (bread) wheat and durum wheat. Bread wheat is classified as one of the most important staple crops worldwide. The demand for wheat is rising each year and is expected to increase from about 680 million tons to around 813 million tons in 2030 in order to meet food and feed demands for the rising human and animal populations.

Crop protection against pests and diseases is a fundamental measure to ensure that global demand is met. Several studies indicated that diseases caused by fungi, bacteria and viruses pose a threat to global food security. Among these diseases, the three rusts are often considered the most important diseases of wheat worldwide. Rust diseases occur in many regions and their ability to form new races that break resistance of cultivars have directed breeders to develop new resistant varieties. As an example, the new emerging Ug99 stem rust races in many countries including SA suggest further breeding and variety exploitation to protect crops and maintain high yield. Therefore, new varieties with durable resistance genes are needed to replace varieties which have become susceptible. FHB is another fungal disease that needs attention because it causes yield loss and reduces wheat quality. Cultivars carrying FHB resistance have poor agronomic traits but could be used as donors for FHB resistance.

The aim of the study was to combine five rust (leaf, stem and stripe rust) and five FHB resistance genes and QTL into a single wheat genotype. Rust resistant donor lines Avocet*YrSp*, Blade, CS*Lr19*-149-229, Kariega, S16(7.3) and S726(3.2) and FHB donor lines CM-820386 and Frontana were used to transfer *Lr19*, *Lr34/Yr18/Sr57*, *Sr26*, *QYr.sgi-2B.1*, and *YrSp* and FHB type I and II resistance to new breeding lines. Combining of genes and QTL was done through conventional crossing and MAS.

In the study, application of conventional breeding and MAS permitted identification of seven genes of interest in the four double cross population individuals S16, S178, S726 and S791 that originated from crosses between Avocet*YrSp*, Blade, CS*Lr19*-149-229 and Kariega during phase one of the study. Line S16(7.3) was selected from individuals of S16 and line S726(3.2) from individuals of S726. Two progenies, S16(7.3)P3-5.1 and S726(3.2)P3-6.1, selected from rust resistant lines S16(7.3) and S726(3.2) respectively, were subjected to rust infections at seedling stage in order to confirm the presence of the accumulated genes identified using MAS.

Phenotypic screening confirmed the genotypic results. As expected the two lines were resistant to leaf rust. Line S16(7.3)P3-5.1 was susceptible to stem rust as predicted by the marker data. However, in contrast with marker data, line S726(3.2)P3-6.1 was susceptible to stem rust. Both phenotypic and genotypic data thus indicated that the *Sr26* gene was present in a heterozygous state in line S726(3.2), Progeny of this line will thus segregate for this trait.

In stripe rust assays S16(7.3)P3-5.1 tested positive for the adult plant resistance gene *QYr.sgi-7D* (*Lr34*) but was susceptible to leaf rust as the gene is best expressed at the adult plant stage. It was expected that none of the progeny lines will display stripe rust resistance during phenotypic screening since based on marker data they only contained APR genes/QTL for stripe rust. However, line S726(3.2)P3-6.1 showed resistance to stripe rust that indicated that the *YrSp* gene was transferred from Avocet*YrSp* to this line although, due to a lack of molecular markers linked to this trait, no active selection for this trait occurred. Phenotypic screening thus helped to identify the *YrSp* gene. This might indicate that other lines from the double cross population may also possess the *YrSp* gene. However, its presence should be evaluated using phenotypic screening since it adds more value to the selected individuals.

Diallele crosses were made between S16(7.3)/CM-82036 and S726(3.2)/Frontana during phase 2 of the study to combine rust and FHB resistance genes/QTL. A double cross population was generated and MAS used for identifying the best lines. In this part of the study multiple resistance was built by combining 10 genes, viz *Lr19* and *Lr34/Yr18*, *Sr26*, *YrSp* and *QYr.sgi-2B.1*, and FHB type I and II resistance using MAS. The best two lines

identified, namely 116.3 and 123.1, tested positive for nine genes/QTL of interest based on marker data within a period of three years. These two lines tested positive for all five rust resistance genes/QTL and for four of the five FHB QTL. The only marker missing in these two lines was the marker linked to the minor FHB QTL on chromosome 7A. However, it tested positive for the four most important FHB QTL transferred from CM-82036 and Frontana, namely the *Fhb1* gene or *Qfhs.ndsu.3BS* QTL on chromosome 3B, the *Fhb2* gene or *Qfhs.ndsu-6B* QTL on chromosome 6B, the *Qfhs.ifa-5A* QTL on chromosome 5A and the *Qfhs.ndsu-3AL* QTL on chromosome 3A. These lines thus contain two FHB QTL conferring type II FHB resistance and two FHB QTL conferring type I resistance. Although this QTL has been confirmed to be present in Sumai 3, its presence was not confirmed in CM-82036. Although markers linked to this QTL were used during this study the presence of this gene was not a given from the start of the study. Furthermore the markers flanking the QTL region was not closely linked to the QTL and will not provide accurate results. Markers for the 7A QTL was furthermore difficult to score in the segregating population and was mostly scored as missing data. Marker data for the 7A QTL was thus not reliable in this study. However, transfer of the QTL on chromosome 7A was not the main focus of the study. All lines containing at least the QTL on chromosomes 3A, 3B and 5A should provide good resistance to both type I and II FHB resistance.

Screening for other markers linked to the QTL on chromosomes 3A, 5A and 7A was difficult probably due to the quality of DNA or primers. Segregating patterns of alleles present in the progeny of the double cross population complicated the marker data. Rust resistant lines that contained genes from CS*Lr19*-149-229 and CM-82036 tested positive or had fragments very close to that of CM-82036 for some FHB screening which made it difficult for screening the F_1 population. Some difficulties were encountered when individuals were screened using the silver staining method but better results were obtained with the Gel Scan 3000. However, MAS was successful to identify the best lines that can be used for further breeding programmes and its use permitted selection of plants with more than one gene without phenotyping within a short period of time.

The current study shows that MAS and conventional breeding can be in cooperated to improve the combining of genes into a single genotype for improved performance. The developed lines containing combined genes have higher levels and/or a wide spectrum of resistance compared to the parental lines. However, it is recommended for future breeding programmes that the developed lines be further tested for the missing markers. Phenotypic screening was done using primary leaves and it is therefore important to further test for APR genes in the field.

The parental lines Avocet/*YrSp*, Blade, CS*Lr19*-149-229, CM-82036 and Frontana are not South African cultivars or lines and only Kariega is a local cultivar. This implies that lines developed from these crosses may not be adapted to local conditions. Pre-breeding programmes to develop doubled haploids from the selected lines are recommended to obtain markers in a homozygous state. These lines can also be used in breeding programmes through backcrossing to improve baking quality and to transfer desirable genes to adapted germplasm.

Breeding for disease resistance using traditional methods is possible but takes a longer time. It is therefore concluded that MAS was useful in this study. Within a period of three years, nine genes/QTL were successfully identified in two double cross lines. Identification of *Lr19* and *Lr34* would have not been possible if only phenotypic screening was used because *Lr34* gene is masked by the presence of *Lr19*. Incorporation of modern and traditional breeding methods is therefore highly recommended because both methods are equally important to the breeders. It is furthermore recommended that *Sr2* resistance should be incorporated into these lines since it provides broad-spectrum and durable protection against stem rust, including to Ug99 and all its derivatives. If this could be achieved, it will be the first time that *Sr2* resistance and type II FHB resistance are combined into a single genotype

Summary

Wheat (*Triticum aestivum*) is one of the most important cereal crops worldwide and cultivated in a wide range of environments. The production of wheat, based on consumption and other demands, needs to be increased to meet the annual requirements for its domesticated uses. Wheat productivity is influenced by biotic and abiotic stresses. Therefore, appropriate, efficient and environmentally friendly control measures to lessen such stresses need to be applied.

Fungal diseases of wheat, including leaf rust, stripe rust, stem rust, powdery mildew and Fusarium head blight (FHB) can cause yield losses. The diseases result in a reduction in grain yield and affect grain quality negatively. The use of resistant cultivars is an effective, economical and environmentally safe approach to lower fungicide dependence and to reduce production losses due to foliar diseases. In this study, we combined rust and FHB resistance genes and/or quantitative trait loci (QTL) and molecular marker analyses were applied using polymorphic microsatellites (simple sequence repeats, SSR).

The aim of the study was to combine five rust resistance genes (against leaf, stem and stripe rust) and five FHB resistance genes/QTL for type I and II resistance into a single wheat genotype with the aid of both marker-assisted and phenotypic selection. Molecular markers used in the study are already being used in commercial breeding programmes in South Africa. The study was divided into two phases. Phase one was aimed at combining rust resistance genes/QTL into a single genotype using parental lines Avocet*YrSp*, Blade, Kariega and CS*Lr19*-149-299 and to select the best lines based on the number and combination of homozygous genes/QTL present. The genes *Sr2*, *Sr26*, *Lr19*, *Lr34/Yr18/Sr57* and QTL *QYr.sgi-2B* were sourced from parental lines and combined in a double cross breeding scheme followed by several rounds of selfing. Selection was done using marker-assisted selection (MAS). The two best rust resistant lines were selected. Line S16(7.3) contained *QYr.sgi-2B.1*, *Lr34/Yr18/Sr57* and *Lr19* in a homozygous state while line S726(3.2) contained *Lr34/Yr18/Sr57* and *Lr19* in a homozygous and *Sr26* in a heterozygous state. Phenotypic screening furthermore indicated the presence of *YrSp* in line S726(3.2). FHB

resistance QTL for the second phase of the project were sourced from Frontana (type I resistance) and CM-82036 (type II resistance). The FHB resistant lines were crossed with the two best rust resistant lines in a double cross breeding scheme and selection was done using molecular markers. The study was conducted over a period of three years.

A total of 954 individuals of a double cross population were screened with five markers associated with rust resistance genes/QTL and five markers associated with FHB resistance QTL. The following markers were used to screen for accumulated rust resistance genes/QTL: cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 and Gwm501 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*) and Sr26#43 (*Sr26*). The markers DuPw227 (3A), Barc133 (3B), Gwm156, Gwm293 and Barc197.2 (5A), Gwm133 and Gwm644 (6B) and Gwm233 (7A) were used to identify individuals containing FHB resistance QTL. The number of resistance markers in the double cross family ranged between one to nine out of the 10 tested markers. The two best lines, 116.3 and 123.1, contained all markers except the marker linked to the minor FHB QTL on chromosome 7A. All tested lines contained *Lr34/Yr18/Sr57* in a homozygous state while all other markers were segregating. The best lines will be self-pollinated for use in future breeding programmes.

Keywords: Fusarium head blight (FHB), marker-assisted selection (MAS), phenotypic screening, quantitative trait loci (QTL), rust, simple sequence repeats (SSR)

Opsomming

Koring (*Triticum aestivum*) is een van die belangrikste graangewasse wêreldwyd en word oor ‘n wye reeks omgewings geproduseer. Die produksie van koring, gebaseer op verbruik en aanvraag, moet verhoog word om in die toenemende jaarlikse behoeftes van plaaslike gebruik te voorsien. Koringproduktiwiteit word deur biotiese en abiotiese stres beïnvloed. Toepaslike, doeltreffende en omgewings-vriendelike beheermaatreëls moet dus toegepas word om hierdie stresfaktore te verlig.

Verskeie swamsiektes van koring, insluitend blaarroes, streeproes, stamroes, poeieragtige meeldou en Fusarium-aarskroei (FAS) kan oesverliese veroorsaak en kwaliteit negatief beïnvloed. Die gebruik van weerstandbiedende kultivars is ‘n effektiewe, ekonomiese en veilige benadering tot minder swamdodertoedienings en ook laer verliese a.g.v. blaarsiektes. In hierdie studie is roes- en FAS weerstandsgene en/of kwantitatiewe eienskaploki (“QTL”) gekombineer en molekulêre merkeranalise toegepas d.m.v. polimorfiese mikrosatelliete (“SSR”).

Die doel van die studie was om vyf roesweerstandsgene (teen blaar-, stam- en streeproes) en vyf FAS weerstandsgene/QTL vir tipe I en II weerstand m.b.v. merker-ondersteunde en fenotipiese seleksie in ‘n enkel koringgenotipe te kombineer. Die molekulêre merkers wat in hierdie studie gebruik is word reeds in kommersiële teelprogramme in Suid-Afrika toegepas. Die studie is in twee fases verdeel. In fase een is roesweerstandsgene/QTL vanaf die skenkerlyne Avocet*YrSp*, Blade, Kariega en CS*Lr19*-149-299 in ‘n enkel genotipe gekombineer. Die beste lyne op grond van die aantal en kombinasie van homosigotiese gene/QTL teenwoordig is geselekteer. Die *Sr2*, *Sr26*, *Lr19*, *Lr34/Yr18/Sr57* gene en *QYr.sgi-2B* QTL is vanaf die skenkerlyne verkry en in ‘n dubbelkruising teelskema gekombineer gevolg deur verskeie rondtes van selfbestuiwing. Seleksie is m.b.v. merker-ondersteunde seleksie gedoen. Die twee beste roesweerstandbiedende lyne is geselekteer. *QYr.sgi-2B.1*, *Lr34/Yr18/Sr57* en *Lr19* was homogeen in lyn S16(7.3) terwyl *Lr34/Yr18/Sr57* en *Lr19* homogeen en *Sr26* heterogeen was in lyn S726(3.2). Fenotipiese toetse het verder aangetoon dat die *YrSp* geen in lyn S726(3.2) teenwoordig was. FAS weerstandsgene/QTL wat

gedurende die tweede fase van die projek gebruik is, is vanaf Frontana (tipe I weerstand) en CM-82036 (tipe II weerstand) verkry. Die FAS weerstandbiedende lyne is met die twee roesweerstandbiedende lyne in ‘n dubbelkruising teelskema gekruis en seleksie is m.b.v. molekulêre merkers gedoen. Die studie is oor ‘n tydperk van drie jaar uitgevoer.

‘n Totaal van 954 individue van die dubbelkruispopulasie is met vyf merkers gekoppel aan roesweerstandsgene en vyf merkers gekoppel aan FAS weerstandsgene/QTL getoets. Die volgende merkers is gebruik om vir die gekombineerde roesweerstandsgene/QTL te toets: cssfr5 (*Lr34/Yr18/Sr57*), Gwm148 en Gwm501 (*QYr.sgi-2B.1*), STSLr19$_{130}$ (*Lr19*) en Sr26#43 (*Sr26*). Die merkers DuPw227 (3A), Barc133 (3B), Gwm156, Gwm293 en Barc197.2 (5A), Gwm133 en Gwm644 (6B) en Gwm233 (7A) is gebruik om individue wat FAS weerstandsgene/QTL bevat te identifiseer. Die aantal merkers gekoppel aan weerstand in die dubbelkruispopulasie het gewissel tussen een en nege uit die 10 getoetste merkers. Die twee beste lyne, 116.3 en 123.1, het al die merkers behalwe die merker gekoppel aan die kleinere QTL op chromosoom 7A bevat. *Lr34/Yr18/Sr57* was homosigoties in al die lyne wat getoets is terwyl al die ander merkers nog steeds gesegregeer het. Die beste lyne sal selfbestuif word vir gebruik in toekomstige teelprogramme.

Sleutelwoorde: Fenotipiese seleksie, Fusarium-aarskroei (FAS), merker-ondersteunde seleksie (“MAS”), mikrosatelliete (“SSR”), kwantitatiewe eienskaploki (“QTL”), roes