Phenotypic Characterisation and QTL Mapping of Adult Plant Resistance to Leaf Rust and Stem Rust of Wheat (*Triticum aestivum* L.)

by

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Thesis Summary

Wheat (*Triticum aestivum* L.) is an important crop in South Africa after maize in total production. It is grown mainly as a cash and food crop predominantly by commercial farmers. There has been a decline in wheat production in South Africa because of a number of production constraints. Leaf rust caused by *Puccinia triticina* Eriks. and stem rust (*P*. *graminis* f. sp. *tritici*) are the most important foliar diseases limiting wheat production in South Africa. Breeding for durable rust resistance is the most economic and environmentally friendly strategy to control the wheat rust diseases. Due to emergence of new and virulent races of the rust pathogens, a continuous search for new sources of effective resistance genes is necessary to develop improved wheat varieties with stable and durable resistance. Hence, the overall aim of this study was to identify promising wheat genotypes with adult plant resistance (APR) to leaf rust and stem rust diseases using a newly developed recombinant inbred line (RIL) population. Furthermore, the study aimed to identify genomic regions possessing candidate resistance genes against leaf rust and stem rust of wheat for marker-assisted resistance breeding. The specific objectives were: 1) to assess APR of selected wheat genotypes to leaf rust and stem rust, as well as to develop segregating populations for resistance breeding, 2) to determine the inheritance of APR in a recombinant inbred line population of a cross between two cultivars 'Popo' and 'Kariega', and to identify wheat breeding lines possessing both leaf rust and stem rust resistance genes, and 3) to undertake a genome-wide scan for identification of quantitative trait loci (QTL) that significantly affect APR to leaf rust and stem rust using a newly developed recombinant inbred line population of wheat developed from a cross of two popular spring wheat cultivars, Popo and Kariega. In addition, to construct a genetic linkage map useful to locate QTL controlling rust resistance and other important agronomic traits in the Popo/Kariega genetic background.

The study was carried out as a series of experiments, each contributing towards a specific objective. Data generated is thus summarised in three research chapters. Chapter 3 addresses objective 1 where eight selected Kenyan cultivars (Gem, Romany, Paka, Fahari, Kudu, Pasa, Ngiri and Popo) with known resistance to stem rust, together with local checks (Kariega, Morocco, McNair and SST88) were

evaluated for leaf rust and stem rust resistance at seedling stage and at adult plant stage across four representative testing environments. Selected diagnostic markers were used to determine the presence of known genes. The selected wheat cultivars were crossed with local checks using a bi-parental mating design. The tested Kenyan wheat cultivars exhibited APR to both stem rust and leaf rust. The presence of two APR genes, i.e., *Sr2/Yr30/Lr27/Pbc1* and *Sr57/Lr34/Yr18/Pm38* was confirmed in some of the tested cultivars. Resistance gene *Lr34* was present in the cultivars Gem, Fahari, Kudu, Ngiri and Kariega, while *Sr2* was present in Gem, Romany, Paka and Kudu. The seedling resistance gene, *Sr35,* was only detected in cultivar Popo. Overall, the first experiment resulted to a development of 909 $F_{6:8}$ recombinant inbred lines as part of the nested mating design.

Objective 2 of the study is summarised in Chapter 4. For this specific objective, a panel of 179 RILs and three susceptible check varieties (Gariep, SST88 and Morocco) were evaluated across four diverse environments for APR to leaf rust and stem rust diseases. The recombinant inbred line population was developed through a cross of two parental cultivars (Popo and Kariega) followed by continuous selfing. The aim of the study was to investigate the inheritance of APR in the RILs, and to identify breeding lines possessing both leaf rust and stem rust resistance. Analysis of variance indicated highly significant ($P < 0.001$) differences among the tested RILs for both pathogens across the testing environments. The broad sense heritability estimates were 0.53 and 0.77 for leaf rust and stem rust, respectively, suggesting that the use of the newly developed RILs in the genetic background of Popo/Kariega can enhance pre-breeding for rust resistance against the two diseases. Twenty-six RILs had mean disease severity scores that were better than the parental lines and showed higher levels of resistance to both pathogens.

Chapter 5 presents the last objective. For this specific objective, a Diversity Array Technology-sequencing (DArT-seq) methodology was used for genetic analysis and to develop a linkage map using a newly developed recombinant inbred line population derived from a cross between two spring wheat cultivars *viz*. Popo and Kariega. The 179 RIL population was evaluated for APR to leaf rust and stem rust under field

conditions across four diverse environments. Quantitative trait loci analysis was performed using single marker analysis (SMA) and inclusive composite interval mapping (ICIM) methods with QTL IciMapping 4.0 software. A high-density genetic map was successfully generated using SNP markers from DArT. This should serve as a useful resource for analysing genome-wide variation of other complex agronomic traits. Quantitative trait loci mapping revealed that resistance to leaf rust and stem rust in the studied population was conditioned mostly by additive genes. In total, 44 putative QTLs (10 for leaf rust and 34 for stem rust) with additive genetic effects were detected on 14 chromosomes explaining 2.45 to 21.21% of the phenotypic variation present in the RILs. A consistent genomic region designated as *QLr.sgi-5A.1* was identified on chromosome 5A conditioning leaf rust resistance. Another consistent QTL designated as *QLr/Sr.sgi-7D.1.3* was identified on chromosome 7D to control both leaf rust and stem rust resistance.

Overall, the main aim and specific objectives of the present study as outlined above were successfully achieved. The study generated valuable genetic resources and candidate APR gene(s) for resistance breeding of wheat against leaf rust and stem rust pathogens. Seeds from the twenty-six best performing lines have been shared with two major South African breeding companies; Sensako and Pannar. The newly found QTL are useful genetic markers to undertake marker-assisted breeding and for mapping of leaf rust and stem rust resistance genes in wheat.

Declaration

I, Sandiswa Figlan, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other scientists' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced.
5. This thesis does not contain text, graphics or tables taken from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference section.

Signed……Date…….

Sandiswa Figlan (Candidate)

As the candidate's supervisor(s), we have approved this thesis for submission**.**

Signed……Date…….

Prof. Hussein A. Shimelis

Signed……Date…….

Prof. Toi J. Tsilo

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Dedication

This thesis is humbly dedicated to my late grandparents ***Ellof Leslie Figlan*** *and* ***Thozama Eugene Figlan****, who taught me the importance of hard work and the value of education.*

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Chapter 5

Abbreviations

ABC	ATP–binding cassette
APR	Adult plant resistance
AFLP	Amplified fragment length polymorphism
ARC-SGI	Agricultural Research Council-Small Grain Institute
BGRI	Borlaug Global Rust Initiative
BSMV	Barley stripe mosaic virus
ºC	Degrees Celsius
CAPS	Cleaved amplified polymorphic sequence
CBI	Crop Breeding Institute
CIMMYT	International Maize and Wheat Improvement Centre
cm	Centimetre
CTAB	Cetyl trimethyl ammonium bromide
DAFF	Department of Agriculture Forestry and Fisheries
DArT	Diversity Array Technology
DArT-seq	Diversity Array Technology-sequencing
DH	Doubled haploid
DNA	Deoxyribonucleic acid
EST	Expressed sequence tag
F_1 to F_6	First filial generation to sixth filial generation
$F_{6:8}$	Lines derived from 6^{th} filial generation, now in 8^{th} filial generation
GBS	Genotype-by-sequencing
hrs	Hours
HIGS	Host induced gene silencing
ICIM	Inclusive composite interval mapping
KASP	Kompetitive allele specific PCR
LSD	Least significant difference
LOD	Logarithm of odds
MAS	Marker assisted selection
ml	Millilitre (10^{-3} L)
mm	Millimetre
NGS	Next generation sequencing

ng/µl	Nano-grams per microlitre
NIL	Near isogenic line
%	Percent
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pgt	*Puccinia graminis tritici*
Pst	*Puccinia striiformis tritici*
Pt	*Puccinia triticina*
PPRI	Plant Protection Research Institute
PROC GLM	Procedure – general linear model
QTL	Quantitative trait loci
RIL	Recombinant inbred line
RNA	Ribonucleic acid
dsRNA	Double stranded RNA
siRNA	Small interfering RNA
mRNA	Messenger RNA
RNA-i	RNA-interference
RNA-seq	RNA-sequencing
r/s	Revolutions per second
SADC	Southern African Development Community
SARF	Sum of adjacent recombination frequencies
SMA	Single marker analysis
SNP	Single nucleotide polymorphism
sp.	Species in singular form
spp.	Species in plural form
SSR	Simple sequence repeat
TBE	Tris-borate-ethylenediamine tetraacetic acid (Tris/Borate/EDTA)
w/v	Mass per volume ratio
USDA-ARS	United States Department of Agriculture-Agricultural Research Service
VIGS	Virus induced gene silencing

Thesis Introduction

Background

The present challenges of agriculture are to meet the ever increasing demand for food, feed and bio-energy in the face of a constantly increasing world population and global climate change. It is forecasted that the world population will be around 7.7 billion people by 2020 and this figure is expected to rise to over 10 billion by the year 2050 (Shiferaw et al., 2011). Nevertheless, agricultural productivity is estimated to be growing at a slower rate of approximately 1.8% annually (Altman, 1999). Agricultural production and productivity is constrained by an array of stress factors including abiotic, biotic and socio-economic constraints. The relative importance of these constraints and their effect on crop yields varies among years and cropping regions. Integrated crop improvement is expected to play a crucial role in enhancing crop productivity through breeding of improved crop cultivars better adapted to the changing climate and tolerant to biotic and abiotic stresses (Newton et al., 2011).

Wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) provide at least 30% of food calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al., 2011). Approximately over 600 tonnes of wheat is produced and harvested worldwide annually. In 2015/2016 season, the European Union, was the world's largest producer of wheat, followed by China, India, Russia and the USA producing 157.98, 130.19, 88.94, 61 and 55.84 million metric tonnes, respectively. Sub-Saharan African countries including South Africa have been lagging behind in wheat production. These countries have failed to meet local or regional demands for wheat. South Africa is one of the leading wheat producers in sub-Saharan Africa. Wheat is the second most important grain crop widely grown across the Western Cape, Northern Cape, Free State, North West, Mpumalanga, KwaZulu-Natal and Limpopo Provinces in the country. However, wheat production in South Africa has declined progressively over the past 10 years, with producers switching to more profitable crops like canola, oat, maize and soybean, mainly due to various biotic and abiotic stress factors affecting production and productivity. In the 2014/2015 crop season, the area planted with wheat in South Africa was at a record low level of only

476, 570 hectares (Esterhuizen, 2016) compared to 500, 000 hectares in 2013 and 974, 000 hectares in 2002 (Agricultural Statistics, 2014). A declining land area for wheat cultivation suggests that producers are disinterested in wheat production due to the low profitability of this sector (Lemmer et al., 2012).

Constraints to wheat production

The major constraints to wheat production in South Africa include pests, diseases, drought and soil infertility. The most common fungal diseases of wheat include the wheat rusts, *Fusarium* head blight and powdery mildew (Duveiller et al., 2007). Wheat rusts including stem rust caused by *Puccinia graminis* f. sp. *tritici*, leaf rust (*P. titicina* Eriks.) and stripe rust (*P. striiformis* f. sp. *tritici*) are amongst the most damaging diseases of wheat, and have historically caused devastating yield losses worldwide. However, precise data on actual yield losses caused by wheat rusts on farmers' fields, especially in developing countries, are often unavailable.

Wheat rust diseases continue to pose a constant threat to sustainable wheat production, leading to food insecurity on a global scale (Pretorius et al., 2007). The wheat rusts severely affect the crop through inhibiting the efficiency of photosynthesis (Livne, 1964) and aggressively killing the foliar parts under high disease pressure. In turn, this leads to reduced kernel formation per head, lower kernel weight and poor grain quality and yield loss (Peturson et al., 1945; Kolmer et al., 2007; Singh et al., 2008). Damage caused by wheat rusts can potentially reduce wheat yields by up to 80% on susceptible cultivars (Roelfs, 1992; McIntosh et al., 1995) when favourable weather conditions are prevalent for infection and disease development. New and aggressively virulent races are emerging to overcome effective resistance in existing wheat cultivars, particularly if resistance is conferred by race-specific genes.

The emergence of a potentially devastating wheat stem rust race, Ug99 has threatened global wheat production. The appearance of this race and other virulent races of rust pathogens have necessitated for the development of wheat cultivars possessing stable, durable and effective stem rust resistance genes (Singh et al.,

2008). Since the first detection of Ug99 in Uganda in 1999 (Pretorius et al., 2000) about 13 variants of the race have been detected in 13 countries (http://rusttracker.cimmyt.org/?page_id=22). The last country added on reporting the Ug99 race is Egypt (Patpour et al., 2016). South Africa was included on the Ug99 list in 2000, and to date there are four races of Ug99 present in the country (Pretorius et al., 2000), designated as TTKSF (2000), TTKSP (2007), PTKST (2010) and TTKSF+ (2010) (http://rusttracker.cimmyt.org/?page_id=1034). This clearly shows that the pathogen is evolving and spreading, necessitating development and deployment of stem rust resistant wheat cultivars to boost productivity.

The leaf rust disease of wheat caused by *P. triticina*, has received relatively less research attention compared to stem rust. However, this pathogen continues to cause annual yield losses of wheat (Kolmer et al., 2007). In southern Africa, the detection of virulent leaf rust races from Zimbabwe and Zambia with similar genetic lineages to those recently discovered in South Africa (Pretorius et al., 2015) have also been alarming to the wheat industry. This suggests high chance of migration of wheat rust races between countries, and highlights the need for integrated and collaborative disease management strategies.

Several strategies are available to control the wheat rusts including cultural, biological, chemical and host plant resistance. These have been practiced by various wheat producers around the world with varying levels of success. In South Africa, wheat producers rely predominantly on the use of fungicides to control the wheat rusts (Boshoff et al., 2003; Pretorius et al., 2007; Terefe et al., 2009). Losses from wheat rusts can be mitigated through timely application of fungicides. However, the low profitability of wheat production cannot sustain such costly inputs mainly under dryland conditions (Lowe et al., 2011). Furthermore, fungicide application is not economic and environmentally friendly, and can lead to development of fungicide resistant races. Enhanced host plant resistance, and the combination of several effective rust resistance genes remains the most profitable and environmentally friendly control strategy (Kloppers and Pretorius, 1997; McCallum et al., 2016). Advanced breeding approaches using host plant resistance and gene pyramiding can enhance durability

of resistance. However, wheat cultivars with multiple and effective rust resistance genes are yet to be developed. Progress in understanding the molecular basis of rust disease resistance at both host and non-host levels will offer further possibilities for resistance breeding using biotechnological approaches. The use of advanced selection methods, high-throughput genetic analysis such as field pathogenomics, transgenics, genome editing and next generation sequencing may enhance the efficiency of breeding for durable rust resistance in wheat. This could subsequently result to a realisation of large proportions of economic returns in investments in international wheat research. A multidisciplinary approach involving pathologists, breeders, geneticists, physiologists and agronomists at different stages of research and development is necessary to develop improved wheat cultivars with stable and durable rust resistance through host plant resistance breeding.

Rationale

Leaf rust and stem rust of wheat are widely reported in South Africa reaching epidemic proportions and causing significant yield losses. The use of fungicides is not economically feasible and not environmentally friendly. Breeding improved wheat cultivars with durable leaf rust and stem rust resistance is the cheapest and economic approach to control the diseases. Several international research groups were established such as the Borlaug Global Rust Initiative (www.globalrust.org) project, together with regional and global research collaborators. Wheat researchers are searching for new and effective resistance sources and/or improving diagnostic procedures. In South Africa, the Agricultural Research Council–Small Grain Institute (ARC-SGI) in collaboration with private wheat breeding companies and Universities have embarked on pre-breeding and breeding of wheat to develop cultivars with effective rust resistance genes using a variety of genetic and genomic resources. Identifying and developing useful DNA markers and mapping candidate genes conferring durable resistance to the two rust diseases will enable wheat breeders to apply marker-assisted selection (MAS) for rapid development of disease resistant cultivars and for food security and improved livelihoods.

Aims and objectives of the study

The overall aim of this study was to identify promising wheat genotypes with adult plant resistance to leaf rust and stem rust diseases using a newly developed recombinant inbred line (RIL) population. Furthermore, the study aimed to identify genomic regions possessing candidate resistance genes against leaf rust and stem rust of wheat for marker-assisted resistance breeding.

The specific objectives of the study were:

1. to assess adult plant resistance (APR) of selected wheat genotypes to leaf rust and stem rust, as well as to develop segregating populations for resistance breeding,
2. to investigate the inheritance of adult-plant resistance in a recombinant inbred line population of a cross between two cultivars 'Popo' and 'Kariega', and to identify wheat breeding lines possessing both leaf and stem rust resistance genes, and
3. to undertake a genome-wide scan for identification of quantitative trait loci (QTL) that significantly affect adult plant resistance to leaf rust and stem rust using a newly developed recombinant inbred line population of wheat developed from a cross of two popular cultivars, Popo and Kariega. In addition, to construct a genetic linkage map useful to locate genes/QTL controlling other important agronomic traits in the Popo/Kariega genetic background.

Thesis outline

The experiments were carried out as a series of tests, each contributing towards a specific objective. Overall, the thesis includes five chapters written in the form of discrete research papers, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters. Chapter 1 is published in the African Journal of Biotechnology (2014, Vol 14, pp: 4188-4199), while Chapter 3 has been accepted for publication (16/09/2016) in the journal - Cereal Research Communications: CRC-D-16-00149 and Chapter 4 is under review in the South African Journal of Plant and Soil: TJPS-2016-0079.

The structure of the thesis is outlined below:

Chapter	Title
-	Thesis Introduction
1	Review: Wheat breeding for durable leaf rust resistance in southern Africa: current status - bottlenecks and future prospects
2	Review: Wheat stem rust in South Africa - current status and future research directions
3	Adult plant resistance of selected Kenyan wheat cultivars to leaf rust and stem rust diseases
4	Adult plant resistance to leaf rust and stem rust of wheat in a newly developed recombinant inbred line population
5	Linkage mapping and quantitative trait loci analysis of genes conferring adult plant resistance to leaf rust and stem rust of wheat
-	An overview of the research findings

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Chapter 1

1. Review: Wheat Breeding for Durable Leaf Rust Resistance in Southern Africa - Current Status, Bottlenecks and Future Prospects

Abstract

Leaf rust, caused by *Puccinia triticina* (*Pt*) is a highly diverse and widespread disease affecting wheat. Although considerable progress has been made to control leaf rust through host plant resistance breeding in southern Africa, frequent evolution of the pathogen population still presents a major challenge to the achievement of durable resistance. Surveillance and monitoring of the pathogen has revealed the occurrence of common races across the region, justifying the need for concerted efforts by countries in the Southern African Development Community (SADC) to use more efficient strategies to manage the disease. Understanding *Pt* genetic variability across the region is crucial to allow deployment of right combinations of resistance (*R*) genes that confer durable resistance to leaf rust. Several molecular markers including single nucleotide polymorphisms (SNPs) and quantitative trait loci (QTLs) that are associated with genes for leaf rust resistance have been reported. This review highlights the variability of the leaf rust pathogen populations, current status, challenges and future prospects in breeding wheat for durable resistance in southern Africa. The potential of utilizing modern breeding technologies including genetic modification and genome editing technologies to produce varieties with broad-spectrum durable leaf rust resistance is discussed, not overlooking the importance of timeous, collaborative and efficient surveillance of the pathogen across the sub-region.

Key words: Breeding; Durable resistance; Pathogen variability; *Puccinia triticina*; Virulence; Wheat

1.1. Introduction

Leaf rust or brown rust, caused by *Puccinia triticina* Eriks. (*Pt*), is widely distributed across the major wheat growing regions worldwide (Saari and Prescott, 1985; Kolmer, 2013). It is considered as one of the most common diseases of bread wheat (*Triticum aestivum*), durum wheat (*T. turgidum* var. *durum*), and triticale (*X Triticosecale*) (Bolton et al., 2008; Huerta-Espino et al., 2011). The pathogen causing leaf rust is an obligate biotrophic fungus mainly infecting the leaves of wheat at various growth stages. It can also infect the leaf sheath and glumes, causing considerable grain yield losses of more than 50% in susceptible cultivars (Hussein et al., 2005; Huerta-Espino et al., 2011). The southern Africa sub-region is the main epidemiological zone of leaf rust, causing most wheat cultivars prone to infection (Saari and Prescott, 1985; Huerta-Espino et al., 2011). Since the 1980s, certain South African wheat cultivars have been reported to be susceptible to leaf rust, with localised epidemics frequently occurring in the winter rainfall regions of the Western Cape Province and irrigated areas in other provinces (Pretorius et al., 2007; Terefe et al., 2009). Recently, low infection levels have been reported in farmers' fields due to lower inoculum levels mainly resulting from repeated fungicide applications (Terefe et al., 2009). However, finding pre-emptive, effective and more sustainable ways to control future leaf rust epidemics should be the main objectives of wheat breeding programmes in southern Africa, than to rely entirely on fungicide applications.

Collections of *Pt* from countries in southern Africa including Malawi, South Africa, Zambia and Zimbabwe, resulted in the identification of over 20 *Pt* physiological races to date (Pretorius et al., 1987; Pretorius and Le Roux, 1988; Pretorius et al., 1990; Pretorius et al., 2015; Terefe et al., 2014a, b, c). Analysis of the collected samples have revealed pathogenic relationships among the major races detected in different parts of southern Africa (Terefe et al., 2014b; Pretorius et al., 2015). The narrow genetic diversity among the *Pt* races suggests their wide distribution in the region presumably evolved from a common pedigree through spontaneous mutations and genetic recombination. Also, the genetic similarities could indicate dispersal of fungal spores over thousands of kilometres across the region by wind and water, as is the case with other regions (Brown and Hovmøller, 2002; Kolmer, 2005). Accidental transfer by means of farm implements, contaminated clothing or goods also play a

major role in the spread of spores across borders and could be one of the contributing factors. The spore dispersal, spontaneous mutations, genetic recombination and pathogen resurgence could also explain the occurrence of new races in areas where they were not detected before or over a long period of time. Such new races often pose challenges because they may acquire fungicide resistance or defeat known leaf rust resistance genes, leading to resistance breakdown. Hence there is a need for regular monitoring and collaborative surveillance of changes in the virulence patterns among leaf rust races in each country and across regions. Essential information gathered through these surveys can guide deployment of appropriate conventional and cutting-edge breeding technologies to produce improved varieties or breeding lines with broad spectrum and durable resistance to old and emerging *Pt* races.

Host-plant resistance has been shown to be a cost effective and environmentally safe control strategy for rusts and other diseases (Johnson, 1981; Johnson, 1984; Kolmer, 1996). In southern Africa, as in many other regions, one of the earlier major challenges was that *Pt* frequently acquired new and aggressive virulence that overcame existing resistance particularly when it was conferred by single, race-specific genes (Pretorius et al., 2007; Terefe et al., 2014b; Pretorius et al., 2015). Also, continual release of wheat cultivars with different race-specific resistance genes could have placed an intensive selection pressure on *Pt*, leading to the diversity of rust populations in the region as observed in North America (Kolmer et al., 2007). However, host-plant resistance has considerable success where continuous surveys for leaf rust races and other rust diseases are integrated with pre-breeding, breeding and post-release management efforts targeting rust resistance as demonstrated by the Borlaug Global Rust Initiative (BGRI) and associated regional and global projects (Evanega et al., 2014). To achieve a sustainable and cost effective control strategy for leaf rust, a multidisciplinary and co-operative research approach involving a number of stakeholders including breeders, geneticists, pathologists, biotechnologists, policy makers and farmers is required. This also includes collaborative research and exchange of resistant germplasm to safeguard border countries in the event of severe leaf rust outbreaks since the urediniospores disperse widely.

This review discusses the variability and distribution of *Pt* races in southern Africa. Focus is placed on describing the predominant races common across the region and the deployed wheat leaf rust resistance genes. A comprehensive summary of effective leaf rust resistance genes available in different parts of the world, which could be pyramided into regional cultivars through advanced genetic and genomic technologies is also provided. Perspectives on the strides taken, and milestones reached through collaborative research towards finding the best strategy to achieve durable leaf rust resistance in southern Africa and major challenges encountered in the process are also discussed.

1.2. Variability and distribution of wheat leaf rust pathogen in southern Africa

Puccinia triticina race populations throughout the world, including in southern Africa, are diverse in terms of their molecular architecture as well as the combinations and complexity of virulence patterns. This is influenced by co-evolution of the pathogen with diverse wheats and genetic recombination of races from various *Triticum* species (Kolmer and Liu, 2000; Liu et al., 2014). The genetic characteristics of all *Pt* populations is typical of diploid/dikaryotic populations, with a life cycle summarised by Bolton et al. (2008). The pathogen maintains high levels of variability through sequential mutations and sexual or asexual recombination which lead to virulence shifts (Bolton et al., 2008). The importance and frequency of somatic recombination as a mechanism to induce variation in *Pt* races is however unclear. Dispersion of rust populations over large distances to new areas/regions where they were not detected before adds to the aforementioned sources of variability. To understand the variability of *Pt* races in the region, surveys and collections of leaf rust samples are conducted annually based on protocols outlined by the Global Cereal Rust Monitoring System (www.rusttracker.cimmyt.org/) by experts from various organisations in southern Africa. These institutions include the Agricultural Research Council-Small Grain Institute (ARC-SGI) and the University of Free State in South Africa, the Chitedze Research Station and the International Maize and Wheat Improvement Centre (CIMMYT) in Malawi, the Crop Breeding Institute (CBI) and Plant Protection Research Institute (PPRI) in Zimbabwe (www.rusttracker.cimmyt.org/). Race analysis and surveys are crucial for generating reports that are then compiled and shared with the wheat breeding

community worldwide through a global rust monitoring system developed by CIMMYT and other partners as part of the BGRI to mitigate the threat of wheat rust disease (www.globalrust.org/).

Race analysis

Traditional and genomic tools can be deployed to distinguish leaf rust physiological races. Differential varieties or lines form a basic and crucial component of race analysis. Ideally, near-isogenic lines (NILs) containing different leaf rust resistance genes/alleles on particular loci can be used to distinguish races by their phenotypic responses to different pathogen strains. Having NILs encompassing single *Lr* genes in a uniform background of a highly susceptible cultivar or line facilitates research in understanding the genetics of host-parasite interactions (McCallum et al., 2016). The use of differential lines to distinguish physiological specialisation of *P. triticina* was pioneered by Mains and Jackson (1926). Improvements were constantly made on the original leaf rust differential set through additions of newly described physiological races. A series of NILs in a Thatcher (*T. aestivum*) background are now routinely used globally (Table 1.1). The original set of NILs was developed by Dr Peter Dyck from the Cereal Research Centre, Agriculture and Agri-Food Canada in Winnipeg following work initiated by Dr Robert G. Anderson (Anderson, 1963). Differential lines are grouped in sets of four. When the four lines are classified for resistance or susceptibility, there are 16 possible combinations. Additional sets are commonly used to supplement the standard differential lines (Huerta-Espino et al., 2011; Terefe et al., 2014a, b, c). The North American nomenclature system for designating virulence combinations of *Pt* cultures is followed in most southern African countries including South Africa, as proposed by the North American Wheat Leaf Rust Research Workers Committee (Kolmer et al., 2010). Pathogenicity of a race is given a four letter code, where each of the first three letters indicate the pathogenicity of the race on one set of four lines. The fourth letter describes the pathogenicity of the race on the set of lines used to supplement the standard differential lines (Kolmer et al., 2010; McCallum et al., 2010). A number of sophisticated and modern tools can also be used to investigate variation in *Pt* populations including the use of DNA based markers and field pathogenomics.

Table 1.1. Common near isogeneic lines (NILs) in Thatcher background encompassing specific *Lr* genes [Adapted from McCallum et al. (2016)]

Lr gene	RL number	Pedigree
Lr1	RL6003	Thatcher*6/Centenario
Lr2a	RL6016	Thatcher*6/Webster
Lr2b	RL6019	Thatcher*6/Carina
Lr2c	RL6047	Thatcher*6/Brevit
Lr3a	RL6002	Thatcher*6/Democrat
Lr3bg	RL6042	Bage/8*Thatcher
Lr3ka	RL6007	Thatcher*6/Klein Anniversario
Lr9	RL6010	Transfer (*Aegilops umbellulata*)/6*Thatcher
Lr10	RL6004	Thatcher*6/Exchange
Lr11	RL6053	Thatcher*6//E-1/Hussar
Lr12	RL6011	Exchange/6*Thatcher
Lr13	RL6001	Prelude*6/Loros
Lr14a	RL6013	Selkirk/6*Thatcher
Lr14b	RL6006	Thatcher*6/Maria Escobar
Lr15	RL6052	Thatcher*6/W1483
Lr16	RL6005	Thatcher*6/Exchange
Lr17a	RL6008	Klein Lucero/6*Thatcher
Lr18	RL6009	Thatcher*7/Africa 43
Lr19	RL6040	Thatcher*7/Translocation 4 (*Lr19* derived from *Agropyron elongatum*)
Lr20	RL6092	Thatcher*6/Timmo
Lr21	RL6043	Thatcher*6/RL5406(Tetra Canthatch/*Aegilops squarrosa* var *meyeri*-RL5289)
Lr22a	RL6044	Thatcher*7/RL5404(Tetra Canthatch/*Aegilops squarrosa* var *strangulata*-RL5271)
Lr22b	Thatcher	Marquis/Iumillo Durum//Marquis/Kanred
Lr23	RL6012	Lee 310/6*Thatcher
Lr24	RL6064	Thatcher*6/3/Agent//2*Prelude/8*Marquis
Lr25	RL6084	Thatcher*7/Transec
Lr26	RL6078	Thatcher*6/St-1-25
Lr28	RL6079	Thatcher*6/C-77-1
Lr29	RL6080	Thatcher*6//CS7D/Ag#11
Lr30	RL6049	Thatcher*6/Terenzio
Lr32	RL6086	Thatcher*6/3/Thatcher/*Aegilops squarrosa*//Mq(K)
Lr33	RL6057	Thatcher*6/PI58548
Lr34	RL6058	Thatcher*6/PI58548
Lr35	RL5711	Marquis-K*8//RL5344/RL5346 (*Triticum monococcum*)
Lr37	RL6081	Thatcher*8/VPM
Lr38	RL6097	Thatcher*6/T7Kohn
Lr44	RL6147	Thatcher*6/*Triticum speltoides* 7831 85GN 438
Lr45	RL6144	Thatcher*6/St-1
Lr52	RL6107	Thatcher*6/V336
Lr60	RL6172	Thatcher*3/V860

Table 1.1. Continued

Lr gene	RL number	Pedigree
Lr63	RL6137	Thatcher*6/TMR5-J14-12-24
Lr64	RL6149	Thatcher*6/8404
Lr67	RL6077	Thatcher*6/PI250413

Use of molecular markers for race diagnostics

DNA based molecular markers such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have long been successfully used in mycology and plant pathology for the differentiation of species within the *Puccinia* genera (Kolmer et al., 1995; Liu and Kolmer, 1998). Through the use of these markers, several studies have characterised leaf rust races from distinct race groups. Kolmer and Liu (2000) distinguished groups of *P. triticina* isolates from international collections using RAPD markers. Results from sixty nine *Pt* isolates collected in Canada, tested for AFLP suggested that populations in North America most likely evolved from a number of introductions of the fungus that differed in molecular background (Kolmer, 2001). However, there are some limitations in using RAPD and AFLP markers mainly because of their dominant nature, hence they cannot differentiate between homozygote and heterozygote genotypes. Co-dominant markers such as locus-specific microsatellites or simple sequence repeats (SSRs) and single nucleotide polymorphism (SNP) were later developed for *P. triticina* to distinguish heterozygote genotypes (Suenaga et al., 2003; Lagudah et al., 2006; Vida et al., 2009; Terracciano et al., 2013).

In southern Africa, the use of DNA-based markers, particularly co-dominant markers such as SSRs and SNPs in race analysis and identification often complements the traditional methodology of phenotyping. A number of leaf rust races have been detected in various locations in southern Africa including Malawi, Mozambique, South Africa, Swaziland, Zambia and Zimbabwe (Table 1.2). Pretorius et al. (1987) detected races PDRS, SBDS and SDDS in the early 1980s in South Africa. The earliest evidence of commonality in southern African *Pt* races was reported by Pretorius and Purchase (1990) from collections conducted in Zimbabwe, Zambia and Malawi. Based on differential *Lr* genes used in this study, SBDS, a race which was also detected in

Zimbabwe in 1986, together with SCDS which was first detected in South Africa in 1988 (Pretorius and Purchase, 1990), were found to be similar except for the virulence of the latter to *Lr24* and *Lr26* genes (Pretorius and Purchase, 1990). SBDS and SDDS were also found to have similar virulence/avirulence profiles, with SDDS being virulent to *Lr24*. Another race, SDDN was detected in the Western Cape Province of South Africa in 2005. This race had genetic similarities with SDDS, SCDS and SFDS which were first detected in South Africa in 1987 (Pretorius and Le Roux, 1988), all sharing virulence to 11 *Lr* genes (Terefe et al., 2014b).

Using SSR markers, the race PDRS was found to be genetically different from 10 other South African races, leading to a speculation that it is a foreign introduction (Visser et al., 2012). Terefe et al. (2014) also investigated the genetic relationship between a new *Pt* race and existing South African races making use of a number of SSR markers. The results from this study suggested that two new races CCPS and MCDS entered South Africa as exotic introductions. The same study revealed that race CCPS shared 71% and 51% genetic similarities to European races CCPSL and CCTSL, respectively. The CCPS race was even closer to MCPSS from the Czech Republic (Terefe et al., 2014b), validating the commonality of some races within regions. Race FBPT was detected in the Western Cape Province in 2010 (Terefe et al., 2014c). In a recent study, similar races (MCDS, TCPS, FBPT and SCDS) have been detected by Pretorius et al. (2015) from 63 isolates obtained in different southern African countries during 2011-2013. MCDS and TCPS were found in Zimbabwe and Zambia whereas FBPT and SCDS were detected in Zimbabwe and Malawi, respectively. MCDS, FPBT and SCDS were also detected in South Africa. Pretorius et al. (2007) revealed that the variation between most of the races identified in southern Africa was associated with virulence against *Lr10, Lr14a*, *Lr17*, *Lr24* and *Lr26* resistance genes.

Table 1.2. Avirulence/virulence profiles of frequent *Puccinia triticina* races detected in different localities in southern Africa from the 1980s[1]

Race (NA notation)	Locality	Avirulence/virulence profile	Reference(s)
PDRS	South Africa, Malawi	*Lr2a, 2b,* ***9****, 15,* ***16****, 17,* ***18****, 23, 26/1, 2c, 3a, 3bg, 3ka, 10, 11, 14a, 20, 24, 28, 30, B*	(Pretorius and Le Roux, 1988; Pretorius and Purchase,1990)
SFDS	South Africa	*Lr3a, 3bg, 3ka,* ***9****, 11,* ***16****,* ***18****, 26, 30/1, 2a, 2b, 2c, 10, 14a, 15, 17, 20, 23, 24, 28, B*	(Pretorius and Le Roux, 1988)
SBDS	South Africa, Zambia, Zimbabwe	*Lr3a, 3bg, 3ka,* ***9****, 11,* ***16****,* ***18****, 24, 26, 20/1, 2a, 2b, 2c, 10, 14a, 15, 17, 20, 23, 28, B*	(Pretorius et al.,1987)
SDDS	South Africa	*Lr3a, 3bg, 3ka,* ***9****, 11,* ***16****,* ***18****, 26, 30/1, 2a, 2b, 2c, 10, 14a, 15, 17, 20, 23, 24, 28, B*	(Pretorius et al., 1987)
SCDS	Malawi, South Africa	*3a, 3bg, 3ka,* ***9****, 11,* ***16****,* ***18****, 24, 30/1, 2a, 2b, 2c, 10, 14a, 15, 17, 20, 23, 26, 28, B*	(Pretorius et al., 1990; Terefe et al., 2014c)
SDDN	South Africa	*Lr3a, 3bg, 3ka,* ***9****, 10, 11,* ***16****,* ***18****, 26, 30/1, 2a, 2b, 2c, 14a, 15, 17, 20, 23, 24, 28, B*	(Pretorius and Bender, 2010)
CCPS	South Africa	*Lr1, 2a, 2b, 2c,* ***9,*** *11,* ***16****,* ***18****, 20, 23, 24/3a, 3bg, 3ka, 10, 14a, 15, 17, 26, 28, 30, B*	(Terefe et al., 2014b; Terefe et al. 2014c)
MCDS	Zimbabwe, Zambia, South Africa	*Lr2a, 2b, 2c, 3ka,* ***9****, 11,* ***16****,* ***18****, 20, 24, 28, 30/1, 3a, 3bg, 10, 14a, 15, 17, 23, 26, B*	(Terefe et al., 2014a)
TCPS	Zimbabwe, Zambia	*Lr****9****, 11,* ***16****,* ***18****, 24/1, 2a, 2c, 3a, 3ka, 10, 14a, 17, 26, 30, B*	
FBPT	Zimbabwe, South Africa	*Lr1, 2a, 2b,* ***9****, 11, 15,* ***16****, , , 20, 21, 23, 24, , 26, , 28, , /2c, 3a, 3ka, 10, 14a, 17, 18, 30, B*	(Pretorius et al., 2015)

[1]This table is not necessarily exhaustive, only *P. triticina* races that have been predominant in southern Africa over the years are highlighted. Good genes that are still effective across regions are in bold.

Prospects of field pathogenomics

The technology that has not been explored to its fullest capacity for leaf rust race analysis is 'field pathogenomics'. This is a relatively new method that uses the latest DNA sequencing technologies to quickly generate high resolution data to describe the diversity within a pathogen population directly from infected samples. A study by Hubbard et al. (2015) using field pathogenomics tools successfully discovered a dramatic shift in wheat yellow rust (*P. striiformis*) population in the United Kingdom, which can similarly be useful for leaf rust studies in southern Africa. With the increasing availability of fully sequenced pathogen genomes, this new high throughput technology will provide new insights into the biology, population structure and pathogenesis of pathogens of interest (Derevnina and Michelmore, 2015).

The *Pt* sequencing project was recently established forming part of the Fungal Genome Initiative at the Broad Institute (www.broad.mit.edu/annotation/genome/puccinia_group/MultiHome.html/). This initiative has provided the opportunity to generate expressed sequence tag (EST) libraries which have contributed to gene discoveries and stage-specific expression analysis (Xu et al., 2011). The generation of ESTs also has several applications including designing molecular markers such as SSRs, designing and constructing expression microarrays, genome annotation and comparative genomics to reveal relatedness between species. Based on information from a combination of EST sequences, *in silico* predictions and RNA-seq transcript analysis, a high quality reference genome sequence of *Pt* is now annotated and publicly available. The availability of genome sequence information of *Pt* will assist researchers to understand the dynamics of host-pathogen interaction, genetic and genomic responses of the pathogen to fungicide use, and the evolution of resistance to essential pesticides, hence increasing our ability to mitigate risks caused by the pathogen. Since shared virulence strategies may be used by different fungi to invade specific plant hosts, comparative analysis of the *Pt* genome with those of other pathogens may also offer a new and powerful approach to identify common and divergent virulence strategies as well as evolutionary history of pathogen lineages. Gardiner et al. (2012) used comparative analysis to identify novel virulence genes in fungi infecting cereals. The power of comparative genomics

has proven useful in identifying potential pathogenicity factors in *Fusarium graminearium* and *Ustilago maydis*. The ever increasing efficacy and decreasing costs of sequencing should motivate more researchers, especially in southern Africa to tap into the field of pathogenomics. This should shed more light on the potential origin, adaptation and interaction of southern African leaf rust races with the host, hence providing effective opportunities for leaf rust management.

1.3. Control strategies of leaf rust

Various control options including biological, cultural, chemical (fungicides) and host plant resistance are available. However, only limited studies are conducted on the biological and cultural control options, leaving the last two options being widely used. Timely and accurate application of fungicides is effective in reducing both the incidence and severity of leaf rust in wheat. In the past years, regular fungicide spraying on commercial wheat fields of the Western Cape Province in South Africa contributed to the leaf rust fungus becoming less prevalent. Fungicides with active ingredients such as epoxiconazole, tebuconazole, pyraclostrobin and trifloxystrobin remain effective for protecting yields of susceptible varieties from leaf rust attack. Nevertheless, fungicide use is not economically and environmentally sustainable, and can pose health hazards to people and animals, as well as phytotoxicity to the crop (Kolmer et al., 2007; Pretorius et al., 2007; McCallum et al., 2016). Further, repeated use of fungicides belonging to the same group may result in resistance build-up among the pathogen strains putting more pressure of formulating new fungicides that can combat new, normally more virulent mutant strains. Thus far, researchers are under pressure to ensure reduction of overreliance on fungicides but at the same time promoting greater yield stability, subsequently generating a large proportion of returns on global economic investments in international wheat research (Chaves et al., 2013). Enhanced host plant resistance, and more importantly, the combination of several effective leaf rust resistance genes remains the most feasible, economic and environmentally friendly approach to ensure durable resistance (Ayliffe et al., 2008; McCallum et al., 2016). Wheat cultivars and breeding lines with multiple leaf rust resistant genes have significantly lower disease levels (German and Kolmer, 1992; Vanzetti et al., 2011; Tsilo et al., 2014), and the use of this kind of resistance has a potential to significantly reduce disease epidemics.

Host plant resistance

Host plant resistance against *Pt* is grouped into two broad categories: 1) vertical resistance which is caused by single or major or race specific or seedling resistance genes and 2) horizontal or partial or polygenic or slow rusting resistance caused by minor or race non-specific or adult plant resistance genes. The proposed mechanisms of action for race-specific and adult plant resistance genes, including their interaction with the pathogen avirulence (*Avr*) genes and the guard hypothesis have been widely discussed in detail in previous reports (Johnson, 1984; Vanzetti et al., 2011; Evanega et al., 2014; Sekhwal et al., 2015). Durable resistance breeding programmes should be guided by accurate information of the mechanism and impact of resistance conferred by target genes because it is often the mode of resistance that determine the durability of resistance together with the right combination of resistance genes.

At present, more than 80 different leaf rust resistance genes and alleles that are spread throughout the A, B and D wheat genomes have been identified and catalogued (McIntosh, 1975; Kolmer, 2013). Most of these resistance genes and gene complexes were sourced from relatives of wheat such as *Thinopyrum elongatum* Zhuk. (*Lr19*), *Th. tauschii* (*Lr21*), *Th. elongatum* (*Lr24*), *Secale cereale* L. (*Lr26*), *Aegilops peregrina* (*Lr59*) among others (McIntosh, 1975; Autrique et al., 1995; McIntosh et al., 1995; Marais and Botes, 2003) and near-isogenic lines carrying individual *Lr* genes are available. Several work that involves transferring resistance genes from wild species to bread wheat have been done around the world. Breeding efforts have also been undertaken to introgress these *Lr* genes into wheat breeding lines. Nevertheless, a significant number of these *Lr* genes are race specific and generally conform to the “gene-for-gene” model first proposed by Flor (1946), conferring resistance to pathogen races with corresponding *Avr* gene. They therefore lack durability, which was defined by Johnson (1984) as “the ability of a widely-deployed resistance gene to provide an economic level of protection over an extended period of time”. They are hence frequently defeated by the appearance of new virulent races in the pathogen population through single-step mutations on the *Avr* gene and/or recombination or migration of new races (Samborski, 1985; Bolton et al., 2008).

To illustrate this fact, most cultivars or breeding lines that were developed earlier in southern Africa are no longer used as one of the reasons is because they carried single or race-specific genes like *Lr1, Lr3a, Lr10, Lr13, Lr14a, Lr17b, Lr24, Lr26, Lr27* and *Lr31* which were later defeated by new virulent races. In some cultivars, however, the combination of some of these leaf rust resistance genes remains effective owing to the absence of that specific virulence combination in local races (Pretorius et al., 1996). Constant identification and selection of new sources of resistance therefore remains important in most breeding programmes, given the ability of *Pt* to overcome race specific resistance genes (Samborski, 1985). The identification of new sources with effective and durable resistance genes allows for efficient incorporation of different genes into germplasm pools, consequently helping to avoid releasing cultivars that are genetically uniform (Kolmer, 1996). In this case, local cultivars can be improved by genes and QTLs outsourced from lines showing durable leaf rust resistance from other countries, for instance the Brazilian cultivar, Toropi, which has exhibited durable adult plant resistance since its release in 1965 (Casassola et al., 2015).

In contrast to the seedling resistance genes, resistance conferred by adult plant resistance genes is often undetectable at a seedling stage, but is known to be effective against a wide range of known *Pt* physiologic races. Adult plant resistance genes confer partial resistance, regulating the pathogen's effectiveness by producing fewer and smaller uredinia which are surrounded by chlorosis. Adult plant resistance often provides long-term, and durable resistance. *Lr34*/*Yr18/Pm38* is one of the few cloned and sequenced adult plant resistance genes (Lagudah et al., 2006) in plants conferring durable resistance to leaf rust, stripe rust, stem rust, powdery mildew caused by *Blumeria graminis* and barley yellow dwarf virus (Singh, 1993). It has been reported that adult plant resistance genes may have small to intermediate effects when controlling resistance individually, whilst high levels of resistance are achieved by combining four to five genes with additive effects (German and Kolmer, 1992; Kloppers and Pretorius, 1997; Singh et al., 2000; Vanzetti et al., 2011; Tsilo et al., 2014). Other adult plant resistance genes including *Lr46/Yr29/Pm39*, *Lr67/Yr46*, *Sr2/Yr30* and *Lr68* have been characterised (Singh et al., 1998; Hiebert et al., 2010; Herrera-Foessel et

al., 2012). Table 1.3 summarises some of the effective seedling and adult plant resistance genes that have been deployed in most breeding programmes in southern Africa and around the world. Availability of DNA markers for these genes makes it easier to deploy host-plant resistance effectively, even in the event when race phenotyping is not possible to conduct gene postulations.

Table 1.3. Chromosome location, description, linked DNA markers and references for leaf rust resistance genes still deployed against most *Puccinia triticina* races in southern Africa and around the world

Lr gene	Chromosomal region	General description	Linked DNA marker	Original source	Reference(s)
		Major Genes (race specific resistance)			
Lr9	6BL	Confers resistance to leaf rust.	*cMWG684* *PSR546* *J13*	*Aegilops umbellulata*	(Schachermayr et al., 1994)
Lr19	7DL	Effective against all races of leaf rust in South Africa.	*STSLr19*$_{130}$	*Agropyron elongatum*	(Prins et al., 2001)
Lr39	1DS	Allelic or identical to *Lr21.*		*Triticum tauschii*	
Lr41	1D			*Triticum tauschii*	
		Minor Genes (race non-specific resistance)			
Lr34/Yr18/Sr57	7DS	Offers partial resistance to leaf rust, yellow rust, stem rust, powdery mildew and yellow dwarf virus. Associated with leaf tip necrosis (morphological marker). Cloned.	*csLV34* *Swm10* *cssfr1 to cssfr7*	*Triticum aestivum*	(Lagudah et al., 2006)
Lr37	2AS	Confers resistance to leaf rust.	*Xcmwg682*	*Triticum ventricosum*	(Helguera et al., 2003)
Lr46/Yr29/Pm39	1BL	Confers slow rusting resistance to leaf rust and stripe rust.	*csLV34*	*Triticum aestivum*	(William et al., 2003)
Lr67/Yr46	4DL	Confers slow rusting resistance to leaf rust and stripe rust. Associated with leaf tip necrosis (morphological marker).		*Triticum aestivum*	(Herrera-Foessel et al., 2011)

Table 1.3. Continued

Lr gene	Chromosomal region	General description	Linked DNA marker	Original source	Reference(s)
Lr68	7BL	Confers slow rusting resistance to leaf rust	*Psy1-1* *gwm146* *cs7BLNLRR* *scGS*	*Triticum aestivum*	(Herrera-Foessel et al., 2012)
Sr2	3BS	Associated with pseudo-black chaff (morphological marker).	*csSr2-SNP* *BARC133* *gwm533* *gwm389* *3B042G11* *3B028F08*	*Triticum turgidum*	(Mago et al., 2011; McNeil et al., 2008)

The most useful diagnostic markers are underlined.

1.4. Durable leaf rust resistance

Resistance is considered durable if it remains effective within a cultivar under cultivation for a significant number of years over a substantial area with favourable conditions for the respective pathogen (Johnson, 1981, 1984). Several studies have demonstrated that durability of leaf rust resistance is more likely to be of adult plant type than of seedling type and is often linked to genes or gene loci that confer durable resistance to other rusts and other diseases, as in the case of *Lr34/Yr18/Sr57* (Singh, 1992), *Lr46/Yr29/Pm39* (Kolmer et al., 2015), *Lr67/Yr46* (Herrera-Foessel et al., 2014) and probably *Lr68* (Herrera-Foessel et al., 2012), though the effect of this gene (*Lr68*) on other diseases has not been reported yet. However, successful integration or pyramiding of a number of single *Lr* genes conferring complete resistance with a few minor genes or adult plant resistance genes could result in a significantly broader durable resistance over a significant number of years, a strategy advocated by BGRI. Also, when genes are combined, they often complement each other, giving reactions different from those given by each component gene alone (Kloppers and Pretorius, 1997). Furthermore, the effects of simultaneous mutations in the pathogen on virulence against multiple resistance genes is greatly reduced as compared to single genes, therefore resistance remains effective for longer (Schaefer et al., 1963).

Kloppers and Pretorius (1997) observed active complementation of *Lr34* and *Lr13* with both genes improving resistance on the selected lines even with the presence of races possessing virulence for the *Lr13* gene. German and Kolmer (1992) and Kolmer et al. (2007) also reported prolonged and positive interaction of resistance genes *Lr16*, *Lr23* and *Lr34* in North America. The complementary effect of combining resistance genes was also tested by Pretorius et al. (1996) transferring *Lr21*, *Lr29*, *Lr32*, *Lr34*, *Lr35*, *Lr36*, *Lr37*, *Lr41*, and *Lr42* to South African cv. Palmiet and Karee through the backcrossing method. Likewise, Vanzetti et al. (2011) found that seedling resistance genes such as *Lr16, Lr47, Lr19, Lr41, Lr21, Lr25* and *Lr29* provided durable resistance when combined with APR genes such as *Lr34, Sr2* and *Lr46* in Argentina. A recent study by Silva et al. (2015) demonstrated the relevance of combining *Lr34*, *Lr68* and *Sr2* to increase leaf rust resistance, showing the importance of using APRs as key genes to be deployed in breeding programmes to attain high levels of resistance. Combinations of partial resistance genes in the CIMMYT breeding programme have

conferred adequate resistance to leaf rust in the field for several years (Singh et al., 2005). Breeding lines from this programme are widely used in southern Africa. Local researchers should propose breeding strategies and efficient use of tools to assist in outsourcing and introgressing new sources of rust resistance into suitable backgrounds. Efficient screening of a large number of breeding lines and varieties to determine the presence and frequency of resistance alleles in breeding programmes is also necessary. However, the limited availability of diagnostic molecular markers greatly impedes the screening and gene pyramiding processes. Pre-breeding efforts are well undertaken in many institutions around the world to address the challenges. In South Africa, the University of the Free State, Stellenbosch University and Agricultural Research Council among others have invested significantly on wheat leaf rust research and to pyramid resistance genes. Some of the effective genes used in pyramiding schemes include *Lr9, Lr19, Lr29, Lr34, Lr45, Lr47, Lr51* and *Lr52*.

The development of more precise and affordable molecular markers including KASP (Kompetitive Allele Specific PCR) markers, the use of next generation sequencing (NGS) technologies and QTL mapping have proved crucial in identifying more adult plant resistance genes and other effective race specific genes that can be combined to achieve stable and durable resistance. Several studies supporting the use of these tools, looking at their advantages and occurring gaps are available. A recent NGS based transcript analysis by Satapathy et al. (2014), which revealed the differential responsiveness of pathogen defence related to WRKY genes, with the potential of detecting leaf rust specific resistance genes encourages utilisation of advanced technologies in rust breeding programmes. Further, Li et al. (2014) provides a comprehensive summary of the genomic resources for durable leaf rust resistance breeding including eighty previously reported QTLs, and gives prospects of fine mapping and cloning of these QTLs due to advances in NGS technologies. Therefore, SADC countries which have not yet invested in some of these advanced genomic tools can equally benefit from other partners through regional and global collaborations, improving their capacity to develop cultivars with durable resistance.

1.5. Challenges in breeding for durable leaf rust resistance in southern Africa

As much as rust pathogens in general have great economic importance, limited information is available on the genes and factors required for pathogenesis and virulence. Adding to this, accurate information on the effect of the environment and full knowledge of the identity of effective resistance genes in the host, their mode of action when interacting with the *Avr* gene, and the interaction of slow rusting resistance genes with other resistance genes or gene loci to achieve stable and durable rust resistance across environments is still limited. It has been reported in several studies that temperature variations play a significant role in the expression of many resistance genes. A study by Herrera-Foessel et al. (2012) found that in Ciudad Obregon, Mexico, the effect of *Lr68* was smaller than the effect of *Lr34, Lr67*, or *Lr46* in the 2007-2008 and 2008-2009 season, whilst *Lr68* showed stronger effects than *Lr46* in the 2010-2011 season in the same studied environment. The two cropping seasons varied greatly in temperature as the 2010-2011 season proved to be significantly cooler than the previous seasons, suggesting that *Lr68* may express better at lower temperatures. Studies by Pretorius et al. (1984) and Kolmer (1996) also highlighted the effects of temperature on the expression of the adult plant resistance gene *Lr13*. In the former study, *Lr13* was expressed at 25°C in seedling plants exposed to three isolates of *P. recondita* from Mexico, China and Chile while the latter study showed that isolates from North America have high infection types to seedlings with *Lr13* regardless of temperature. Unavailability of funds to sustain research efforts put in to intensively study and understand both the host as well as the pathogen and its variability, has also created a huge bottleneck in breeding for leaf rust resistance in most southern African countries. Hence, an investment strategy that supports sustained research programmes geared towards identifying and addressing the ever evolving rust races is necessary.

1.6. The future of durable resistance breeding

Experience has taught that there is far more effort required than just relying on the phenotypic and genetic characterisation of individual resistance genes to achieve durable resistance. A more system-oriented approach is needed and this may include developing durable transgenic hosts and silencing of essential genes in the pathogen by expressing small interfering RNAs in the host (HIGS). Modest progress has been

made in engineering durable resistance to wheat rust, especially leaf rust which cause considerable worldwide damage to wheat production. Genes that are responsible for resistance to rust are continually being characterised and mapped in wheat and its relatives with only a few durable leaf rust resistance genes mapped. Adding to this, map based cloning in wheat has become easier with the availability of whole genome sequencing, however, limited work has been done in cloning multiple effective leaf rust resistance genes. Some of the cloned leaf rust resistance genes include *Lr1* (Cloutier et al., 2007)*, Lr10* (Feuillet et al., 2003)*, Lr21* (Huang et al., 2003) and *Lr34* (Lagudah et al., 2006). Cloning more effective resistance genes is needed for incorporation into resistance gene cassettes which could be successful in breeding for durable rust resistance. The advantage of cassettes is that the genes segregate as a unit. The genome editing technology can also prove useful, aiding in sequentially inserting multiple cassettes or incorporating multiple genes including non-host resistance genes at a single target site in the plant genome.

Prospects of host induced gene silencing

The recent discovery of RNA interference (RNAi) technology has provided new opportunities to explore engineering of resistance to some biotrophic pathogens in plants by inducing silencing of genes essential for pathogen virulence. RNAi is an intrinsic cellular mechanism shared by all multicellular eukaryotes with apparent roles in gene regulation and defence against viral infection (Baulcombe, 2004). The mechanism occurs post-transcriptionally and converts double stranded (ds) RNA into short RNA duplexes of 21 to 28 nucleotides in length, followed by the guided cleavage of complimentary messenger (m) RNA by the generated sequence-specific single-stranded RNAs termed short interfering (si) RNAs (Watson et al., 2005; Small, 2007). The RNAi pathway can also be activated by viral RNAs and is a major line of defence against RNA viruses. Virus-induced gene silencing (VIGS) uses viral vectors to produce dsRNA of a target gene fragment and triggers RNAi silencing. The mechanism has been exploited extensively and has become a powerful functional genomic tool to silence any gene of interest by introducing target gene sequences into cells or organisms. Recent studies have demonstrated that the expression of silencing constructs in plants designed on fungal genes can silence the expression of their

target respective genes in invading pathogenic fungi (Nunes and Dean, 2012). The barley stripe mosaic virus (BSMV) is popularly used as a vector in wheat to scrutinise candidate genes for their involvement in rust resistance. Yin et al. (2011) made use of BSMV to express dsRNA from *Puccinia* genes in plants to determine whether silencing can be delivered to the pathogen and suppress expression of the fungal genes. Results from this study clearly showed that some *P. striiformis* genes can be silenced through a host-induced RNAi system. This proves to be a viable technology to analyse gene functions in rust fungi and can be utilised for rust control in southern Africa.

1.7. Conclusions and future prospects

The variability and constant evolution of wheat leaf rust populations exhorts huge pressure on wheat breeders and researchers in general to be constantly vigilant against the emergence of new rust races. This requires timeous monitoring and collaborative surveillance of changes in the virulence patterns among rust pathogens in each country and across regions. Durable host plant resistance to leaf rust is one of the most important traits that breeding programmes should invest in, permitting a reduction in the use of fungicides as well as promoting greater stability of yields. The use of highly sophisticated and high-throughput tools such as field pathogenomics, transgenic solutions, genome editing and next generation sequencing to study both the host and the pathogen will assist in ultimately achieving broad spectrum and durable leaf rust resistance in wheat. This will subsequently result to a realisation of large proportions of returns on global economic investments in international wheat research. A multidisciplinary approach involving pathologists, breeders, geneticists, physiologists and agronomists at different stages of research and development is necessary to develop an improved cultivar with stable and durable leaf rust resistance through host plant resistance approach.

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Chapter 2

2. Review: Wheat Stem Rust in South Africa – Current Status and Future Research Directions

Abstract

Plant diseases are among the major causes of crop yield loss and food insecurity. In South Africa, stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici*. Eriks. & E. Henn. (*Pgt*) is an important disease of wheat. Records of stem rust occurrence in South Africa date back to the late 1720's, when it was first discovered in the south-western wheat growing areas of the Western Cape. Although growing stem rust resistant cultivars is one of the most economic and environmentally feasible strategies, one of the challenges in host-plant resistance is that *Pgt* frequently acquires new virulence to overcome resistance in existing cultivars. There is strong evidence that the pathogen continues to evolve through mutation and genetic recombination. The appearance of stem rust race Ug99 (TTKSK, North American race notation) in East Africa in 1999 and subsequent epidemics in Kenya and Ethiopia was accompanied by the occurrence of four Ug99 variants (TTKSF, TTKSP, PTKST and TTKSF+) in South Africa. These have resulted in a renewed interest in understanding the status of *Pgt* races and stem rust resistance in the world as well as the need for a new host-plant resistance strategy. The current review summarises up-to-date literature on the prevalence of stem rust races in South Africa, and also draws attention to the resistance genes and strategies currently deployed to combat this disease. The aim of the review is to provide perspectives on research milestones and guide future research programs for reducing losses incurred by the stem rust disease of wheat in South Africa.

Keywords: Durable resistance; Pathogen variability; *Puccinia graminis* f. sp*. tritici.*

2.1. Introduction

Wheat is one of the most important grain crops in South Africa with an estimated annual production of 1.7 to 2 million tons between 2013 and 2014 (South African Grain Information Service 2014 - http://www.sagis.org.za/Flatpages/Oesskattingdekbrief.htm). Compared to the global wheat market, South Africa is a minor player producing less than 0.4% of the world total. South Africa is a net importer of wheat, since in a country where wheat prices are trading at import parity levels, it is more economical to import wheat products than to produce them locally (Louw et al., 2013). According to the Department of Agriculture, Forestry and Fisheries (DAFF) wheat market value chain profile (2011/2012), South Africa is the largest producer of wheat in the Southern African Development Community (SADC) region and the fourth largest producer on the African continent. Approximately 27% of the total cultivated area is under irrigation with the remaining being dry land cultivation (SAGIS 2014 - http://www.sagis.org.za/Flatpages/Oesskattingdekbrief.htm). Spring wheat cultivars are suited to the winter rainfall areas whilst the winter and intermediate types are suited to the summer rainfall areas of South Africa (Jordaan, 2002). The wheat growing areas are also differentially suitable for the development of stem, stripe and leaf rust diseases of wheat caused by *Puccinia graminis* Pers. f. sp. *tritici* (*Pgt*), *P. striiformis f*. sp. *tritici* (*Pst*) and *P. triticina* Eriks (*Pt*), respectively (Pretorius et al., 2007).

This review focuses specifically on stem rust as one of the diseases that continue to incite renewed interest from plant breeders, geneticists and pathologists as a result of pathogen variability, manifested by the frequent appearance of new virulent races. The review is more focused on Africa with special emphasis on South Africa with the aim of tracking the evolution and spread of the Ug99 races in the country, and also on providing a general description of the pathogen, the damage it causes and mitigations strategies.

2.2. Stem rust pathogen: morphology, reproduction and mode of dispersal

Puccinia graminis has an intricate life cycle that includes both sexual and asexual reproduction cycles. The fungus is heteroecious with uredinial (asexual) and telial stages occurring on wheat and other species of *Poaceae*, while its pycnial (sexual)

and aecial stages occur on alternative hosts including *Berberis* spp. and *Mahonia* spp. (Campbell and Long, 2001). *Berberis* plants have not yet been found in South Africa, it is thus believed that sexual reproduction does not occur in the country. The sexual stage on wheat host begins with the production of urediniospores (Roelfs, 1985). Uredinia are produced after a successful infection of a grass host by *P. graminis.* These occur most frequently on the leaf sheaths of a wheat plant, but can also occur on true stem tissues, leaves, glumes and awns (Singh et al., 2011). The urediniospores produced during the crop season are oval and red-orange in colour varying from 15-20 µm by 40-60 µm in size and are easily shaken off a plant (Knott, 1989; Singh et al., 2006, 2011). Stem rust infection appears as elongated blister-like pustules with the uredinia being about 3 mm by 10 mm in size. As the infected plant develops, the uredia also age, converting from producing urediniospores to black teliospores, hence the disease is also known as black rust (Murray et al., 1998). The teliospores, unlike the urediniospores, are firmly attached to the plant tissue (Singh et al., 2011). The urediniospores are dispersed by wind and water and can spread over vast distances. In addition to natural dispersal mechanisms, accidental transfer by means of farm implements, contaminated clothing or goods may also contribute to the spread of spores (Singh et al., 2008). The speed of stem rust infection depends on the virulence of the rust races, susceptibility of cultivar as well as favourable environmental conditions such as temperature and humidity.

2.3. Effects of stem rust on grain yield and end-use quality of wheat

Wheat rusts are one of the major biotic stress factors leading to serious economic losses in South Africa and the world (Singh et al., 2006; Pretorius et al., 2007; Sharma et al., 2013). *Pgt* is highly aggressive making it the centre of attention of wheat breeders, geneticists, pathologists and farmers. An apparently healthy looking crop, three weeks prior to harvesting can be severely devastated and destroyed by the explosive build-up of stem rust if sufficient inoculum arrives from a heavily infected wheat crop from a nearby field (Leonard and Szabo, 2005; Singh et al., 2006). When wheat stems are severely infected, nutrient flow to the developing heads is interrupted, resulting in shrivelled grain. In addition, stems weakened by rust infection are prone to lodging, causing yield loss (Knott, 1989). Studies show an average grain yield loss of 35% around the world for a range of different cultivars owing to stem rust disease

(Pretorius, 1983). This loss can increase to a total loss depending on the growth stage of the host when infection starts (Roelfs, 1985; Leonard and Szabo, 2005).

2.4. Stem rust in South Africa

Effective control of stem rust requires a co-ordinated effort, including race monitoring, collection and characterisation of sources of resistance, and resistance breeding (Boshoff et al*.,* 2000). The pathotyping of stem rust in South Africa was initiated in the 1920s. This resulted in standard races 34 and 21 being the first to be identified in 1922 and 1929, respectively, and have been the most prevalent races (Pretorius et al*.,* 2007). However, from 1960 to 1980 there was renewed interest in *Pgt*, leading to more regular surveys, the establishment of an improved differential set to characterize races, and a structured approach towards identifying effective sources of resistance (Pretorius et al*.,* 2007). In 1980 the Agricultural Research Council-Small Grain Institute (ARC-SGI) in Bethlehem, South Africa, initiated annual rust surveys which coincided with the mandatory inclusion of stem rust resistance genes in all newly released cultivars (Pretorius et al*.,* 2007). The surveys were conducted to assist wheat breeding programmes with information on effective resistance genes.

The annual stem rust surveys were extended in 2013 to include the genetic characterization of all field isolates using simple sequence repeat markers (SSRs). However, due to the very high genetic similarity between the members of the Ug99 lineage, SSR analysis cannot distinguish between them. For that reason, Dr LJ Szabo at the USDA-ARS, St Paul, Minnesota, USA developed single nucleotide polymorphism (SNP) markers based on TaqMan technology (L J Szabo, unpublished results). A combined SNP and SSR analysis are thus currently employed to identify all the field isolates from the surveys. Preliminary results indicated that combined with the phenotypical data, a very accurate assessment of the diversity of the local *Pgt* population can be made.

In South Africa, the first documented epidemic of wheat stem rust occurred in the south-western wheat growing areas of the Western Cape province in 1726 (Pretorius

et al., 2007). Owing to expanding wheat production in the country, epidemics became a recurring phenomenon and turned out to be particularly devastating in the winter-rainfall production regions of the Western and Eastern Cape, as well as in the summer-rainfall regions of the Free State. The last epidemic occurred in 1985 on *Sr24*-derived wheat cultivars in the Albertinia area of the Western Cape (Boshoff et al., 2000). Although the *Sr24* stem rust resistance gene was effective against most races of *Pgt* and was used in commercial wheat cultivars worldwide (Jin et al., 2008), detection of the races 2SA100 and 2SA101 (Agricultural Research Council of South Africa race notation) that were virulent to *Sr24* substantially increased the vulnerability of South African wheat cultivars (Le Roux and Rijkenberg, 1987). Over 30 *Pgt* races of wheat and triticale have been characterised in South Africa since the early 1980s (Table 2.1). *Pgt* continues to acquire virulence against resistance genes through genetic recombination, mutation and new introductions from other countries. Growing a single cultivar over a large area also contributes to the development of new virulent races. For example, SST44 carrying *Sr24* was widely used in the 1980s and the prevalence of race 2SA100, which is virulent to *Sr24*, increased dramatically (Le Roux, 1985). The timely detection of stem rust races with new virulence is hence important to resistance breeding as information on the virulence profiles of new races would enable wheat geneticists and breeders to utilise effective resistance genes in their breeding programs.

Table 2.1**.** Avirulence/virulence patterns of the stem rust races identified in South Africa

Races	Avirulence/virulence genes
2SA2	*Sr5, Sr6, Sr9b, Sr9e, Sr17, Sr24, Sr30, Sr36/ Sr7b, Sr9g*
2SA4	*Sr8b, Sr9g, Sr21, Sr24, Sr27, Sr31, Sr36/Sr5, Sr6, Sr7b, Sr8a, Sr9b, Sr9e, Sr11, Sr17, Sr23, Sr30, Sr37, Sr44, SrGt*
2SA6	*Sr9e, Sr24, Sr36/Sr5, Sr6, Sr7b, Sr9b, Sr9g, Sr17, Sr30*
2SA10	*Sr6, Sr7b, Sr9b, Sr9e, Sr17, Sr24, Sr30, Sr36/Sr5, Sr9g*
2SA18	*Sr7b, Sr9e, Sr24, Sr30, Sr36/Sr5, Sr6, Sr9g, Sr17*
2SA20	*Sr9e, Sr24, Sr30/Sr5, Sr6, Sr7b, Sr9b, Sr9g, Sr17, Sr36*
2SA32	*Sr5, Sr6, Sr7b, Sr9b, Sr9g, Sr17, Sr24, Sr30*
2SA33	*Sr7b, Sr9e, Sr9g, Sr17, Sr24, Sr30/Sr5, Sr6, Sr9b, Sr36*
2SA36	*Sr9e, Sr9g, Sr23, Sr24, Sr27, Sr30, Sr37, Sr44, SrGt/Sr5, Sr6, Sr9b, Sr11, Sr17, Sr36*
2SA39	*Sr5, Sr9b, Sr9e, Sr17, Sr24, Sr30/Sr6, Sr7b, Sr9g*
2SA43	*Sr24, Sr36/Sr5, Sr6, Sr7b, Sr9b, Sr9e, Sr9g, Sr17, Sr30*
2SA45	*Sr9e, Sr9g, Sr24, Sr36/Sr5, Sr6, Sr7b, Sr9b, Sr17, Sr30*
2SA48	*Sr9e, Sr9g, Sr24, Sr30/Sr5, Sr6, Sr7b, Sr9b, Sr17, Sr36*
2SA49	*Sr9e, Sr9g, Sr24/Sr5, Sr6, Sr7b, Sr9b, Sr17, Sr30, Sr36*
2SA51	*Sr9b, Sr9e, Sr9g, Sr17, Sr24, Sr30, Sr36/Sr5, Sr6, Sr7b*
2SA52	*Sr9e, Sr24, Sr30, Sr36/Sr5, Sr6, Sr7b, Sr9b, Sr9g, Sr17*
2SA53	*Sr24/Sr5, Sr6, Sr7b, Sr9e, Sr9g, Sr17, Sr30, Sr36*
2SA54	*Sr7b, Sr9e, Sr9g, Sr24, Sr36/Sr5, Sr6, Sr9b, Sr17, Sr30*
2SA55	*Sr5, Sr6, Sr7b, Sr8b, Sr9b, Sr9e, Sr9g, Sr17, Sr21, Sr24, Sr27, Sr30, Sr31, Sr36, Sr38, SrEm, SrKw, SrSatu, SrTmp/Sr8a, Sr9a, Sr9d, Sr10, Sr11, Sr44, SrMcN*
2SA88	*Sr13, Sr15, Sr21, Sr22, Sr24, Sr25, Sr26, Sr27, Sr29, Sr31, Sr32, Sr33, Sr35, Sr36, Sr39, Sr43, SrAgi, SrEm, SrKw, SrSatu/Sr5, Sr6, Sr7a, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9f, Sr9g, Sr10, Sr11, Sr12, Sr14, Sr16, Sr17, Sr19, Sr20, Sr23, Sr30, Sr34, Sr38, SrLc*
2SA88+	*Sr24, Sr27, Sr31, SrKw, SrSatu, SrTmp/Sr6, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr30, Sr88, Web, SrMcN*
2SA99	*Sr5, Sr6, Sr9b, Sr9e, Sr21, Sr24, Sr27, Sr31, Sr36, Sr38, SrKw, SrSatu/Sr7b, Sr8a, Sr9g, Sr11, Sr17, Sr30*
2SA100	*Sr8b, Sr9e, Sr9g, Sr21, Sr27, Sr30, Sr36, Sr37, Sr44, SrGt/Sr5, Sr6, Sr7b, Sr8a, Sr9b, Sr11, Sr17, Sr23, Sr24*
2SA101	*Sr9e, Sr30, Sr36/Sr5 Sr6, Sr7, Sr9b, Sr9g, Sr17, Sr24*

Table 2.1. Continued

Races	Avirulence/virulence genes
2SA102	*Sr5, Sr6, Sr7b, Sr8b, Sr9b, Sr9e, Sr11, Sr17, Sr21, Sr23, Sr24, Sr30, Sr31, Sr36, Sr37, SrGt/Sr8a, Sr9g, Sr27, Sr30, Sr44*
2SA103	*Sr5, Sr6, Sr9b, Sr9e, Sr9g, Sr11, Sr17, Sr23, Sr24, Sr36, Sr37, SrGt/Sr27, Sr30, Sr44*
2SA104	*Sr5, Sr6, Sr7b, Sr8b, Sr9b, Sr9e, Sr17, Sr21, Sr24, Sr30, Sr31, Sr36, Sr38, SrEm, SrSatu, SrTmp/Sr8a, Sr9a, Sr9d, Sr9g, Sr10, Sr11, Sr27, Sr44, SrKw, SrMcN*
2SA105	*Sr5, Sr6, Sr7b, Sr8b, Sr9b, Sr9e, Sr17, Sr21, Sr24, Sr30, Sr31, Sr36, Sr38, SrEm, SrTmp/Sr8a, Sr9a, Sr9d, Sr9g, Sr10, Sr11, Sr27, Sr44, SrKw, SrSatu, SrMcN*
2SA106	*Sr21, Sr27, Sr31, Sr36, Sr44, SrEm, SrKw, SrSatu, SrTmp/Sr5, Sr6, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr24, Sr30, Sr38, SrMcN*
2SA107	*Sr13, Sr14, Sr21, Sr22, Sr25, Sr26, Sr27, Sr29, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr42, Sr43, SrSt 44, SrEm, SrTmp, SrSatu/SrSr5, Sr6, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr16, Sr17, Sr24, Sr30, Sr31, Sr34, Sr38, Sr41, SrMcN*

The *Sr31* resistance gene that was transferred from rye to wheat was once one of the most effective and durable resistance genes. Durable resistance is a mechanism conferring effective resistance to a cultivar for long period of time during its widespread cultivation in a favourable environment for a disease (Johnson, 1984). Nevertheless, the first *Pgt* race with virulence to *Sr31* was reported in 1999 in Uganda, East Africa (Pretorius et al*.,* 2000). The race was later designated as TTKS by Wanyera et al. (2006) using the North American nomenclature system. When a fifth set of differential lines was added, this race was re-named TTKSK (Jin et al*.,* 2008, 2009). During 2003 and 2004, the majority of Kenyan cultivars and a large portion of CIMMYT wheat germplasm with gene *Sr31* planted in Kenya were susceptible to stem rust disease (Wanyera et al., 2006). Since the discovery of TTKSK, seven other variants of the Ug99 lineage have been detected in different parts of Africa (Singh et al., 2011). The evolution and occurrence of Ug99 variants have further broken down most of widely deployed stem rust resistance genes existing in commercial cultivars (Haile and Röder, 2013). The introduction of new cultivars could also contribute to the evolution of new stem rust races.

In total, four of the eight Ug99 race group members (TTKSF, TTKSP, PTKST and TTKSF+) were found in South Africa, as well as in several other southern African countries (Mukoyi et al*.,* 2011). The first South African member of the Ug99 lineage was TTKSF (2SA88). A genetic study indicated strong genetic resemblance to TTKSK (Visser et al*.,* 2009), except for avirulence towards *Sr31* (Pretorius et al*.,* 2007). This proved that TTKSF did not develop from the South African race 3SA43 as was previously suggested (Pretorius et al*.,* 2007). This was the first genetic study confirming that the South African stem rust population changes due to foreign races being introduced into the country.

The second South African Ug99 group member TTKSP (2SA106) with virulence to *Sr24* was detected in 2007 in the Western Cape, South Africa (Terefe et al*.,* 2010). TTKSP is phenotypically similar to PTKST except for avirulence to *Sr31* and similar to TTKSF except for virulence to *Sr24* (Visser et al*.,* 2011). The *Sr24* gene is one of the genes that were initially identified as conferring resistance to Ug99 (Jin et al*.,* 2008).

Virulence of TTKSP to *Sr24* was of particular concern in South Africa as more than 20% of the commercial cultivars and elite germplasm had *Sr24* as a major resistance gene (Le Roux and Rijkenberg, 1987).

In November 2009, the third Ug99 race PTKST (2SA107) was detected at two locations (Cedara and Greytown) in Kwa-Zulu Natal Province. PTKST is virulent to both *Sr24* and *Sr31* (Pretorius et al*.,* 2010), but unlike TTKSK, it is avirulent to *Sr21* (Terefe et al*.,* 2010). Races with similar virulence phenotypes as 2SA107 were previously known in Kenya and Ethiopia which suggest that PTKST, just like TTKSF might have entered South Africa through an introduction from one of the East African countries (Terefe et al*.,* 2010). The final South African member of the Ug99 lineage is TTKSF+ (2SA88+) that was identified in December 2010 at Afrikaskop in the eastern Free State (Pretorius et al., 2012). It is thought to represent a local adaptation from TTKSF with virulence on cv. Matlabas.

The similarity of *Pgt* races in Zimbabwe, South Africa, and Mozambique thus supports cross boundary introductions of races (Mukoyi et al., 2011). As such, all stem rust introductions and new races that evolved locally could have a substantial impact on resistance breeding strategies in all neighbouring countries.

2.5. Genes deployed for stem rust resistance in South Africa

The rapid adaptation and distribution of Ug99 and its variants incited a renewed interest leading to a robust search and deployment of new resistance genes effective against stem rust races. The gene deployment strategy is important and should be considered in all breeding programs around the world. This strategy has mostly been advocated in recent years by the Borlaug Global Rust Initiative (http://www.globalrust.org/), a group of rust pathologists, breeders, geneticists and policy makers. For gene deployment, there are more than 57 different stem rust resistance genes catalogued and identified to date (McIntosh et al*.,* 2011; Haile and Röder, 2013). Most of these resistance genes and gene complexes were sourced from relatives of wheat (McIntosh et al*.,* 2003; Jin et al*.,* 2007; Liu et al., 2014) and near-isogenic lines carrying individual

Sr genes are available in several wheat backgrounds. These resistance genes are spread throughout the three wheat genomes (A, B and D). Multiple resistance alleles are known to exist at the *Sr7*, *Sr8*, and *Sr9* loci. Of these multi resistance loci, the *Sr9* locus carries a large number of alleles (McIntosh et al., 2003; Tsilo et al., 2007; Rouse et al., 2014) and more alleles or closely linked stem rust genes with resistance to Ug99 races have also been reported at this locus (Hiebert et al., 2010; Zurn et al., 2014). Recently, a wheat stem rust resistance gene *SrWeb* subsequently designated as *Sr9h*, an allele at the *Sr9* locus has been identified from cultivar Webster, also present in Ug99 resistant cultivar Gabo 56 (Rouse et al., 2014).

In general, host-plant resistance against *Pgt* is characterised in two broad categories: resistance genes effective at seedling (major genes) and adult plant stage (minor genes) respectively. Apart from the *Sr2*, *Sr55*, *Sr56, Sr57* and *Sr58* genes, about 53 of the designated *Sr* genes are single-locus major genes conferring resistance at all stages of plant development. In many cases this resistance is short lived due to the emergence of new virulent races (Singh et al*.,* 2008; Jin et al*.,* 2009; Njau et al*.,* 2010). In South Africa, most wheat cultivars have not been intensively characterised for stem rust resistance. Wheat cultivars released between the late 90's to early 2000, however, had major genes for resistance to locally prevalent *Pgt* races which became defeated with time as more virulent races were reported (Pretorius et al*.,* 2007). The only race specific resistance genes that are known to be deployed include *Sr9e*, *Sr24*, *Sr27, Sr36* and *Sr39* found in different cultivars (Le Roux and Rijkenberg, 1987; Smith and Le Roux, 1992). Amongst those still effective is *Sr39* which was transferred from the wild relative *Aegilops speltoides* L. (Kerber and Dyck, 1990). Nevertheless, this gene has not been used extensively in wheat breeding worldwide because of the negative effects associated with *Ae. speltoides* chromatin; however, Niu et al*.* (2011) and Mago et al*.* (2009) have developed a set of recombinants with reduced *Ae. speltoides* fragments addressing the problem of linkage drag associated with the *Sr39* gene and the seed source is made available. Rouse et al. (2014) demonstrated that a race specific gene *Sr9h* in cultivar Gabo 56 confers resistance to *Pgt* race TTKSK. A robust allelism test determined that the *Sr9h* resistance gene is a new allele of *Sr9*, adding to the six alleles already present at this locus.

The adult plant resistance (APR) gene that has been deployed in South Africa is *Sr2* (Pretorius and Brown, 1999), a recessive gene that provides a slow rusting response at adult plant stage and has been used worldwide (Singh et al*.,* 2006). *Sr2* was originally selected by McFadden in the 1920s from Yaraslav emmer wheat (Dubin and Brennan, 2009) and was mapped on chromosome 3BS of wheat close to the *csSr2* marker (Mago et al., 2011). Resistance given by this gene is unique in that it is pleiotropic, conferring resistance to other diseases including leaf rust, stripe rust and powdery mildew (McIntosh et al*.,* 1995). One of the first cultivars confirmed to carry *Sr2* called Palmiet, was released in 1985 in South Africa. It contributed significantly to the reduction of stem rust levels until its withdrawal from production in 1999 (Pretorius et al*.,* 2012).

It was recently shown that approximately 50% of the historic South African wheat cultivars carried the *Sr2* gene (P. Ntshakaza, unpublished data). The frequency of *Sr2* in recent commercial cultivars, however, appears to be low based on the use of the *Sr2* marker *csSr2* with only three cultivars PAN 3377, Inia and Steenbras having the gene (Pretorius et al*.,* 2012). The loss of *Sr2* in commercial cultivars could be attributed to the fact that breeding for durable stem rust resistance may have not been the main focus of breeders (J. Smith and S.C. Smith, unpublished data). Hence, many commercial cultivars are susceptible to *Pgt* races 2SA102 and 2SA103. Pretorius et al*.* (2012) also showed that very few recent cultivars have resistance to all South African *Pgt* races as 88% of entries tested susceptible at seedling stage to at least one of the tested races. The status of stem rust resistance in commercial cultivars will probably change in the near future, as current and future breeding efforts aim to re-introduce *Sr2* in combination with other effective stem rust resistance genes back into commercial cultivars (Table 2.2). Nevertheless, the *Sr2* gene does not provide sufficient level of protection when deployed singly; it interacts with other genes that express intermediate levels of resistance to condition acceptable levels (Bansal et al*.,* 2014). Identification of other APR genes such as *Sr55* (*Lr67/Yr46/Pm46*) (McIntosh et al., 2012), *Sr56* (Bansal et al*.,* 2014), *Sr57* (*Lr34/Yr18/Pm38*) (Lagudah et al*.,* 2006) and *Sr58* (*Lr46/Yr29/Pm39*) (McIntosh et al., 2012) is well appreciated in breeding for durable stem rust resistance in wheat, nonetheless efforts to characterise more of these APR genes should be considered.

Table 2.2. Effective and ineffective stem rust resistance genes against common races in South Africa as detected in the fields during evaluations.

Sr genes	
Effective	Ineffective
Sr2, Sr13, Sr14, Sr22, Sr25, Sr26, Sr29, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr42, Sr43	*Sr5, Sr6, Sr7a, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9f, Sr9g, Sr10, Sr11, Sr12, Sr15, Sr16, Sr17, Sr18, Sr19, Sr20, Sr21, Sr23, Sr24, Sr27, Sr30, Sr31, Sr34, Sr38, Sr41, Srwld-1*

2.6. Development of stem rust resistant germplasm in South Africa: current and future directions

Despite the occurrence of stem rust in South Africa, information on the genetic basis of stem rust resistance in leading South African wheat cultivars and breeding lines is limited. Understanding the status of stem rust in South Africa and neighbouring countries (Lesotho, Zimbabwe and Botswana) should provide useful and detailed information on which genes were already used, so that new sources of resistance could be introduced and deployed strategically. More importantly, breeders should have this information to be able to target specific genes as they routinely re-cycle germplasm and breeding lines in their breeding programs.

As a result of renewed interest on host resistance, different research groups in South Africa comprised of farmers, plant pathologists, geneticists and breeders together with international collaborators are exploring ways to combat stem rust disease by searching for new sources of resistance, consequently aiming at producing germplasm with durable resistance. As confirmed in the past, the conventional methods of phenotyping wheat to search and pyramid genes into a single genotype, have proven to be difficult and time consuming; hence, it is usually not feasible for a regular breeding program to apply all necessary rust races in selecting lines with multiple rust resistance genes. Even so, multi-race tests are commonly performed in the glasshouse on seedlings and important adult plant resistance may not be recognised. It is also possible that multiple-race selection does not identify which genes are effective; hence a more advanced technique is needed to identify and diagnose each effective resistance gene.

Marker-assisted selection (MAS) has been proven to be a powerful tool to facilitate the development of cultivars with multiple resistance genes or gene pyramids (Dubcovsky, 2004; Bernardo et al., 2013; Haile and Röder, 2013). Molecular markers linked to effective stem rust resistance genes can be used to predict the presence of specific genes with high accuracy without the need for disease evaluation, thus helping with the transfer of several genes into adapted material (Tsilo et al., 2008, 2009; Pumphrey, 2012; Bernardo et al*.,* 2013). Several laboratories, worldwide, have made concerted efforts to develop and identify molecular markers that are linked to effective race-specific and non-race specific rust resistance genes against *Pgt* and with reference to the Ug99 race group (Table 2.3). Nevertheless, some of the markers linked to effective resistance genes are not suitable for routine applications of MAS in breeding programs. Efforts of optimising DNA markers and developing the most diagnostic markers are on-going processes in many laboratories. With the availability of next-generation sequencing platforms, more diagnostic resistance gene markers will be made available for high-throughput screenings and application of MAS in breeding for stem rust resistance. Cloning of the identified resistance genes is extremely important, not only can it lead to developing excellent markers but can also assist in detecting important resistant gene families. To date, only a few stem rust resistance genes have been cloned, namely *Sr33* (Periyannan et al., 2013) and *Sr35* (Saintenac et al*.,* 2013), making it easier to apply resistance gene-derived markers. Moreover, fixing of these incorporated resistance genes into suitable genetic backgrounds and subsequent development of inbred lines can still take a considerable number of years before a new cultivar is ready for field trials. The doubled haploid (DH) technique can shorten the inbreeding process to a single cycle, without compromising the integrity and the agronomical quality of the lines (Wessels and Botes, 2014).

Table 2.3. Chromosomal location, description, linked DNA markers and references for wheat stem rust resistance genes effective against *Puccinia graminis* f. sp. *tritici* races with special reference to Ug99 resistance in Africa.

Sr gene	Chromosomal location	General description	Linked DNA markers[1]	Original source	References
		Major genes (race-specific resistance)			
SrWeb (*Sr9h*) *and SrWLR*	2BL	✓ Allelism tests demonstrated that *SrWeb* from cultivar Webster is *Sr9h* adding to six alleles already present at this locus. ✓ Effective against Ug99 race TTKSK.	*GWM47* *IWA6121* *XwPt-3132* *IWA6122* *IWA7620* *IWA8295* *IWA8362*	*Triticum aestivum*	(Hiebert et al., 2010; Zurn et al., 2014; Rouse et al., 2014)
Sr13	6AL	✓ Confers moderate resistance to TTKS. ✓ Frequent gene in durum varieties.	*BARC104* *WMC580* *DUPW167* *CK207347* *CD926040* *BE403950*	*Triticum turgidum*	(Simons et al., 2011)
Sr22	7AL	✓ Confers resistance to Ug99 and other important races. ✓ The region surrounding this gene was modified to minimize effects of yield penalty and delay in heading date.	*WMC633* *CFA2019* *BARC121* *IH81-BM* *IA81-AG* *cssu22*	*Triticum monococcum*	(Khan et al., 2005; Olson et al., 2010; Periyannan et al., 2011)
Sr24	1BS	✓ Confers resistance to TTKS but not its variants. ✓ Useful due to its linkage to *Lr24*.	*SCS73719* *Sr24#12*	*Agropyron elongatum*	(Mago et al., 2005; Olson et al., 2010)
Sr25	7DL, 7AL	✓ Confers a high level of resistance only in some genetic backgrounds. ✓ Sources with EMS-induced mutation on yellow pigment gene are available.	*BF145935*	*Thinopyrum ponticum*	(Knott 1980; Liu et al., 2010)
Sr26	6AL	✓ Confers resistance to Ug99 and other races. ✓ Sources with short chromatin fragment on chromosome 6AL are available. ✓ Multiplexing two primer sets to detect co-dominancy.	*Sr26#43* *BE51879*	*Thinopyrum ponticum*	(Mago et al., 2005; Dundas et al., 2007; Liu et al., 2010)

Table 2.3. Continued

Sr gene	Chromosomal location	General description	Linked DNA markers[1]	Original source	References
Sr27	3A	✓ Effective against Ug99. ✓ Has not been used in wheat improvement.	-	*Secale cereale*	(Singh et al., 2011)
Sr28	2BL	✓ Confers APR for most known races. ✓ Seedling stage resistance for races BCCBC, TTKSK and TTKST.	*WMC322* *WPT7004* *wpt7004*	*Triticum aestivum*	(Rouse et al., 2012)
Sr32	2A, 2B, 2D	✓ Effective against Ug99 lineage. ✓ Several recombinant translocation stocks with reduced *Aegilops speltoides* chromatin harbouring *Sr32* are available.	*csSr32#1* *cssu22* *csSr32#2*	*Aegilops speltoides*	(Mago et al., 2013)
Sr33	1DS	✓ Confers only moderate levels of resistance. ✓ Cloned gene	*BE405778* *BE499711*	*Aegilops tauschii*	(Sambasivam et al., 2008; Periyannan et al., 2013)
Sr35	3AL	✓ Effective against race TTKSK (Ug99) and its variants (TTKST and TTTSK. ✓ Cloned gene	*AK332451* *AK331487* *GWM271* *WMC559* *BARC51* *CFA2170* *CFA2076* *BE423242* *BF485004* *AK3358187* *BE405552*	*Triticum monococcum*	(Zhang et al., 2010; Saintenac et al., 2013)
Sr36	2BS	✓ Confers a high level of resistance to Ug99 and some of its variants.	*GWM319* *WMC477* *STM773-2*	*Triticum timopheevi*	(Bariana et al., 2001; Tsilo et al., 2008)
Sr37	4BL	✓ Effective gene against Ug99. ✓ Recombinants with smaller alien segments were produced using the *ph1b* system, however, inheritance of resistance in these recombinants has been unstable to date.	-	*Triticum timopheevii*	(McIntosh and Gyarfas, 1971)

Table 2.3. Continued

Sr gene	Chromosomal location	General description	Linked DNA markers[1]	Original source	References
Sr39	2B	✓ Moderately to highly resistant to Ug99 in seedling tests. ✓ Multiple stocks with reduced *Aegilops speltoides* chromatin segments harbouring *Sr39* on chromosome 2BS are available to prevent effects due to linkage drag.	*Sr39#22r* *Sr39#50s* *WMC471* *RWGS27* *RWGS28* *RWGS29*	*Aegilops speltoides*	(Mago et al., 2009; Niu et al., 2011)
Sr40	2BS	✓ Confers resistance to Ug99. ✓ Four recombinants with reduced *Triticum timopheevii* chromatin are available to prevent effects of linkage drag.	*Sr39#22r* *Xwmc344* *Xwmc474* *Xgwm374* *Xwmx474* *Xwmc661*	*Triticum timopheevii* ssp. *armeniacum*	(Wu et al., 2009)
Sr42	6DS	✓ Confers resistance to TTKSK and variants TTKST and TTKSK. ✓ Mapped to the same position on 6DS as *SrtMp* and *SrCad* indicating that they are the same genes.	*BARC183* *GPW5182* *FSD_RSA*	*Triticum aestivum*	(Ghazvini et al., 2012)
Sr43	7D	✓ Confers resistance to TTKSK, TTKST, TTTSK. ✓ Recently, two putative translocation lines with reduced alien chromatin have been developed.	*Xcfa2040*	*Aegilops elongatum*	(Niu et al., 2014)
Sr44	7DS	✓ Moderately to highly resistant to Ug99 in seedling tests.	*BF145935*	*Thinopyrum intermedium*	(Liu et al., 2010)
Sr45	1DS	✓ A locus more proximal to *Sr33*. ✓ Confers moderate levels of resistance.	*ccsu45*	*Aegilops tauschii*	(Periyannan et al., 2014)

Table 2.3. Continued

Sr gene	Chromosomal location	General description	Linked DNA markers[1]	Original source	References
		Adult Plant Resistance genes (race-non-specific resistance)			
Sr2	3BS	✓ Conferred durable resistance against all virulent races of *Pgt* worldwide for more than 50 years combined with other genes. ✓ Deployed in many wheat cultivars worldwide. ✓ Pseudo-black chaff (morphological marker).	*GWM533* *GWM389* *BARC133* *csSr2-SNP* *3B042G11* *3B028F08* *STM559TGAG*	*Triticum turgidum*	(McNeil et al., 2008; Mago et al., 2011)
Sr55 (*Lr67/Yr46/Pm46*)	4DL	✓ Confers adult plant resistance to stem rust and powldery mildew	*Xgwm165* *Xgwm192* *Xcfd71* *Xbarc98* *Xcfd23*	*Triticum aestivum*	(Herrera-Foessel et al., 2011)
Sr56	5BL	✓ Confers adult plant resistance to stem rust	*sun209* *sun320*	*Triticum aestivum*	(Bansal et al*.,* 2014)
Sr57 (*Lr34/Yr18/Pm38)*	7DS	✓ Confers adult plant resistance to stem rust ✓ Under appropriate conditions it may confer resistance in seedlings to certain rust races	*csLV34*	*Triticum aestivum*	Lagudah et al., 2006
Sr58 (*Lr46/Yr29/Pm39)*	1BL	✓ Confers adult plant resistance to stem rust	*Xwmc44* *Xgw259* *Xbarc80* *Xgwm259*	*Triticum aestivum*	(William et al., 2003)

[1] The most useful markers are underlined.

At the Agricultural Research Council–Small Grain Institute, MAS is routinely used to pyramid stem rust resistance genes into adapted germplasm in order to deploy a durable rust resistance strategy as proposed by the Borlaug Global Rust Initiative. The targeted genes include *Sr2* (3BS), *Sr25* (located on 7Ae#1 of *Thinopyrum ponticum* that was translocated onto 7DL chromosome), *Sr26* (located on 6Ae#1 of *T. ponticum* that was translocated onto 6AL chromosome) and *Sr39* (from chromosome 2BS of *Aegilops speltoides*). These genes are introgressed into the widely used South African bread wheat cultivars as a means to increase broad spectrum resistance. The genes used in this pyramiding scheme have been engineered to reduce the likelihood of linkage drag and originated from sources developed by the late Dr Douglas R. Knott from the University of Saskatchewan (sources of *Sr25* with EMS-induced mutation on yellow pigment gene) (Knott, 1980), Dr Ian Dundas from the University of Adelaide (reduced fragments of *Sr26*) (Dundas et al., 2004) and Dr Steven S. Xu from the United State Department of Agriculture–Agricultural Research Service (reduced fragment of *Sr39*) (Niu et al., 2011). Several of other diagnostic/ most useful markers (Table 2.3) are used to select resistance genes.

The Plant Breeding Laboratory of the University of Stellenbosch has also clearly demonstrated and reaffirmed the importance of a breeding scheme where conventional breeding strategies are integrated with MAS and DH techniques in order to speed up cultivar development. In a study by Wessels and Botes (2014) from the University of Stellenbosch, several rust resistance genes were successfully incorporated into a population of inbred lines within two seasons. The study has also shown the importance of the incorporation of locally available resistance genes into a genetic background that is suitable for South African agroclimatic conditions. Other pre-breeding and breeding efforts at the University of Free State and two South African private breeding companies are underway to increase durable stem rust resistance using a variety of sources and resistance genes. With advancements in technology, adoption of high throughput MAS by all breeding programs will make it easier to continuously integrate new sources of resistance into the existing gene pool, thereby sustaining the stem rust control strategy.

2.7. Conclusions and recommendations

Norman Borlaug, the Nobel laureate, who is widely considered as the father of wheat improvement in the 1960s, once noted that ‘rust never sleeps’. Although farmers controlled the stem rust fungus with chemicals, the approach is not a long term solution and can raise costs on economy and environment. Host plant resistance, and more importantly, the combination of several stem rust resistant genes remain the most feasible, economic and environmentally safe approach. For this reason, it is clear that South African breeders and pathologists need to continuously replace susceptible cultivars with ones with durable stem rust resistance by introgressing new resistance genes.

Race characterisation using both phenotypic and genotypic data has improved our understanding of the dispersal mechanisms and evolution of stem rust across regions. The deployment of resistance genes in South Africa has also progressed from one gene per cultivar to multiple genes to offer a broad spectrum and durable resistance to stem rust. In this way, the chances of a pathogen defeating virulence for multiple genes are very low. And this may also lead to lower levels of stem rust populations; hence, reducing the chances of further spread of races to other wheat production areas around the world that currently do not have the races present in South Africa and other parts of Africa.

Efforts for effective implementation of integrated disease management practices and gene stewardship in the SADC region, as well as international collaborative initiatives from farmers, breeders and scientists should be adopted to decrease the chance of new races evolving and/or getting introduced. Emphasis should be placed on the promotion of preventive approaches such as releasing cultivars with durable resistance or multiple resistance genes, rapid seed multiplication systems, intensive surveillance approaches, institutional coordination and contingency planning for effective emergency response capability. There is hence a need to rally for more research funds to be allocated for the sustained and improved productivity of wheat at local, regional and continental basis. This includes an intensive study and understanding of both the host and pathogen genetics and variability. A recent study

by Pardey et al. (2013) concludes that farmers should play a pro-active role in the research programs, and understand that maintaining yield and growth rates necessary to meet anticipated future demands will require a sustained effort to plant wheat varieties that are resistant to existing races of rust. Pardey et al. (2013) undoubtedly shows that this will necessitate an investment strategy that supports sustained research programs geared towards identifying and addressing evolving stem rust threats across the globe.

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Chapter 3

3. Adult Plant Resistance of Selected Kenyan Wheat Cultivars to Leaf Rust and Stem Rust Diseases

Abstract

Phenotypic and genotypic evaluation of wheat genetic resources and development of segregating populations are pre-requisites for identifying rust resistance genes. The objectives of this study were to assess adult plant resistance (APR) of selected wheat genotypes to leaf rust and stem rust and to develop segregating populations for resistance breeding. Eight selected Kenyan cultivars with known resistance to stem rust, together with local checks were evaluated for leaf rust and stem rust resistance at seedling stage and also across several environments. Selected diagnostic markers were used to determine the presence of known genes. All eight cultivars were crossed with local checks using a bi-parental mating design. Seedling tests revealed that parents exhibited differential infection types against wheat rust races. Cultivars Paka and Popo consistently showed resistant infection types at seedling stage, while Gem, Romany, Pasa, Fahari, Kudu, Ngiri and Kariega varied for resistant and susceptible infection types depending on the pathogen race used. The control cultivars Morocco and McNair consistently showed susceptible infection types as expected. In the field, all cultivars except for Morocco showed moderate to high levels of resistance, indicating the presence of resistance genes. Using diagnostic markers, presence of *Lr34* was confirmed in Gem, Fahari, Kudu, Ngiri and Kariega, while *Sr2* was present in Gem, Romany, Paka and Kudu. Seedling resistance gene, *Sr35*, was only detected in cultivar Popo. Overall, the study developed 909 $F_{6:8}$ recombinant inbred lines (RILs) as part of the nested mating design and are useful genetic resources for further studies and for mapping wheat rust resistance genes.

Keywords: Adult plant resistance; Leaf rust; Population development; Stem rust; Wheat

3.1. Introduction

A number of wheat diseases cause significant yield loss and increased cost of production. Stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*) and leaf rust by *P. triticina* Eriks. (*Pt*) are the most widely distributed and yield limiting diseases in South Africa (Pretorius et al., 2007). The recent detection of new virulent leaf rust races such as CCPS, MCDS and FBPT (Pretorius et al., 2015) and four highly virulent Ug99 variants including TTKSF, TTKSP, PTKST and TTKSF+ (http://rusttracker.cimmyt.org/?page id=22) present significant threat to wheat production in South Africa, necessitating integrated and collaborative management strategies of the diseases.

Host-plant resistance to wheat diseases is the most effective, environmentally and economically sustainable approach to reduce yield losses compared to the use of crop protection chemicals (Pretorius et al., 2007; McCallum et al., 2016). Breeding of wheat cultivars with durable resistance to rust diseases is the best control strategy (Kolmer, 1996; Singh et al., 2005). Therefore, identification of sources of resistance and candidate genes remains important for breeding purposes.

Disease resistance can be broadly categorised as race-specific or race non-specific. Race-specific (vertical resistance) is conditioned by major genes and can be detected at a seedling or adult plant stage. Race non-specific resistance (horizontal resistance) is controlled by minor genes and remains effective against all races of the pathogen and is expressed at an adult plant stage, as partial and slow rusting resistance (Parlevliet and van Ommeren, 1975; Johnson, 1984; Singh, 1992; Kolmer, 1996). Race-specific resistance has been widely employed by various wheat improvement programmes. However, this form of resistance is frequently defeated by the appearance of new and virulent races of the pathogen population through a single-step mutation and/or sexual recombination events (Kolmer, 2005; Terefe et al., 2014; Kolmer and Acevedo, 2016). Race non-specific resistance confers long-term and durable resistance (Singh et al., 2000, 2005). However, progress on breeding for durable resistance is slow, requiring identification and pyramiding of novel, adult plant resistance (APR) genes into a desirable genotype. So far, few APR genes have been characterised and catalogued in wheat (Krattinger et al., 2009; Herrera-Foessel et al., 2011, 2012; Bansal et al., 2014). Screening for APR genes requires proper

phenotyping to correctly detect their expression in the field. Often genotypes with APR may show susceptibility at the seedling stage, but adult plants show low disease severity. Efficient characterisation of APR genes requires development of segregating populations that can be used to dissect and identify genomic regions controlling such resistance. There is evidence that most APR genes are effective across a number of pathogens, e.g. *Sr2/Yr30/Lr27/Pbc1*, *Sr55/Lr67/Yr46/Pm46/Ltn3, Sr57/Lr34/Yr18/Pm38/Ltn1* and *Sr58/Lr46/Yr29/Pm39/Ltn2* (William et al., 2006; Lillemo et al., 2008; Krattinger et al., 2009; Mago et al., 2011a; Herrera-Foessel et al., 2014; Kolmer et al., 2015). Several Kenyan cultivars have been shown to be good sources of APR genes controlling stem rust (Knott, 1968; Njau et al., 2009, 2010; Onguso and Njau, 2015; Bajgain et al., 2016).

The objectives of this study were to assess leaf rust and stem rust resistance of selected Kenyan cultivars, evaluating the presence of APR genes, and to develop a nested bi-parental mapping population that could be used for genetic studies and for validation of APR genes.

3.2. Materials and Methods

Plant material and seedling screening

Eight Kenyan bread wheat cultivars (Gem, Romany, Paka, Fahari, Kudu, Pasa, Ngiri and Popo) with known stem rust resistance (Njau et al., 2009; Bajgain et al., 2016), together with four local checks (Kariega, Morocco, McNair and SST88) were used for the study. Seeds of the cultivars were obtained from the National Small Grain Germplasm Collection of the Agricultural Research Council-Small Grain Institute (ARC-SGI) in Bethlehem. The genotypes were screened in a glasshouse for seedling resistance to leaf rust and stem rust at ARC-SGI. Cultivars together with rust susceptible checks SST88 and McNair were sown in sterilised soil in 10 cm diameter plastic pots under a temperature-controlled seedling room at 20 to 25ºC. Five seeds from each of the cultivars were sown, with three replicates per genotype. After emergence, plants were fertilised twice with 10 g l^{-1} Multifeed water soluble fertiliser (19:8:16 NPK plus micronutrients).

The genotypes were tested using six South African stem rust races (TTKSF+: 2SA88+, TTKSF: 2SA88, BPGSC+*Sr27*: 2SA102, BPGSC+*Sr27*+*Kiwiet*+*Satu*: 2SA105, TTKSP: 2SA106 and PTKST: 2SA107) and four leaf rust races (PDRS: 3SA133, SDDN: 3SA144, CCPS: 3SA145 and MCDS: 3SA146). Some of the races used in the study are most prevalent in the South African Development Community (SADC) region. The urediniospores of the leaf rust and stem rust races were obtained from regularly maintained stocks stored in a -80°C freezer at ARC-SGI. Plants were infected using the established inoculation protocol (Pretorius et al., 2000). Infection types were scored 14 days post inoculation using a scale of 0 - 4 according to Stakman et al. (1962) for stem rust and Long and Kolmer (1989); Pretorius et al. (2000) for leaf rust; where a score of 0 represents immune with no sign of infection, 0; nearly immune with a few faint flecks, ; very resistant with no uredinia but hypersensitive necrotic or chlorotic flecks are present, 1; resistant with small uredinia often surrounded by necrosis, 2; moderately resistant with small to medium uredinia often surrounded by necrosis, 3; moderately susceptible with medium sized uredinia without chlorosis or necrosis and 4 represents very susceptible with large uredinia without chlorosis or necrosis.

Field screening for adult plant resistance

The genotypes were evaluated under field conditions for resistance to leaf rust and stem rust. Evaluations were conducted across two wheat-growing locations in South Africa, (Cedara situated in KwaZulu-Natal Province in 2014 and 2015) and (Tygerhoek, in Western Cape Province in 2015). The locations differed in soil conditions, temperature and moisture and are hot spot areas for wheat rust pathogens and are annually surveyed for rust races. Hence, the locations were selected based on the known prevalence of the two diseases in the past. The cultivars were replicated five times in each environment. Approximately 20 seeds from each cultivar were sown in hill plots. Rust susceptible cultivars Morocco, SST88 and McNair were planted as spreader rows around the experimental area to ensure disease initiation and spread. All cultural practices such as fertilisation, irrigation and other management practices were followed according to the recommendation of the specific areas.

Field infection response of the cultivars were visually assessed. Disease severity was recorded using a modified Cobb scale of 0 to 10 (Peterson et al., 1948; Tsilo et al.,

2014); where scores of 0 to 2 represent highly resistant, 3 resistant, 4 to 5 moderately resistant, 6 to 7 moderately susceptible, 8 susceptible and 9 to 10 highly susceptible. The disease severity of leaf rust and stem rust was recorded only once in each season on adult plants when the disease symptoms were fully developed on the susceptible checks Morocco, SST88 and McNair displaying 80% or higher disease severity. The cultivars were screened in the field mainly to identify cultivars with adult plant resistance (APR).

3.3. Data Analysis

Disease severity score for each cultivar from all the environments were collected to determine the overall response to leaf rust and stem rust. Analysis of variance using the severity scores of test genotypes was performed using the general linear model procedure (PROC GLM) in SAS software (version 9.3, SAS Institute Inc, Cary NC, USA).

Homogenisation of leaf samples and DNA extraction

Three to four leaves from each of the test cultivars were harvested from ten-day old seedling plants for DNA sampling. Leaves were placed in 2 ml Eppendorf tubes and stored in a -80ºC freezer prior to DNA extraction. To homogenise the samples during DNA extraction, two round stainless-steel ball bearings (5 mm in diameter) were added to the frozen leaf material in 2 ml Eppendorf tubes. The frozen leaf material was homogenised using Qiagen's Tissue Lyser for 1 min at 30 r/s. Genomic DNA was isolated using the modified CTAB method (Porebski et al., 1997). The quality, purity and quantity of the extracted DNA was tested using the Nano-Drop 1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA).

Genotyping

DNA samples from all cultivars were screened using diagnostic markers based on protocols for marker-assisted selection (http://maswheat.ucdavis.edu/). Table 3.1 shows information on the markers used in this study and related references. The *Sr31* marker was included in the screening, though no longer effective, mainly to test if the cultivars carried the gene. PCR products were resolved on 1% (w/v) agarose- tris-borate-

ethylenediaminetetraacetic acid (TBE) gel stained with a fluorescent DNA stain GelGreen™.

Table 3.1. Description of DNA markers used in this study

Gene	Primer name*	Sequence 5' to 3'	Number of nucleotides	Type of marker	Amplification product sizes (bp)	Reference
Sr2	*csSr2-F*	CAAGGGTTGCTAGGATTGGAAAAC	24	CAPS	172	(Mago et al., 2011b)
	csSr2-R	AGATAACTCTTATGATCTTACATTTTTCTG	30			
Sr22	*WMC633-F*	ACACCAGCGGGGATATTTGTTAC	23	SSR	117	(Periyannan et al., 2011)
	WMC633-R	GTGCACAAGACATGAGGTGGATT	23			
Sr35	*CFA2170-F*	TGGCAAGTAACATGAACGGA	20	SSR	~180/190	(Babiker et al., 2009)
	CFA2170-R	ATGTCATTCATGTTGCCCCT	20			
Lr9	*J13-F*	CCACACTACCCCAAAGAGACG	21	STS	1110	(Schachermayr et al., 1994)
	J13-R	TCCTTTTATTCCGCACGCCGG	21			
Lr19	*Gb-F*	CATCCTTGGGGACCTC	16	STS	130	(Prins et al., 2001)
	Gb-R	CCAGCTCGCATACATCCA	18			
Lr34	*csLV34F*	GTTGGTTAAGACTGGTGATGG	21	STS	150	(Lagudah et al., 2009)
	csLV34R	TGCTTGCTATTGCTGAATAGT	21			
	L34PLUSR	GCCATTTAACATAATCATGATGGA	24	STS	517	
	L34DINT9F	TTGATGAAACCAGTTTTTTTTCTA	24	STS		
	L34MINUSR	TATGCCATTTAACATAATCATGAA	24	STS	523	

*Information obtained from MAS Wheat (http://maswheat.ucdavis.edu/)

Development of a nested bi-parental population

A nested bi-parental population was developed involving crosses of eight Kenyan cultivars (Gem, Romany, Paka, Fahari, Kudu, Pasa, Ngiri, Popo) with two of the four local checks (Kariega and Morocco) (Figure 3.1). The cultivars used for the crosses

were chosen because of their known resistance to wheat diseases, acceptable end-use quality and yield. Kenyan cultivars also carry many *Sr* genes (McIntosh et al., 1995; Njau et al., 2009, 2010; Bajgain et al., 2016), hence the strategic inclusion of these cultivars in the study. Table 3.2 shows the characteristics of each cultivar used to develop the nested bi-parental population.

Seeds were planted in sterilised soil in 40 cm diameter plastic pots placed in a temperature-controlled greenhouse at 18-25ºC and natural photoperiods (14 - 16 hrs). Pollen from each of the eight Kenyan cultivars was used to pollinate the emasculated Kariega and Morocco heads (the eight Kenyan cultivars were used as males, and the two checks as females). All F_1 seed from each successful combination/population were planted in pots in the glasshouse, producing the F_2 seed. Approximately 300-400 F_2 seeds from each population was planted in trays in the greenhouse and upon harvest, a single seed was randomly selected from each F_2 plant and used to produce F_3 generation. A single seed descent selection protocol was followed for three additional generations, producing F_6 seeds. After the last self-pollination cycle, every line was bulk harvested twice in the field (Bethlehem and Cedara) for multiplication purposes to produce $F_{6:8}$ seeds.

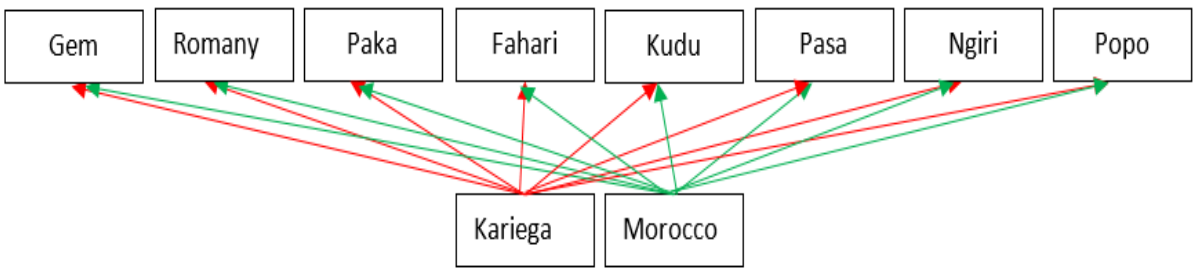

Figure 3.1. Scheme showing crosses of 10 wheat cultivars to develop bi-parental populations

Table 3.2. Origin, pedigree, agronomic characteristics and reported stem rust reaction (Ug99) of cultivars in the study

	Parent	Origin	Pedigree	Characteristic	FR-(stem rust)*
Male	Gem	Kenya (1964)	BT908/FRONTANA//CAJEME 54	-	1.0
	Romany	Kenya (1966)	COLOTANA 261-51/YAKTANA 54A	Hard, Red grain, Spring wheat with genes: *Lr23, Lr10, Lr13, Sr5, Sr6, Sr7a, Sr9b, Sr30, Yr6, vrn1, Vrn2, vrn3, Glu-A1b, B1b, D1a, Glu-A3c, B3d, D3b, Glu-A3c, B3e, D3b, ne1, Ne2*	5.3
	Paka	Kenya (1975)	WISCONSIN-245/II-50-17//CI-8154/2*TOBARI-66	Hard, Red grain, Spring wheat with genes: *Glu-A1a, B1b, D1d*	3.7
	Fahari	Kenya (1977)	TOBARI-66/3/SRPC-527-67//CI-8154/2*FROCOR	Hard, Red grain, Spring wheat with genes: *csLV34b, Ltn; Glu-A1b, B1b, D1d*	3.7
	Kudu	Kenya (1966)	KENYA-131/KENYA-184-P	Hard, Red grain, Spring wheat with genes: *Sr2, Glu-A1b, B1i, D1d*	3.7
	Pasa	Kenya (1989)	BUCK BUCK/CHAT	Soft, White grain, Spring wheat with genes: *Glu-A1b, B1c, D1d*	5.3
	Ngiri	Kenya (1979)	SANTACATALINA/3/MANITOU/4/2*TOBARI-66	Hard, Red grain, Spring wheat with genes: *csLV34b, Ltn; Glu-A1b, B1b, D1d*	2.3
	Popo	Kenya (1982)	KLEIN-ATLAS/TOBARI-66//CENTRIFEN/3/BLUEBIRD/4/KENYA-FAHARI	Hard, Red grain, Spring wheat with genes: *Glu-A1b, B1i, D1d*	2.3

Table 3.2. Continued

	Parent	Origin	Pedigree	Characteristic	FR-(stem rust)*
Female	Kariega	South Africa (1993)	(SST-44[CI13523(Agent)/3*T4 (Anza)]//K-4500.2/(SIB)SAPSUCKER	With genes: *Lr3bg, Lr34*, *Yr18; Glu-A1b, Glu-B1i, Glu-D1d; Glu-A1b, Glu-B1i, Glu-D1a*	-
	Morocco	Obscure but considered a North African cultivar	-	Early maturing, Soft, White grain, Spring wheat	-

*Field reaction information obtained from Njau et al. (2009) and Bajgain et al. (2016) using a scale of 0-10.

3.4. Results

Stem rust reaction of wheat cultivars at seedling stage

All wheat cultivars displayed differential infection types to the stem rust races used. Overall, cultivars displayed susceptible reactions at seedling stage to the two diseases with the exception of Paka and Popo which consistently showed resistant infection types (IT of ; to 2). All other cultivars (Gem, Romany, Pasa, Fahari, Kudu, Ngiri and Kariega) displayed ITs of 3 to 4 depending on the race used, whilst Morocco and susceptible check McNair consistently showed highly susceptible infection types (IT of 4) (Table 3.3).

Table 3.3. Seedling infection types (IT) of wheat cultivars to various stem rust races prevalent in South Africa

	Cultivar	Stem rust races#					
		TTKSF+	TTKSF	BPGSC+*Sr27*	BPGSC+*Sr27*+*Kiwiet*+*Satu*	TTKSP	PTKST
Kenyan cultivars	Gem	3	3+	3	3	3	3
	Romany	3	3+	3	3	4	4
	Pasa	3	3	3	3	3	3
	Fahari	4	3+	4	3	3	3
	Kudu	3	3	3	3	3	3+
	Paka	1	1	;1	1	;1	1
	Ngiri	3	3+	3	3	3	3
	Popo	2	2	1+	2	1+	1+
	Kariega*	3	3	3	3	3	3
	Morocco*	4	4	4	4	4	4
	McNair*	4	4	4	4	4	4

*Checks, #A scale of 0-4 was used according to Stakman et al. (1962)

Stem rust reaction of wheat cultivars at adult plant stage

The selected cultivars except for Morocco also displayed moderately resistant to resistant reactions to stem rust at adult plant stage (Table 3.4). Cultivars Morocco and McNair consistently showed highly susceptible reactions across testing environments. Analysis of variance indicated highly significant differences between the genotypes, environment and genotype-by-environment interaction (P = 0.0001).

Table 3.4. Stem rust disease severity of wheat cultivars across environments

	Cultivar	Stem rust field screening#				
		Tygerhoek 2015	Cedara 2014	Cedara 2015	Mean	Std Dev
Kenyan cultivars	Gem	3	3	3	3	0
	Romany	1	5	3	3	1.79
	Pasa	5	6	6	5.67	0.52
	Fahari	3	4	4	3.67	0.52
	Kudu	2	3	3	2.67	0.52
	Paka	4	5	4	4.33	0.52
	Ngiri	3	3	2	2.67	0.52
	Popo	2	2	3	2.33	0.52
	Kariega*	4	5	5	4.67	0.52
	Morocco*	9	10	9	9.33	0.52
	McNair*	9	10	10	9.67	0.52

*Checks, #A scale of 0-10 was used according to modified Cobb scale Peterson et al. (1948) and Tsilo et al. (2014)

Leaf rust reaction of wheat cultivars at seedling stage

Similarly, all wheat cultivars displayed differential infection types to the leaf rust races used. Cultivars displayed resistant to susceptible infection types at seedling stage. Gem, Paka, and Popo are the only genotypes that consistently showed resistant infection types (IT of 2 or lower), with Pasa showing resistant to moderately resistant infection types (IT 2 to 2+). Other cultivars (Romany, Fahari, Kudu, Ngiri and Kariega) showed both resistant or susceptible infection types (IT of 2 to 4) depending on the test race used, whilst Morocco and McNair consistently showed highly susceptible infection type (IT of 4) (Table 3.5).

Table 3.5. Seedling infection types (IT) of wheat cultivars to various leaf rust races prevalent in South Africa

	Cultivar	Leaf rust races#			
		PDRS	SDDN	CCPS	MCDS
Kenyan cultivars	Gem	2	2	2	2
	Romany	3	3	3	3
	Pasa	2	2	2	2+
	Fahari	3	3+	4	3
	Kudu	3	3	3+	2
	Paka	1	1	;1	1
	Ngiri	3	3+	3	2
	Popo	;1	2	1	2

Table 3.5. Continued

Cultivar	Leaf rust races#			
	PDRS	SDDN	CCPS	MCDS
Kariega*	3	3	2	3
Morocco*	4	4	4	4
McNair*	4	4	4	4

*Checks, #A scale of 0-4 was used according to Stakman et al. (1962)

Leaf rust reaction of wheat cultivars at adult plant stage

All test genotypes except Morocco and SST88, displayed moderately resistant to resistant reactions at adult plant stage (Table 3.6). Morocco and SST88 consistently showed highly susceptible reactions in all field environments. Analysis of variance indicated highly significant differences between the genotypes, environment and genotype-by-environment interaction (P = 0.0001).

Table 3.6. Leaf rust disease severity of wheat parental cultivars across environments

	Cultivar	Leaf rust field screening#				
		Tygerhoek 2015	Cedara 2014	Cedara 2015	Mean	Std Dev
Kenyan cultivars	Gem	2	2	3	2.33	0.52
	Romany	1	3	2	2	0.89
	Pasa	4	6	4	4.67	1.03
	Fahari	3	2	5	3.33	1.37
	Kudu	2	2	2	2	0
	Paka	4	5	5	4.67	0.52
	Ngiri	3	5	4	4	0.89
	Popo	2	3	3	2.67	0.52
	Kariega*	5	5	5	5	0
	Morocco*	9	10	9	9.33	0.52
	SST88*	9	10	10	9.67	0.52

*Checks, #A scale of 0-10 was used according to modified Cobb scale Peterson et al. (1948) and Tsilo et al. (2014)

Identification of leaf rust and stem rust resistance genes using molecular markers

Genotyping with marker J13 and marker Gb linked to leaf rust seedling resistance genes *Lr9* and *Lr19*, respectively, did not yield any positive PCR product in all cultivars. For stem rust, as expected the results indicated that none of the parental cultivars contain the gene *Sr22* when screened with marker WMC633. Cultivar Popo was screened positive for *Sr35*. The markers linked to pleiotropic and adult plant resistant

genes *Lr34/Sr57* and *Sr2/Lr27* were also tested. Of the selected cultivars, five (Gem, Fahari, Kudu, Ngiri and Kariega) tested positive for *Lr34/Sr57*, four (Gem, Romany, Kudu and Paka) tested positive for *Sr2/Lr27* while two (Gem and Kudu) carried both APR genes (Table 3.7).

Table 3.7. Presence of leaf and stem rust resistance genes in selected wheat cultivars

	Cultivar	Marker screening						
		Sr2/Lr27	*Sr22*	*Sr31*	*Sr35*	*Lr9*	*Lr19*	*Lr34/Sr57*
Kenyan cultivars	Gem	+	-	-	-	-	-	+
	Romany	+	-	-	-	-	-	-
	Pasa	-	-	-	-	-	-	-
	Fahari	-	-	-	-	-	-	+
	Kudu	+	-	-	-	-	-	+
	Paka	+	-	-	-	-	-	-
	Ngiri	-	-	-	-	-	-	+
	Popo	-	-	-	+	-	-	-
	Kariega*	-	-	-	-	-	-	+
	Morocco*	-	-	-	-	-	-	-
	Control (+ve)	+	+	+	+	+	+	+

*Cultivars used as local checks, + denotes present, - denotes absent

Development of a nested bi-parental population

Several lines were lost over the cycles of single seed descent selection and populations with less than 100 lines were not included in the panel, resulting to the final number of 7 bi-parental populations with a total of 909 $F_{6:8}$ recombinant inbred lines (RILs) (Table 3.8).

Table 3.8. Nested bi-parental population developed and advanced to $F_{6:8}$ generation

Cross	Designated name	No. of RILs
Popo/Kariega	TF4	179
Popo/Morocco	TF5	121
Romany/Kariega	TF6	109
Paka/Kariega	TF7	124
Gem/Kariega	TF11	128
Fahari/Morocco	TF13	132
Fahari/Kariega	TF14	116
	Total	909

3.5. Discussion

Previous studies have shown that Kenyan cultivars are good sources of stem rust resistance (Knott, 1968; Njau et al., 2009), specifically the resistance expressed at an adult plant stage. This has been attributed mainly to the presence of *Sr2* gene complex, either as the sole source of resistance or in combination with other minor and major genes. In the current study, the results of seedling tests against 6 stem rust races predominant in South Africa showed that most Kenyan cultivars displayed highly susceptible infection types at a seedling stage, with the exception of Paka and Popo. The same susceptibility was reported against Ug99 races (TTKS and TTKSK) at seedling stage (Njau et al., 2009; Bajgain et al., 2016). However, all these Kenyan cultivars showed good level of resistance at seedling stage to all predominant races of stem rust in the U.S.A (Njau et al., 2009; Bajgain et al., 2016) and not against African races (results of all studies including the current), implying that these cultivars, with the exception of Paka and Popo, did not express effective race-specific genes to South African races including the Ug99 race group.

Nevertheless, all eight of these Kenyan cultivars displayed high levels of resistance to stem rust in three environments in South Africa. Similar trends were previously observed, with Kenyan cultivars (including all those used in the current study) showing susceptible infection types at the seedling stage and maintaining low severity to stem rust in the field across a range of environments (Singh et al., 2008; Njau et al., 2009; Onguso and Njau, 2015), confirming that these cultivars have race non-specific resistance to stem rust. As with Paka and Popo, it would mean that these two cultivars also have race-specific resistance gene(s) that are effective against the prevalent stem rust races in South Africa. The presence of *Sr35* was only confirmed in Popo using the *Sr35* linked marker. The same results were obtained by Bajgain et al. (2016) and Prins et al. (2016). Although marker data was in agreement in these studies, the seedling phenotype of Popo did not give similar results. This could be explained by use of different source of seed as cultivar Popo in the aforementioned studies showed seedling susceptibility to races within the Ug99 lineage which is not supported by the presence of *Sr35* (a race-specific gene effective against several Ug99 races) (Njau et al., 2009; Bajgain et al., 2016; Prins et al., 2016).

Since all eight Kenyan cultivars were confirmed to have APR resistance to stem rust in multiple environments in South Africa, North America, and Kenya (Njau et al., 2009; Bajgain et al., 2016; present study), they certainly carried multiple useful APR genes to stem rust. To date, more than five APR genes have been identified and validated by several studies (*Sr2/Yr30/Lr27/Pbc1, Sr55/Lr67/Yr46/Pm46/Ltn3, Sr57/Lr34/Yr18/Pm38/Ltn1, Sr58/Lr46/Yr29/Pm39*/*Ltn2* and *Lr68*) and confer resistance to not only stem rust but also to other diseases such as leaf rust, yellow rust and powdery mildew of wheat (William et al., 2006; Lillemo et al., 2008; Krattinger et al., 2009; Herrera-Foessel et al., 2011; Mago et al., 2011a; Herrera-Foessel et al., 2014; Kolmer et al., 2015). Of the reported genes, our results were in agreement with that of Bajgain et al. (2016) that Gem, Kudu, Paka and Romany all carry the *Sr2* gene. In our study, four of these cultivars Gem, Fahari, Kudu, and Ngiri tested positive for *Sr57/Lr34* using the the allele specific markers *(cssfr1, cssfr2, cssfr3 and cssfr4)*. The *Sr57* results were different from those of Bajgain et al. (2016) as they found none of these cultivars tested positive for *Sr57*. Due to different levels of APR resistance present in these eight cultivars based on results from multi-environment testing trials (Njau et al., 2009; Bajgain et al., 2016; and the current study), new APR genes could be present in these cultivars in combination with race-specific resistance genes. Efforts were made to map the APR genes using these cultivars in a nested mating design with LMPG-6 as a common parent (Bajgain et al., 2016), and several QTL regions were identified as significant, some co-localizing with known location of defeated genes and some as new genes.

The five APR genes for wheat stem rust resistance have also been shown to confer resistance to leaf rust at adult plant stage. Most APR genes show susceptible reactions to leaf rust at seedling stage (Kolmer, 1996; Tsilo et al., 2014). Since only Gem, Pasa, Paka and Popo showed resistance to all four predominant races of leaf rust in South Africa at seedling stage, these cultivars carry effective race-specific resistance genes to leaf rust. Using diagnostic markers, all the eight cultivars proved not to carry *Lr9* and *Lr19* resistance genes. Hence, it is speculated that these cultivars may have uncharacterised resistance genes to leaf rust or other characterised leaf rust resistance genes not tested in this study. For APR genes, only four cultivars Gem, Fahari, Kudu, and Ngiri tested positive for *Lr34*/*Sr57* using the allele specific markers (*cssfr1*, *cssfr2*, *cssfr3* and *cssfr4*) as mentioned before. It will be interesting to see if

other new APR genes for stem rust are also responsible for leaf rust resistance observed in the current study. This is highly possible as most of these APR genes have pleiotropic effects, and also do not confer adequate levels of resistance when present singly (Kolmer, 1996; Singh et al., 2013; Silva et al., 2015). The nested bi-parental population developed using the eight Kenyan cultivars in the backgrounds of Kariega and Morocco will therefore provide a powerful tool for identifying the 'unconfirmed or unknown' APR genes or QTLs controlling resistance to leaf rust and stem rust and possibly other economically important traits. It will be interesting to see if QTLs that will be identified using the nested bi-parental population developed in the current study will be the same QTLs detected by Bajgain et al. (2016), who used the same Kenyan cultivars in a different background of LMPG-6.

3.6. Conclusions

This study has shown that the tested Kenyan wheat cultivars exhibit adult plant resistance not only to stem rust but also to leaf rust. Screening with molecular markers only confirmed the presence of two APR genes, i.e., *Sr2/Yr30/Lr27/Pbc1* and *Sr57/Lr34/Yr18/Pm38* in some of the cultivars, hence the consideration to deploy the varieties in constructing a nested bi-parental population that will add value in the identification and validation of a new set of APR genes that are present in these cultivars. The principal effect of APR genes in controlling slow rusting in the studied cultivars highlighted the value of having them as sources of durable resistance. The genetic resource developed in this study will be a useful tool for identifying the 'unconfirmed or unknown' APR gene(s) controlling leaf rust and stem rust diseases.

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Chapter 4

4. Adult Plant Resistance to Leaf Rust and Stem Rust of Wheat in a Newly Developed Recombinant Inbred Line Population

Abstract

Leaf rust caused by *Puccinia triticina* and stem rust by *P. graminis* are the most destructive diseases of wheat (*Triticum aestivum* L.) resulting in substantial yield losses globally. New and aggressively virulent pathotypes are emerging to overcome effective resistance in existing wheat cultivars, particularly when resistance is conferred by race-specific genes. A continuous search for new sources of effective rust resistance genes is necessary to develop improved wheat varieties with stable and durable resistance. The aim of this study was to investigate the inheritance of adult-plant resistance in a recombinant inbred line (RIL) population of a cross between 'Popo' and 'Kariega', and to identify wheat breeding lines possessing both leaf and stem rust resistance. A panel of 179 RILs, two parental varieties and three checks 'Gariep', 'SST88' and 'Morocco' were evaluated in the field, across four diverse environments, for resistance to leaf rust and stem rust diseases. Disease response ranged from highly resistant to highly susceptible reactions with severity scores reaching 100%. Analysis of variance indicated highly significant ($P < 0.001$) differences among the tested RILs for both pathogens across the testing environments. The broad sense heritability estimates were 0.53 and 0.77 for leaf rust and stem rust, respectively. Twenty-six RILs had average severity scores that were better than the parental varieties, and showed higher levels of resistance to both pathogens. Due to the moderate to high heritability estimates for leaf rust and stem rust resistance, the use of the newly developed RILs in the genetic background of Popo/Kariega enhances pre-breeding for rust resistance against the two diseases. Genes controlling such resistance should be explored to elucidate and exploit quantitative trait loci (QTLs) for breeding.

Keywords: Adult Plant Resistance; Durable resistance; Leaf rust; Stem rust; Wheat

4.1. Introduction

Leaf rust of wheat caused by *Puccinia triticina* Eriks. (*Pt*) and stem rust (*P. graminis* Pers. f.sp. *tritici.* Eriks. & E. Henn) (*Pgt*) severely affect the wheat crop through damaging its respiratory system (Livne, 1964) and aggressively killing the foliar parts under high disease pressure. In turn, this leads to stunted growth and reduced photosynthesis with resultant reduction of kernels per head, lower kernel weight and poor grain quality and yield loss (Peturson et al., 1945; Kolmer et al., 2007; Singh et al., 2008). Damage caused by wheat rusts can potentially reduce wheat yields up to 80% on susceptible cultivars (Roelfs, 1992; McIntosh et al., 1995) when favourable weather conditions are prevalent for disease infection and development. Since the first detection of a highly virulent strain of stem rust in Uganda in 1999 which is referred to as Ug99 (Pretorius et al., 2000), about 13 variants within the Ug99 race group have been detected in 13 countries (http://rusttracker.cimmyt.org/?page_id=22). The last country to be added on the Ug99 list is Egypt (Patpour et al., 2016), showing that this group of Ug99 races is spreading. South Africa was long added on the Ug99 list in 2000, and to date there are four races of Ug99 present in the country (Pretorius et al., 2000). This clearly shows that the pathogen is evolving and spreading. On the other hand, the detection of virulent leaf rust races from Zimbabwe and Zambia with similar genetic lineages to those recently discovered in South Africa (Pretorius et al., 2015) has also been alarming to the wheat industry. This suggests high chance of migration of wheat rust races between countries, and highlights the need for integrated and collaborative management strategies.

Several disease management strategies including cultural, biological, chemical and host plant resistance have been practiced by various wheat producers around the world with varying levels of success. In South Africa, wheat producers rely predominantly on the use of fungicides to control wheat rusts (Boshoff et al., 2003; Pretorius et al., 2007; Terefe et al., 2009). While it may be possible to mitigate losses through timely application of fungicides, the low profitability of wheat production cannot sustain such costly inputs under many dryland conditions (Lowe et al., 2011). Furthermore, fungicide application is not economic and environmentally friendly, and can lead to development of fungicide resistant pathotypes.

Wheat rust populations are characterised by their ability to maintain high levels of variability through sequential mutations and sexual or asexual recombination, leading to constant and unpredictable virulence shifts (Ayliffe et al., 2008; Bolton et al., 2008; Singh et al., 2008). This phenomenon makes it difficult for plant breeders and wheat producers to keep up with the constant pathogen changes, creating an obstacle for a complete eradication of rust diseases. Enhanced host plant resistance, and the combination of several effective rust resistance genes remains the most profitable and environmentally friendly control strategy (Kloppers and Pretorius, 1997; McCallum et al., 2016).

Host plant resistance could be race specific or race non-specific. Race-specific resistance genes are expressed in all stages of the plant growth. However, this kind of resistance is not durable (Johnson, 1984), devoid of providing an economic level of protection over an extended period of time. Conversely, adult plant resistance (APR) also known as race-non-specific resistance is expressed only at an adult plant stage. APR is generally characterised by lesser and slower pathogen growth without a necrotic response, sometimes referred to as “slow rusting”. To date, only five APR genes (*Sr2*, *Sr55*, *Sr56*, *Sr57*, and *Sr58*) have been discovered. Several of the APR genes confer minor effects with 5-20% reduction in disease severity. Durable resistance to wheat rust is based on the expression of multiple APR genes in combination or with other race-specific resistance genes to offer a long lasting resistance to a number of races of a pathogen (Kloppers and Pretorius, 1997; McCallum et al., 2016).

In light of the rapid evolution and spread of new virulent races of wheat rusts, and the frequent failure of cultivars with limited resistance to rust diseases, it is deemed important to identify new sources of resistance and/or develop breeding lines and cultivars with multiple adult plant resistance genes. Hence, the aim of this study was to investigate the inheritance of adult-plant resistance in a recombinant inbred line (RIL) population of a cross between Popo and Kariega, and also to identify wheat breeding lines possessing both leaf and stem rust resistance.

4.2. Materials and Methods

Development of a RIL population

A population of 179 recombinant inbred lines was developed from a cross between two spring wheat cultivars (Popo and Kariega). The two cultivars were chosen because of their varying levels of resistance to wheat rusts, acceptable grain quality, bread making quality and acceptable yield levels. Popo (KLEIN-ATLAS/TOBARI-66//CENTRIFEN/3/BLUEBIRD/4/KENYA-FAHARI) is a Kenyan hard red spring cultivar that was released in 1982. This cultivar was selected based on the moderate to high levels of APR exhibited during screening in the Njoro stem rust nursery in Kenya (Njau et al., 2009; Macharia, 2013). Kariega (SST-44[CI13523(Agent)/3*T4 (Anza)]//K-4500.2/(SIB)SAPSUCKER) is a South African cultivar released in 1993 by the Agricultural Research Council–Small Grain Institute (ARC-SGI). Kariega has excellent bread making quality and served as a standard check for bread wheat quality in the country. It was widely grown in South Africa during 1999 to 2009 (Tolmay et al., 2016). Kariega is susceptible to most South African *Pgt* races, however, it has high levels of adult plant resistance to stripe rust caused by *P. striiformis* (Ramburan et al., 2004; Prins et al., 2005, 2011).

The RILs were developed following a single seed descent method, starting with a single F_1 seed which was selfed to produce hundreds of F_2 seeds. Each F_2 seed was planted in pots in the glasshouse and upon harvest, a single seed was randomly selected from each F_2 plant producing the F_3 seed. The single seed descent was followed for three additional generations, ultimately producing F_6 seeds. After the last selfing, every line was bulk harvested twice for multiplication purposes to produce $F_{6:8}$ seed for field experiments. Several lines were lost over the cycles of single seed descent, resulting to the final number of 179 $F_{6:8}$ lines.

Study sites, field planting and assessment of leaf rust and stem rust infection responses

All the RILs, the parental lines (Popo and Kariega) and standard check varieties (Gariep, SST88 and Morocco) were evaluated under field conditions for resistance to leaf rust and stem rust. Evaluations were conducted across three wheat-growing

locations in South Africa (Bethlehem, Cedara and Tygerhoek) during 2014 and 2015 growing seasons. This provided a total of six environments. The details of the environments are summarised in Table 4.1.

Table 4.1. Descriptions of the environments used in the study

Environment Code	Site (Province)	Season (Year)	Geographic position			Annual rainfall	Temperature (°C)		Soil type
			Longitude	Latitude	Altitude †(m.a.s.l.)	(mm)	Min.	Max.	
Tygerhoek 2014	Western Cape	2014	19.90939	-34.1623	183	63.9025	11.4367	23.7567	Glenrosa
Tygerhoek 2015		2015				31.2775	11.0667	24.1625	
Cedara 2014	Kwa-Zulu Natal	2014	30.26498	-29.5419	1068	71.3517	9.8792	24.4325	Griffin
Cedara 2015		2015				49.1067	10.4283	24.4375	
Bethlehem 2014	Free State	2014	28.29733	-28.1627	1680	59.4350	6.7750	22.5900	Plinthustalfs
Bethlehem 2015		2015				43.5608	7.6390	24.1820	

†m.a.s.l. = metre above sea level

Each year, the growing seasons were as follows: from early May to late November at Tygerhoek; late September to early March at Cedara; and early July to late January at Bethlehem. The sites are hotspot areas for wheat rust pathogens. Hence, the locations were selected based on the reported prevalence of the two diseases in the past.

The two parental lines and three checks were replicated five times in each environment, while a single replication of each RIL was evaluated. Approximately 20 seeds from each entry were sown in hill plots using an augmented design. At Tygerhoek, five field standard checks (susceptible and resistant) were planted in five rows at the beginning, middle and at the end of the experimental entries to increase the uniformity of rust inoculum across the trial. At Cedara and Bethlehem, two rust-

susceptible cultivars (Morocco and McNair) were planted as spreader rows around the experimental area to ensure disease initiation and spread. All cultural practises such as fertilisation, irrigation and other management practices were followed according to the recommendations of the specific areas.

Field infection response of the test materials (two parental lines, 179 RILs and the three check varieties) were visually assessed. Disease severity was recorded using a quantitative scale of 0 to 10 according to the modified Cobb scale (Peterson et al., 1948). A score of 0 to 2 represents highly resistant; 3: resistant; 4 to 5: moderately resistant; 6 to 7: moderately susceptible; 8: susceptible and 9 to 10 represents highly susceptible showing 100% of the leaf or stem area covered with the disease. The disease severity for leaf and stem rust was recorded only once in each season when the disease symptoms were fully developed with the susceptible checks displaying 80% or higher disease severity. Stem rust disease severity was only recorded in two environments (Tygerhoek 2014 and 2015) due to non-occurrence of natural infection in the other four environments.

4.3. Data Analysis

Disease score data were analysed to determine frequency distribution, analysis of variance, principal components and correlation coefficients. Data analysis was performed using SAS software (version 9.3, SAS Institute Inc, Cary NC, USA) and GenStat software 18th edition. Analysis of variance was performed using the general linear model procedure (PROC GLM) in SAS. Since the leaf and stem rust evaluations were made on a single replication on the RIL population in all the environments, the significance of main effects of the genotype and environment were tested using the genotype-environment mean square (MS_{ge}). The genotype-environment interaction was tested for its significance effect using the error mean square that was estimated from five genotypes (3 checks and 2 parents) that were replicated five times within the test environments according to an augmented design (Federer, 1961). Significant difference between the means of the parental lines was tested using the Fisher's protected least significant difference (LSD) ($P < 0.05$). Variance components for genotype and genotype-environment were used to obtain estimates of the broad

sense heritability (h^2_B) (Tsilo et al., 2014) as follows: $h^2_B = \sigma^2_g/[\sigma^2_g + (\sigma^2_{ge}/e) + (\sigma^2_e/re)]$ or 1-[MS_g/MS_{ge}], where MS_g and MS_{ge} represent the genotype and genotype-environments mean squares respectively, σ^2_g is the genotypic variance = ($MS_{genotype}$ - MS_{ge})/re, σ^2_{ge} is the G x E interaction variance = (MS_{ge} – MS_e)/r, σ^2_e is the error variance = MS_e, r is number of replicatons and e is the number of environments. The phenotypic distribution of rust ratings based on the mean of four environments for leaf rust and two environments for stem rust was tested for normality using the Shapiro-Wilk statistic (Mallard et al., 2005). To display the relationship between the two rusts severity scores on the RIL population and also the environments, a biplot representation diagram was used. A principal component analysis was performed using the multivariate analysis using Genstat 18th Edition. The relationship between the RILs and the rust severity scores in the different environments was displayed using a point vector plot, with the points representing the different genotypes and directional vectors representing the two rusts severity in different environments. The angles between the vectors represent the correlation pattern between the severity scores on different environments.

4.4. Results

Leaf rust reaction of RILs and check genotypes

As expected, in all the environments the five checks (2 parental lines and 3 checks) showed significant differences ($P < 0.0001$) for leaf rust severity. Mean comparison involving the two parental lines (Popo and Kariega) in all the testing environments for leaf rust showed significant differences (LSD=2.1151) at 5% level of probability. The mean severity of Popo was 2.08 for leaf rust at the adult plant stage, whereas Kariega had a value of 4.89. In all the environments, a wide range of leaf rust disease severity (31% to 63%) was observed for the RILs (Appendix 1). Analysis of variance indicated highly significant differences ($P < 0.0001$) among the 179 RILs and the test environments (Table 4.2).

Table 4.2. ANOVA of genotype and environment effects and proportion of phenotypic variation for leaf and stem rust severity obtained across the testing environments among the recombinant inbred line population.

	Experimental lines						Parents and checks					
Disease	Source of variation	*df*	MS	*F*	*P*	h^2_B	Source of variation	*df*	MS	*F*	*P*	h^2_B
Leaf rust	RILs	167	12.138	2.29	0.0001		Parents and checks (PCs)	4	45.8881	7.23	0.0001	
	Environment	3	200.662	37.8	0.0001		Environment	3	4.3459	0.68	0.5658	
	Error	270	5.308				PCs*Environment	11	12.4114	1.96	0.0556	
						0.53	Error	47	6.3461			
												0.59
Stem rust	RILs	113	9.205	1.77	0.0074		Parents and checks (PCs)	4	90.8351	36.31	0.0001	
	Environment	1	63.879	12.31	0.0009		Environment	1	0.0061	0	0.961	
	Error	61	5.191				PCs *Environment	4	8.8188	3.52	0.0164	
						0.77	Error	34	2.502			
												0.88

RIL = Recombinant Inbred Line, *df* = degrees of freedom, MS = Mean Square, h^2_B = broad sense heritability. Genotype (RILs and PCs) and genotype by environment (PCs*Environment) effects were considered significant if $P \leq 0.05$

The observed severity scores ranged from highly resistant to highly susceptible reaction (Figures 4.1a and 4.1b, respectively). Frequency distributions of RILs for leaf rust severity showed continuous and skewed distribution when entries were evaluated across individual environments (Figure 4.2). Severity scores of some RILs were found at both tails of the normal distribution curve when compared to the parental lines, indicating the presence of transgressive segregation in Popo/Kariega population as well as inheritance of resistance from both parents. In Cedara 2014, under high infection pressure, the phenotypic distribution was skewed towards the susceptible phenotype (Figure 4.1b). The pooled mean value of the RILs from all environments indicated continuous distribution. However, most lines were still skewed towards resistance. The broad sense heritability for leaf rust was 0.53.

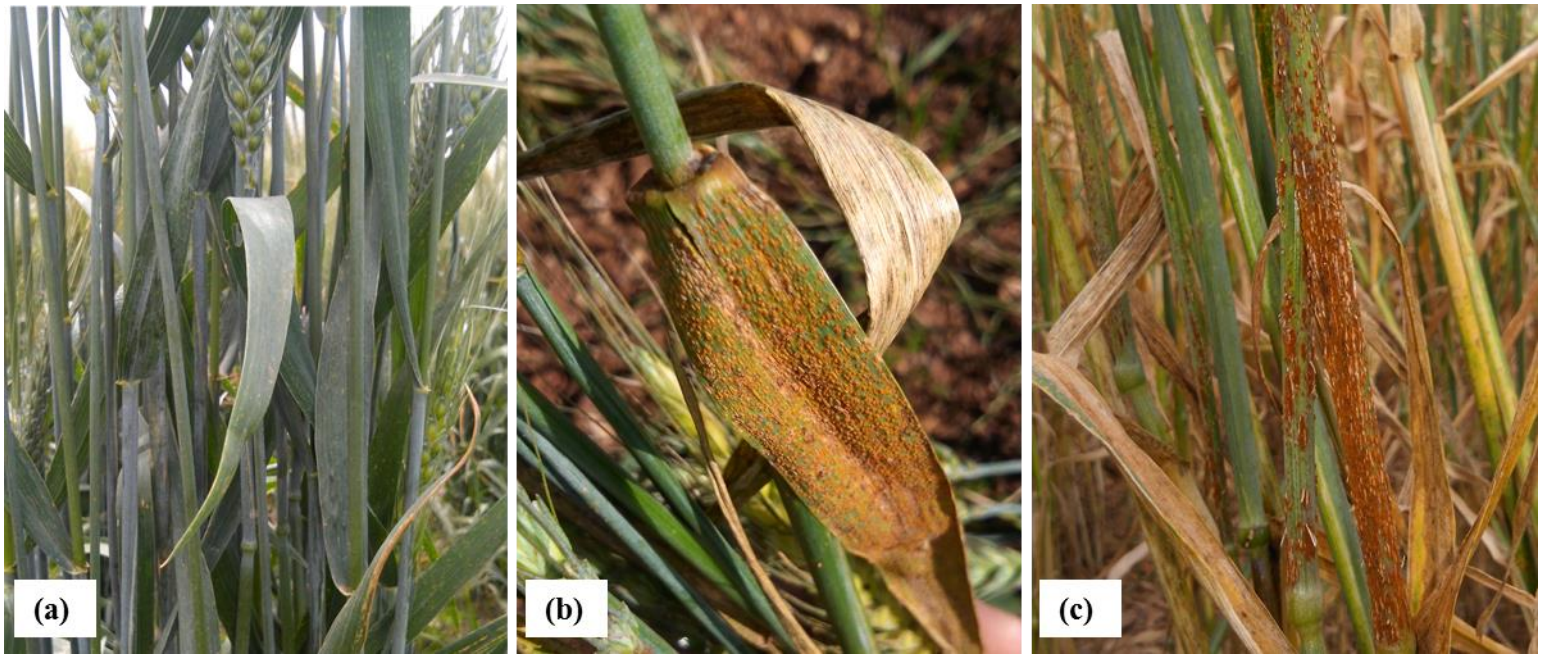

Figure 4.1. Popo/Kariega recombinant inbred lines showing varying rust reactions at Tygerhoek 2014. (a) RIL113 showing immune response, (b) RIL17 showing highly susceptible response to leaf rust (severity score of 8 to 9), and (c) RIL17 showing highly susceptible response to stem rust (severity score of 8 to 9).

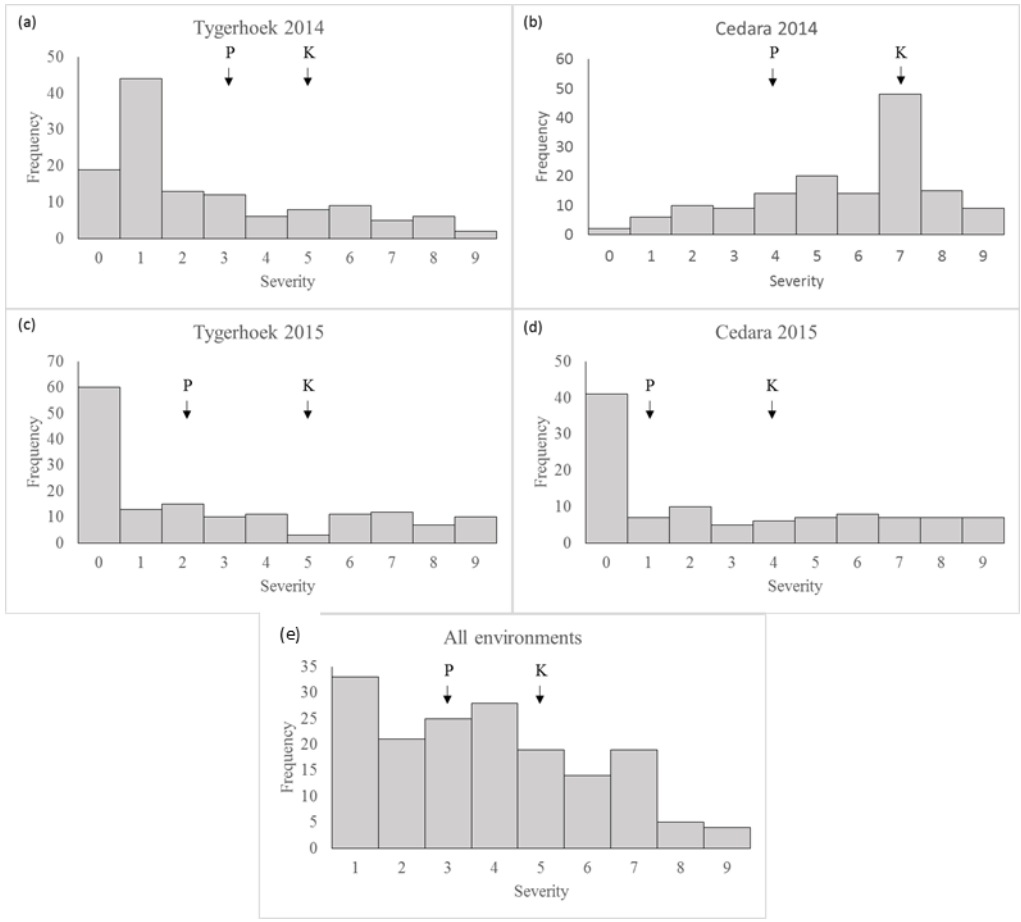

Figure 4.2. Phenotypic distribution of RILs derived from a Popo/Kariega (P/K) cross for leaf rust severity across four environments: (a) Tygerhoek 2014, (b) Cedara 2014, (c) Tygerhoek 2015, (d) Cedara 2015, and (e) Pooled means for all environments

Stem rust reaction of RILs and check genotypes

The five checks (2 parental lines and 3 checks) showed significant differences ($P < 0.0001$) for stem rust severity. Similarly, comparison of the two parental lines (Popo and Kariega) in the two test environments for stem rust showed significant differences (LSD=1.6214) at the 5% probability level. The mean severity of Popo was 2 at the adult plant stage, while Kariega had a rating of 5.9. In Tygerhoek during 2014 and 2015, the mean disease severity ranged between 29% to 43% for the RILs (Appendix 1).

Analysis of variance indicated highly significant differences ($P < 0.01$) among the 179 RILs and the two testing environments (Table 4.1). The observed severity ranged from highly resistant to highly susceptible reaction (Figures 4.1a and 4.1c, respectively). The frequency distribution of RILs for stem rust resistance also showed continuous and skewed distribution in the two studied environments with more RILs showing high resistance. However, severity scores for some RILs were found at both tails of the normal distribution curve when compared to the parental lines (Figure 4.3), yet suggesting the presence of transgressive segregation in Popo/Kariega population as well as inheritance of resistance from both parents. The pooled mean severity of the RILs from the two study environments yielded continuous distribution. The broad sense heritability for stem rust was 0.77, which was comparatively higher than leaf rust resistance.

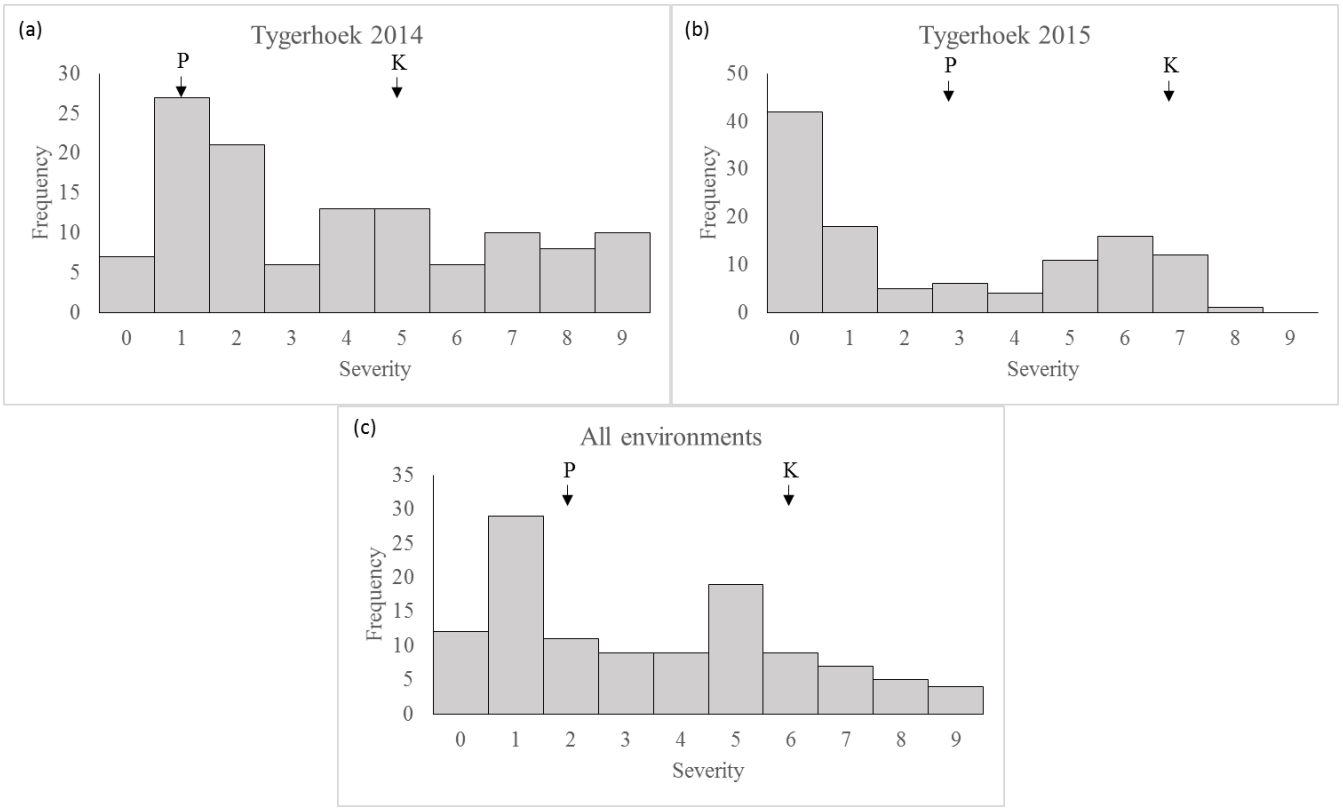

Figure 4.3. Phenotypic distribution of RILs derived from a Popo/Kariega (P/K) cross for stem rust severity in two environments: (a) Tygerhoek 2014, (b) Tygerhoek 2015, and (c) Pooled means for the two environments.

Correlation of leaf and stem rust severity across testing environments

A phenotypic association study among the 179 RILs was conducted using principal component analysis (PCA) (Figure 4.4), mainly to detect similarities in the line's responses to leaf and stem rust in the studied environments, as well as to determine the extent and direction of correlations between the two rusts. The PCA separated the RILs into two distinct principal components; 1 and 2 which correspondingly explained 45.34% and 16.04% of the total variation. A group of individual RILs highly resistant to both rusts was clearly separated from other lines in the population on the PCA biplot. Supporting the PCA results, the mean for the resistant parent was estimated resulting to a disease severity of 2 on a scale from 0 to 9 for both traits (Table 4.3). The same value was used in selecting best performing RILs showing mean severity scores equal or lower than the resistant parent. In total, 26 RILs were selected with mean severity scores of 0 to 2 for both leaf and stem rust (Table 4.3). The same lines were found to group together on the PCA biplot diagram. The phenotypic rust scores in the four environments demonstrated strong correlations with the highest correlation observed between Tygerhoek 2014 leaf rust severity scores and Tygerhoek 2014 stem rust scores ($P < 0.001$). This was followed by Tygerhoek 2014 stem rust scores and Cedara 2014 leaf rust severity scores as shown in Table 4.4.

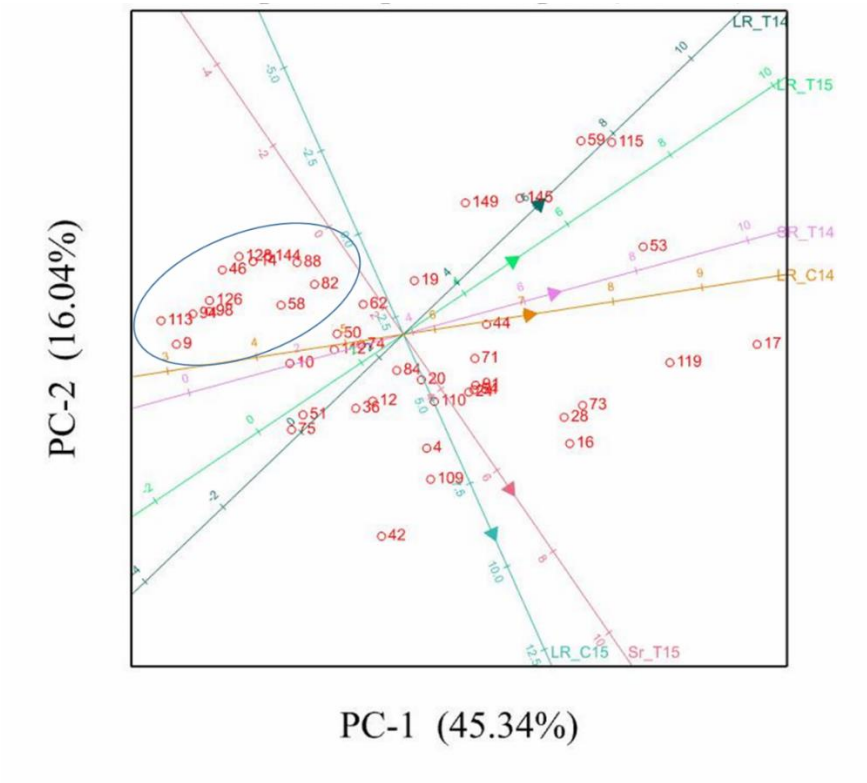

Figure 4.4. Association between 179 Popo/Kariega recombinant inbred lines on the basis of the first two principal components (PC-1 and PC-2) obtained from a principal component analysis based on leaf and stem rust severity scores in four environments. LR_T14 = Leaf rust in Tygerhoek 2014; LR_T15 = Leaf rust in Tygerhoek 2015; SR_T14 = Stem rust in Tygerhoek 2014; LR_C14 = Leaf rust in Cedara 2014; SR_T15 = Stem rust in Tygerhoek 2015 and LR_15 = Leaf rust in Cedara 2015. The genotypes are indicated by numbers.

Table 4.3. Best performing RILs displaying leaf rust and stem rust resistance with mean severity values of 0 to 2 averaged across environments compared with parental lines (Popo and Kariega).

	Mean rust severity score			Mean rust severity score	
Genotype	Leaf rust	Stem rust	Genotype	Leaf rust	Stem rust
RIL1	1.5	1	RIL82	2	0
RIL2	0	1	RIL85	0.33	0.5
RIL3	0	0.5	RIL93	1	1
RIL6	1.5	0.5	RIL94	1	0.5
RIL9	1	0	RIL98	1	0
RIL13	0	2	RIL102	0.67	1
RIL14	0.33	2	RIL113	0.5	0
RIL18	0.5	0.5	RIL125	1.25	1
RIL22	2	1	RIL126	1.25	0
RIL58	2	1	RIL128	0.67	0.5
RIL61	1	0.5	RIL133	0	0
RIL65	0	2	RIL134	0	1
RIL78	1.67	0	RIL147	0	0
Parental lines*					
Popo	2.08a	2.00a			
Kariega	4.89b	5.90b			

* Means in a column followed by different letters are significantly different at $P \leq 0.05$

Table 4.4. Correlation coefficients of leaf rust and stem rust severity scores for 179 Popo/Kariega recombinant inbred line population across environments.

Disease		Leaf rust				Stem rust
	Environment	Cedara 2014	Cedara 2015	Tygerhoek 2014	Tygerhoek 2015	Tygerhoek 2014
Leaf rust	Cedara 2014	-				
	Cedara 2015	0.1908	-			
	Tygerhoek 2014	0.4001*	0.1641	-		
	Tygerhoek 2015	0.3576*	0.2139	0.3686*	-	
Stem rust	Tygerhoek 2014	0.4353*	0.3422*	0.6925**	0.3552*	-
	Tygerhoek 2015	0.3805*	0.3057*	0.2296	0.1918	0.3753*

* and ** denotes significance correlations at $P \leq 0.05$, (**) and t $P \leq 0.001$, respectively

4.5. Discussion

This study describes the use of the observable phenotype to screen and identify sources potentially carrying effective resistance genes at an adult plant stage. These sources can add great value in programmes breeding for stable and durable resistance. Several studies have shown that field screening and selection for rust resistant sources can be labour intensive and costly, and the observable phenotype is largely influenced by the environment (Lillemo et al., 2008; Baye et al., 2011; Gratani, 2014; Tsilo et al., 2014). The severity of the infection pressure is also dependent upon the temperature and humidity while the screening results and reproducibility is highly subject to errors in classification.

In this study, substantial costs associated with field screening and planting space were cut by only replicating the checks. The genotype-environment interaction of the lines was tested for significance using the error mean square that was estimated from five check genotypes (3 checks and 2 parents) that were replicated five times within environments. Significant phenotypic variation for leaf and stem rust resistance on the Popo/Kariega RIL population and checks was clearly observed. The results of this study also showed significant differences in the environments, which suggests that environmental factors, especially temperature and humidity affect the severity response of the different genotypes. For example, the amount of precipitation received in each season as well as the annual total precipitation per site were different (Table 4.1). However, results from blocking within an environment showed insignificant differences within blocks (data not shown) which suggests that the infection was uniform across the experimental trial within the environment and blocking was not necessary. It was also not surprising that the genotype effects were highly significant because the parental lines were selected to provide clear variation in disease response but complement each other in important traits. The transgressive segregation for lower severity scores for some lines in the population is strongly indicative of the inheritance of resistance genes from both parents. The distribution of the disease severity scores for the two traits also clearly showed the influence of many loci, mainly of small effect.

A number of studies have exploited the cultivar Kariega as a parent in mapping populations constructed to elucidate the genetic basis of resistance (Ramburan et al., 2004; Prins et al., 2005). The cultivar carries an important gene complex *Lr34/Yr18/Pm18* on 7D*,* encoding an ATP-binding cassette (ABC) transporter and seedling leaf rust resistance genes *Lr1* and *Lr3a*. The *Lr34* gene has been cloned and is a "slow rusting" gene that contributes to partial levels of rust resistance in many wheat cultivars around the world. The gene has been shown to be completely linked to *Yr18* for partial yellow rust resistance, *Pm18* for partial powdery mildew and *Sr57* for partial stem rust resistance, and it is also associated with leaf-tip necrosis phenotype (Juliana et al., 2015). Several studies have demonstrated the ability of the *Lr34* gene to enhance the effectiveness of other leaf rust genes (German and Kolmer, 1992; Singh, 1992; Pretorius and Roelfs, 1996) and permit the expression of resistance to certain stem rust races normally inhibited by a suppressor gene, thereby resulting in enhanced stem rust resistance (Dyck et al., 1966; Kerber and Aung, 1999). The Kenyan cultivar Popo on the other hand has been shown to exhibit moderate to high levels of APR during screening in the Njoro stem rust nursery in Kenya (Njau et al., 2009). The high level of APR resistance indicate that Popo could contain an APR gene, though not confirmed. Kenyan lines are also known to be carriers of many *Sr* genes discovered to date (McIntosh et al., 1995; Njau et al., 2009; Macharia, 2013; Bajgain et al., 2016). The possible inheritance of the *Lr34* gene from Kariega, the gene(s) controlling APR from Popo together with several genes of minor effects by some of the Popo/Kariega RIL population members could explain the high resistance response observed in the selected 'best performing lines', mainly because of the durable and additive nature of these genes. The APR genes work in synergy, boosting other resistance genes to provide stable, durable and possibly broad-spectrum resistance (Ellis et al., 2014).

4.6. Conclusions

The significant difference among the genotypes for leaf and stem rust resistance and the clear separation of resistant lines on the PCA enabled for a selection of a subset of the best performing lines. These 26 best RILs can be valuable genetic resources for breeding against leaf and stem rust and can be used in pre-breeding programmes aiming at developing germplasm with stable and durable resistance. Nevertheless,

further studies are needed to understand the genetic basis resulting to the observed resistant phenotype, and the interaction with the environment in the selected lines. With the developments made in molecular marker techniques and the advent of next generation sequencing technologies, it will not be a difficult task to determine the genetic diversity present in the selected lines. Bi-parental mapping and QTL analysis can also be conducted in the whole population to determine or identify significant QTLs controlling the phenotypic variance and the percentage of the phenotypic variance contributed by each QTL. The results of this study are invaluable in breeding for resistance and contribute immensely in the surge to fight the 'ever evolving' and 'never sleeping' wheat rusts.

4.7. References

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Appendix 1**.** Mean and standard deviation (averaged from all the environments) of all 179 RILs from a Popo/Kariega cross

	Leaf rust severity			Stem rust				Leaf rust severity			Stem rust				Leaf rust severity			Stem rust		
Genotype	N	Mean	SD	N	Mean	SD	Genotype	N	Mean	SD	N	Mean	SD	Genotype	N	Mean	SD	N	Mean	SD
1	2	1.50	0.71	2	1.00	1.41	28	3	5.00	2.65	2	7.50	2.12	56	1	0.00				
2	2	0.00	0.00	2	1.00	1.41	29	3	5.00	0.00				57	2	1.00	1.41			
3	1	0.00		2	1	0.71	31	2	6.00	1.41	1	6.00		58	4	2.00	2.71	2	1.00	1.41
4	4	4.00	3.16	1	3.00		32	2	7.00	1.41	2	3.50	4.95	59	4	5.00	4.24	2	5.00	5.66
5	1	7.00					33	3	1.33	1.53	1	3.00		60	2	0.00	0.00			
6	2	1.50	2.12	2	0.50	0.71	34	2	2.50	3.54				61	2	1.00	1.41	2	0.50	0.71
8	2	7.00	1.41	2	1.00	1.41	35	4	7.75	0.96				62	4	2.75	2.06	2	3.00	1.41
9	4	1.00	0.82	2	0.00	0.00	36	4	2.25	2.06	2	4.00	2.83	63	1	0.00				
10	4	2.75	2.75	2	0.50	0.71	38	2	8.50	0.71				64	2	5.00	5.66	2	3.50	4.95
11	2	7.00	0.00				39	4	5.75	1.89	1	8.00		65	1	0.00		2	2.00	2.83
12	4	5.00	3.16	2	1.00	1.41	40	3	6.33	3.06				66	1	2.00		2	2.50	2.12
13	1	0.00	.	2	2.00	2.83	41	1	5.00					67	1	6.00		1	1.00	
14	3	0.33	0.58	2	2.00	2.83	42	4	3.00	3.46	1	7.00		68	2	0.50	0.71			
15	3	6.00	4.36	2	4.00	5.66	43	2	4.50	6.36				69	2	1.00	1.41			
16	3	6.33	4.62	2	5.00	0.00	44	4	6.00	2.94	2	2.50	2.12	70	4	7.25	2.06	1	9.00	
17	3	8.33	0.58	2	8.00	1.41	45	4	3.75	2.99	1	3.00		71	4	4.75	3.20	1	0.00	
18	2	0.50	0.71	2	0.50	0.71	46	4	1.00	1.41	1	0.00		72	4	2.25	1.26	1	7.00	
19	4	3.50	2.38	2	3.00	2.83	47	1	4.00					73	4	5.25	2.99	1	7.00	
20	4	3.75	3.20	2	4.00	4.24	48	3	8.67	0.58	1	9.00		74	4	3.75	2.99	2	1.00	1.41
21	4	3.75	2.63	1	5.00		49	3	3.33	1.53	1	5.00		75	4	2.25	2.63	2	2.00	1.41
22	3	2.00	2.65	1	1.00		50	4	3.50	2.65	2	2.00	1.41	76	3	2.67	0.58	2	4.50	3.54
23	3	4.00	3.46				51	4	2.50	2.38	2	2.00	0.00	77	2	1.00	1.41			
24	4	6.25	3.59	2	1.50	0.71	52	1	0.00					78	3	1.67	1.53	1	0.00	
25	3	5.00	3.46	2	7.00	2.83	53	3	5.33	3.79	2	7.50	0.71	79	2	4.00	2.83			
26	2	3.00	4.24	2	0.50	0.71	54	4	4.00	2.71	2	5.00	1.41	80	2	4.50	3.54			
27				2	4.50	3.54	55	3	3.00	1.73	2	2.50	0.71	81	4	2.00	2.71			

Appendix 1**.** Continued

Leaf rust severity				Stem rust			Leaf rust severity				Stem rust			Leaf rust severity				Stem rust		
Genotype	N	Mean	SD	N	Mean	SD	Genotype	N	Mean	SD	N	Mean	SD	Genotype	N	Mean	SD	N	Mean	SD
82	4	2.00	3.37	1	0.00		108	2	3.50	4.95	1	6.00		136	2	4.00	0.00			
83	3	3.33	2.89	2	3.50	3.54	109	3	4.67	3.21	2	4.50	2.12	137	1	7.00		1	1.00	
84	3	2.33	4.04	2	5.00	5.66	110	4	2.50	3.11	2	6.00	0.00	138	4	4.75	3.30			
85	3	0.33	0.58	2	0.50	0.71	111	4	7.00	1.83	1	8.00		139	3	4.00	3.00			
86	3	7.00	2.65				112	4	2.00	3.37	1	4.00		140	1	0.00				
87	2	0.00	0.00	2	4.00	4.24	113	4	0.50	1.00	2	0.00	0.00	141	1	7.00		1	5.00	
88	4	2.50	2.38	2	1.00	1.41	114	1	4.00					143	2	1.50	2.12			
89	4	4.25	2.06	1	5.00		115	3	5.67	4.93	2	5.00	4.24	144	4	2.25	2.63	2	1.00	1.41
90	3	5.33	1.53				117	2	2.50	2.12	2	0.50	0.71	145	4	6.25	2.36	2	2.00	2.83
91	3	5.00	2.65	1	7.00		118	3	5.00	3.61	2	5.50	3.54	146	4	3.25	2.99	1	6.00	
92	4	4.00	4.08	1	2.00		119	3	8.00	1.00	2	6.00	1.41	147	1	0.00		1	0.00	
93	3	1.00	1.00	1	1.00		120	4	2.00	2.16	1	9.00		148	2	7.00	0.00			
94	3	1.00	1.73	2	0.50	0.71	121	3	1.67	2.89				149	3	3.00	4.36	2	2.50	2.12
95	2	2.00	0.00				122	2	2.00	2.83				150	1	8.00		2	2.00	2.83
96	4	5.00	2.83	1	7.00		123	2	3.00	4.24	1	5.00		151	2	3.00	2.83	2	5.00	0.00
97	3	3.33	3.51				124	1	7.00		2	4.50	0.71	152	3	3.33	4.04	1	4.00	
98	3	1.33	2.31	1	0.00		125	4	1.25	2.50	1	1.00		153	1	7.00				
99	1	9.00	.				126	4	1.25	1.89	1	0.00		154	2	4.00	4.24	1	7.00	
100	3	2.67	2.52	1	0.00		127	1	0.00					155	1	4.00		2	5.00	0.00
101	1	6.00	.				128	3	0.67	1.15	2	0.50	0.71	156	2	3.50	2.12	1	2.00	
102	3	0.67	1.15	1	1.00		129	1	5.00		1	6.00		157	1	7.00		1	6.00	
103	3	1.33	2.31				131	2	7.00	1.41				158	1	0.00				
104	2	7.50	0.71	1	5.00		132	2	5.50	2.12	1	1.00						1	1.00	
105	3	5.00	2.00	2	5.00	2.83	133	1	0.00		1	0.00		160	3	5.33	4.04	1	0.00	
106	3	4.00	2.00	1	5.00		134	1	0.00		1	1.00		161	2	2.00	2.83	1	9.00	
107	3	3.33	3.51				135	3	2.33	2.31	2	6.00	1.41	162	3	2.33	4.04			

Appendix 1**.** Continued

Genotype	Leaf rust severity		
	N	Mean	SD
163	3	3.33	3.06
164	2	4.50	2.12
165	3	2.00	1.73
166	1	0.00	
170	1	0.00	
171	2	6.50	0.71
172	2	5.50	2.12
173	2	6.50	0.71
175	1	3.00	
176	1	0.00	
177	3	2.33	4.04
178	2	3.50	4.95

Best performing lines are highlighted. SD = Standard Deviation.

Chapter 5

5. Linkage Mapping and Quantitative Trait Loci Analysis of Genes Conferring Adult Plant Resistance to Leaf Rust and Stem Rust of Wheat

Abstract

Linkage mapping and quantitative trait loci (QTLs) analyses of economic traits are prerequisites for genomic-assisted breeding. In this study, a Diversity Array Technology-sequencing (DArT-seq) platform was used for genetic analysis and to develop a linkage map using a newly developed recombinant inbred line (RIL) population derived from a cross between two spring wheat (*Triticum aestivum* L.) cultivars *viz*. 'Popo' and 'Kariega'. The RIL population was evaluated for adult plant resistance (APR) to leaf rust and stem rust under field conditions across two diverse sites (Cedara and Tygerhoek) during 2014 and 2015, providing four testing environments. A genetic map was developed spanning a total genetic distance of 4990.61 cM (2150.46 cM for A genome, 1731.81 cM for B genome and 1108.34 cM for D genome) and containing a total of 1065 informative single nucleotide polymorphism (SNP) marker loci representing the wheat genome, with an average interval of 4.69 cM per marker. Quantitative trait loci analysis was performed using single marker analysis (SMA) and inclusive composite interval mapping (ICIM) methods with QTL IciMapping 4.0 software based on stepwise regression linear model. A total of 44 putative QTLs (10 for leaf rust and 34 for stem rust) with additive genetic effects were detected on 14 chromosomes explaining 2.45 to 21.21% of the phenotypic variation present in the RILs. Fourteen QTLs were located on the A genome, while 24 on the B genome and 6 on the D genome of wheat. One consistent genomic region designated as *QLr.sgi-5A.1* contributed by Kariega was identified on chromosome 5A controlling leaf rust at the Tygerhoek testing site during 2014 and 2015. Another QTL, *QLr/Sr.sgi-7D.1.3*, also derived from Kariega was detected under the two testing sites (Cedara 2015 and Tygerhoek 2015), controlling both leaf rust and stem rust and explaining 4.78% and 3.20% of the phenotypic variation, respectively. The identified QTLs can possibly be explored as genetic markers to be used in marker-assisted breeding of wheat, emphasising leaf rust and stem rust resistance.

Keywords: Adult plant resistance; Inclusive composite interval mapping; Leaf rust; Stem rust; Quantitative trait

5.1. Introduction

Leaf rust caused by *Puccinia triticina* Eriks. and stem rust (*P. graminis* f. sp. *tritici*,) are the most devastating diseases of wheat (*Triticum aestivum* L.) globally. Dissection of candidate resistance genes and identification of new genomic regions harbouring quantitative trait loci (QTL) enhances breeding efficiency of wheat. This is particularly important with leaf rust and stem rust resistance genes conferring adult plant resistance (APR). Adult plant resistance is conditioned by polygenes and characterised by a lower infection frequency, longer latent period, reduced urediniospore production and smaller uredinial size (Caldwell, 1968; Singh et al., 2005). Most genes conferring APR are race non-specific with slow rusting characteristics. Slow rusting is a resistance mechanism that, in most cases does not completely halt fungal infection, but slows the infection process, consequently reducing the disease rate and final severity in the field (Parleviet and van Ommeren, 1975). This form of resistance has been shown to be partial but durable (Johnson, 1984; Singh, 1992; Kolmer, 1996). One of the aims of most wheat breeding programs is to develop new genetic resources with durable and broad spectrum resistance to wheat diseases including leaf rust and stem rust.

Adult plant resistance is controlled by the additive effects of minor genes conferring race non-specific resistance. When present singly, APR genes do not provide adequate levels of resistance, especially under high disease pressure. Several studies have demonstrated that combining four to five slow rusting additive genes with small to intermediate effects, provides up to near-immune levels of resistance (Singh et al., 2000; Silva et al., 2015). Although APR has been widely reported in plant breeding programmes, the genes underlying this form of resistance are not well understood, hence not well-characterised. Cloning of the *Lr34/Yr18* locus, conferring leaf rust and yellow rust resistance at adult plant stage, has provided initial insights and better understanding of the genetic nature of APR genes (Krattinger et al., 2009). To date, only a few APR genes controlling leaf rust and stem rust have been identified and catalogued in wheat as compared to major genes or seedling resistance genes (McIntosh and Yamazaki, 1973; Herrera-Foessel et al., 2012; Bansal et al., 2014).

Identification of stable and durable QTLs conferring APR across different environments and mapping populations is necessary to control wheat diseases. Stable QTLs/genes are useful for achieving disease resistance in breeding programs. With the advent of DNA sequencing tools, and more recently, the rapid advances in next generation sequencing (NGS) technologies (Vance et al., 2016), it has been possible to sequence the whole-genome of plant genetic resources. This has assisted to identify haplotype blocks or single nucleotide polymorphic (SNP) signatures that are significantly correlated with quantitative trait variation (Davey et al., 2011; Edwards et al., 2013). The high-throughput NGS technologies have led to the development of a second generation of genome-wide markers based on sequence information amenable for application in plant breeding programs (Elshire et al., 2011; Kumar et al., 2012). The NGS technologies are widely applied in plant breeding and comparative genomic studies (Sikhakhane et al., 2016). Genotyping-by-sequencing (GBS) has been adopted in genomic selection of crop plants (Poland and Rife, 2012; Peterson et al., 2014). This sequencing strategy employs a reduced genome representation achieved through restriction enzyme digestion of target genomes, followed by PCR amplification using DNA barcoded adapters. Consequently, this approach allows for the discovery of large numbers of high density SNP markers for exploring significant levels of genetic diversity (Davey et al., 2011; Elshire et al., 2011). Application of the GBS technology for SNP discovery and genotyping in crop plants including wheat has been successfully demonstrated by several studies (Deschamps et al., 2012; Poland et al., 2012; Li et al., 2015; Bajgain et al., 2016; Kim et al., 2016).

A GBS platform known as DArT-seq was developed by the Diversity Array Technology (DArT), Canberra, Australia (http://www.diverityarrays.com/), providing an opportunity to select genome fractions corresponding predominantly to variations in genome sequences. This approach is based on SNP-based marker platform which combines the DArT marker system (Kilian et al., 2012) with Illumina short-read sequencing method (http://www.illumina.com). Restriction enzymes used for DArT-seq separate repetitive fractions from low copy sequences which are highly informative for marker discovery (Sansaloni et al., 2011; Kilian et al., 2012). The high density markers from the DArT-seq platform are important in building saturated genetic linkage maps which

enable trait-based genetic analysis, especially when identifying closely linked markers in highly dense genetic maps.

The GBS platform is the most powerful tool in genetic analysis of APR genes of leaf rust and stem rust resistance breeding of wheat. Therefore, the objectives of this study were: 1) to undertake a genome-wide scan for identification of QTLs that significantly affect adult plant resistance to leaf rust and stem rust of a newly developed recombinant inbred line (RIL) population of wheat developed from a cross of two cultivars, Popo and Kariega, and 2) to construct a genetic linkage map useful to locate genes/QTLs controlling other important agronomic traits in the Popo/Kariega genetic background.

5.2. Materials and Methods

Population development

A previously developed $F_{6:8}$ population of 179 RILs, derived from a cross of two spring wheat cultivars Popo and Kariega was used for this study (see Chapter 4, Section 4.2).

Evaluation of leaf rust and stem rust resistance

Leaf rust and stem rust reactions of 170 RILs (excluding nine lines which failed to germinate or provided inconclusive data) were evaluated. The RILs were established across two wheat growing sites, Cedara and Tygerhoek, during 2014 and 2015 providing four testing environments *viz*. Cedara 2014, Cedara 2015, Tygerhoek 2014 and Tygerhoek 2015, in that order. The sites are hotspot areas for wheat rust pathogens including leaf rust and stem rust. Hence, the locations were selected based on the reported prevalence of the two diseases in the past. Leaf rust was scored across the four environments, while stem rust was rated across two environments (Tygerhoek 2014 and Tygerhoek 2015). Disease severity was recorded using a quantitative scale of 0 to 10 according to the modified Cobb scale (Peterson et al., 1948). A score of 0 to 2 represents highly resistant; 3 resistant; 4 to 5 moderately resistant; 6 to 7 moderately susceptible; 8 susceptible and 9 to 10 highly susceptible

based on the leaf or stem area covered with the disease. The disease severity for leaf and stem rust was recorded only once in each season when the disease symptoms were fully developed with the susceptible checks displaying 80% or higher disease severity (see Chapter 4, Section 4.2).

Preparation of leaf samples for DNA extraction

Three to four young leaves from each of the 170 RILs and two parents Popo and Kariega were harvested from ten-day old seedling plants, placed in 2ml epperndorf tubes and stored in a -80 freezer prior to DNA extraction. To homogenise the samples during DNA extraction, two round stainless-steel ball bearings (5 mm in diameter) were added to the frozen leaf material in 2 ml eppendorf tubes. The frozen leaf material was homogenised using Qiagen's Tissue Lyser for 1 min at 30 r/s.

DNA extraction and DArT-seq

Total genomic DNA was extracted from the 170 RILs and parental cultivars Popo and Kariega using the DArT protocol (http://www.diversityarrays.com/). The quality, purity and quantity of the extracted DNA was tested using the NanoDrop 1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). The A260/A280 and A260/A230 absorbance ratios was used to determine contaminations of protein and polyphenolic/polysaccharide compounds respectively. For genotyping, 500-1000 ng of restriction grade DNA samples, suspended in TE buffer with a final concentration of 50-100 ng/µl were shipped to Diversity Arrays Technology, Pty Ltd, Yarralumla, ACT, Australia for genome profiling using a GBS platform known as DArT-seq.

The DArT-seq system produces two kinds of markers, classical SNP and present/absent variation, also called SilicoDArTs (http://www.diversityarrays.com/dart-application-dartseq-data-types). For this particular study, SNP markers were used for genetic linkage map construction and QTL analysis. From a subset of SNP markers received from DArT, a filtering process to remove all redundant and non-informative

SNPs was followed whereby SNP markers that presented multiple genetic positions were removed. Moreover, SNPs with over 10% missing data or heterozygous alleles were also deleted.

Construction of a genetic linkage map

Inclusive composite interval mapping (ICIM) on the IciMapping 4.0 (http://www.isbreeding.net/) software was used for constructing a linkage map using a minimum logarithm of odds (LOD) value of 3.0. A Popo/Kariega linkage map was constructed using 1065 informative SNP marker loci derived from the DarT-seq GBS platform. Genetic distances between markers were calculated based on the Kosambi function (Kosambi, 1943). Ordering within linkage groups was conducted with nnTwoOpt function (an efficient approximate algorithm for solving traveling salesmen problems in which nearest neighbours are used for tour construction improvement) and rippling with the sum of adjacent recombination frequencies (SARF).

QTL analysis

Single environment QTL scanning was performed by single marker analysis (SMA) and inclusive composite interval mapping (ICIM) through stepwise regression by considering all marker information simultaneously.

5.3. Data Analysis

The severity data were incorporated into the established SNP-based linkage map and this information was subsequently used for individual environment QTL mapping. For ICIM, the function ICIM-ADD (inclusive composite interval mapping of additive and dominant QTLs) was used to identify marker trait associations. The walking speed chosen for all QTLs was 1.0 cM, and the probability in stepwise regression (*P* value inclusion threshold) was 0.001; this threshold corresponded to a highly conservative test for declaring the presence of QTLs. The threshold LOD scores were calculated using 1000 permutations with a type 1 error of 0.05. Significant QTLs were declared if the LOD threshold was greater than the calculated value at $\alpha = 0.05$. ICIM tests both

additive and epistatic effects, and it avoids the possible increase of sampling variance and the complicated background marker selection process that is a common problem in composite interval mapping. For additive mapping, ICIM has increased detection power, reduced false detection rate, and less biased estimates of QTL effects compared to CIM.

5.4. Results

SNP marker distribution in the linkage map

After a filtering process of all redundant and non-informative markers, 1072 out of 8339 SNP markers were used for the construction of a genetic linkage map. Of the 1072 SNP markers, 7 were not linked to the 21 wheat chromosomes, resulting to a total of 28 linkage groups. All 21 wheat chromosomes were represented by the linkage groups. The SNP markers covered 4990.61 cM of the wheat genome (Table 5.1), with a mean interval of 4.69 cM per marker. Most SNPs were assigned to the A and B genomes, with 520 (2150.46 cM) and 410 (1731.81 cM) SNPs, respectively, while the D genome harboured 135 SNPs (1108.34 cM) (Figure 5.1).

Table 5.1. Single nucleotide polymorphism (SNP) markers distributed on different chromosomes in the genetic linkage map

Linkage group*	Chromosome	Length (cM)	No. of SNPs
1	1A	176.08	79
2	1B	304.29	52
3	1D	171.11	36
4	2A	335.96	96
5	2B	309.28	37
6	2D	65.32	14
7	3A	432.28	61
8	3B	404.36	115
9	3D	218.87	15
10	4A	259.18	62
11	4B	169.95	9
12	4D	2. 20	2
13	5A	303.36	73
14	5B	264.26	59
15	5D	101.47	5

Table 5.1. Continued

Linkage group*	Chromosome	Length (cM)	No. of SNPs
16	6A	225.08	78
17	6B	105.29	31
18	6D	183.08	22
19	7A	418.52	71
20	7B	174.38	107
21	7D	366.29	41
Total	21	4990. 61	1065

* Linkage groups with unlinked markers are not included in this table

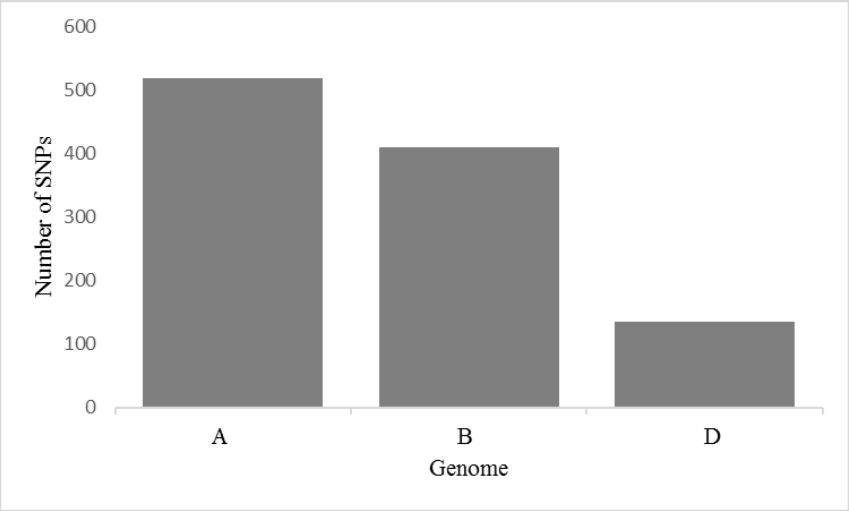

Figure 5.1. Number of single nucleotide polymorphism (SNP) markers assigned on A, B and D genome of wheat

Mapping of resistance QTL to leaf rust

The ICIM method of QTL mapping detected 10 putative minor QTLs with additive genetic effects involved in resistance to leaf rust when evaluated across four different environments at a threshold of LOD > 2.5 (Table 5.2). Of the 10 QTLs, four were contributed by Popo and the remaining six contributed by Kariega. QTL designated as *QLr.sgi-5A.1* on chromosome 5A was found to be common in two environments (Tygerhoek 2014 and 2015). The other QTLs were found to be inconsistent and varied with the test environments. The detected QTLs ranged in their LOD scores from 2.67 to 6.25, each of which explaining 2.63 to 10.48% phenotypic variation. Only one additive QTL (*QLr.sgi-7D.2**) was detected through SMA method on chromosome 7D in one environment (Tygerhoek 2015) which was contributed by Kariega. The same

QTL was detected through ICIM method. No QTL for leaf rust was simultaneously detected in all the test environments.

Table 5.2. Additive quantitative trait loci (QTLs) for adult plant resistance to leaf rust detected in the Popo/Kariega population of 170 recombinant inbred lines by inclusive composite interval mapping (ICIM) and single marker analysis (SMA) across four environments.

				Single or flanking markers				
Environment	QTL[a]	Chr	Pos (cM)	Left marker	Right marker	LOD[b]	PVE (%)[c]	Add[d]
Cedara 2014	*QLr.sgi-3A.1*	3A	305.00	SNPmarker1294_3A	SNPmarker1298_3A	3.01	6.14	-0.57
	QLr.sgi-4A.2	4A	101.00	SNPmarker1927	SNPmarker1932_4A	3.47	6.98	-0.61
Tygerhoek 2014	*QLr.sgi-5A.1*[e]	5A	93.00	SNPmarker2239	SNPmarker1136	4.12	10.06	2.21
Cedara 2015	*QLr.sgi-3A.2*	3A	342.00	SNPmarker1301	SNPmarker1218_3A	3.28	4.58	2.53
	QLr.sgi-3D	3D	84.00	SNPmarker1812_3D	SNPmarker1813_3D	3.30	4.40	-2.51
	QLr.sgi-7B	7B	70.00	SNPmarker3383_7B	SNPmarker3390_7B	2.67	4.48	-2.60
	QLr/Sr.sgi-7D.1.3[¥]	**7D**	**216.00**	**SNPmarker3536**	**SNPmarker3538**	**6.26**	**4.78**	**2.65**
Tygerhoek 2015	*QLr.sgi-2A*	2A	130.00	SNPmarker943_2A	SNPmarker946_2A	2.90	2.64	0.78
	QLr.sgi-5A.1[e]	5A	100.00	SNPmarker2239	SNPmarker1136	4.82	10.48	1.57
	QLr.sgi-7A	7A	273.00	SNPmarker3029_7A	SNPmarker3026	4.25	5.75	1.15
	QLr.sgi-7D.2	7D	257.00	SNPmarker3516_7D	SNPmarker3522_7D	4.86	18.37	2.07
	*QLr.sgi-7D.2**	7D	242.81	SNPmarker3516_7D		4.80	14.06	1.15

[a]QTLs are denoted according to McIntosh et al. (2003)

[b]LOD=Logarithm of odds, where values are the peak logarithm of odds score for the given QTL

[c]PVE=Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL

[d]ADD=Addtitive, where values indicate the additive effect of the QTL; negative value means that the allele was contributed by Popo

[e]Common QTL (shared between two environments)

[¥]QTL in bold control both leaf rust and stem rust

*QTL identified from single marker analysis

Chr=Chromosome; Pos=Position; cM=Centi Morgan

Mapping of resistance QTL to stem rust

The ICIM and SMA methods of QTL mapping detected 34 putative minor QTLs with additive effects involved in resistance to stem rust in the two test environments at a threshold of LOD > 2.5 (Table 5.3). Of the identified QTLs, 14 were contributed by Popo and the remaining 20 contributed by Kariega. The QTLs ranged in their LOD scores from 2.97 to 16.7, each explaining 2.45 to 21.21% phenotypic variation. The SMA method detected 17 additive QTLs involved in resistance to stem rust in one environment (Tygerhoek 2014) and five of these QTLs were contributed by Popo and four by Kariega. Loci *QSr.sgi-1B.2** and *QSr.sgi-1B.3** detected through SMA are speculated to be the same as *Qsr.sgi-1B.1* detected through ICIM method. No QTL for stem rust was simultaneously detected in all the test environments.

Table 5.3. Additive quantitative trait loci (QTLs) for adult plant resistance to stem rust detected in the Popo/Kariega population of 170 recombinant inbred lines by inclusive composite interval mapping (ICIM) and single marker analysis (SMA) in two environments.

Environment	QTL[a]	Chr	Pos (cM)	Single or flanking markers		LOD[b]	PVE (%)[c]	Add[d]
				Left marker	Right marker			
Tygerhoek 2014	*QSr.sgi-1B.1*	1B	60.00	SNPmarker493_1B	SNPmarker440_1B	6.10	21.21	1.23
	*QSr.sgi-1B.2**	1B	59.97	SNPmarker438_1B		7.10	3.16	1.41
	*QSr.sgi-1B.2**	1B	59.97	SNPmarker493_1B		7.10	3.16	1.41
	*QSr.sgi-1B.3**	1B	60.45	SNPmarker440_1B		8.42	3.66	1.52
	*QSr.sgi-1B.3**	1B	60.45	SNPmarker471_1B		8.42	3.66	1.52
	*QSr.sgi-1B.4**	1B	64.87	SNPmarker568		4.91	2.28	1.20
	*QSr.sgi-1B.5**	1B	66.64	SNPmarker573_1B		3.82	1.81	1.07
	*QSr.sgi-1B.6**	1B	71.49	SNPmarker269_1B		3.92	1.86	-1.08
	*QSr.sgi-1B.7**	1B	31.32	SNPmarker443		4.47	2.10	-1.16
	*QSr.sgi-1B.7**	1B	31.32	SNPmarker562		4.47	2.10	-1.16
	*QSr.sgi-1B.8**	1B	32.94	SNPmarker503		6.29	2.85	-1.36
	*QSr.sgi-1B.9**	1B	33.37	SNPmarker501		3.94	1.86	-1.08
	*QSr.sgi-1B.9**	1B	33.37	SNPmarker505		4.75	2.21	1.18
	*QSr.sgi-1B.9**	1B	33.37	SNPmarker550		4.64	2.17	1.17
	*QSr.sgi-1B.10**	1B	34.01	SNPmarker507		5.84	2.67	1.30
	*QSr.sgi-1B.11**	1B	34.42	SNPmarker685_1D		4.78	2.23	1.19
	*QSr.sgi-1B.11**	1B	34.42	SNPmarker683_1D		5.43	2.50	1.27
	*QSr.sgi-7D.1**	7D	103.33	SNPmarker3510_7D		4.64	2.17	1.17
	QSr.sgi-7D.2	7D	109.00	SNPmarker3510_7D	SNPmarker3492_7D	3.13	15.11	1.03
Tygerhoek 2015	*QSr.sgi-1A.1*	1A	122.00	SNPmarker169_1A	SNPmarker166_1A	8.22	3.20	2.57
	QSr.sgi-1A.2	1A	175.00	SNPmarker663	SNPmarker656	12.19	3.20	-2.58
	QSr.sgi-1B.18[e]	1B	79.00	SNPmarker281_1B	SNPmarker308_1B	9.70	3.15	-2.55
	QSr.sgi-1B.19[e]	1B	81.00	SNPmarker307_1B	SNPmarker294_1B	9.76	3.03	-2.50
	QSr.sgi-1B.20	1B	84.00	SNPmarker304	SNPmarker342_1B	8.80	3.19	2.57

Table 5.3. Continued

Environment	QTL[a]	Chr	Pos (cM)	Single or flanking markers		LOD[b]	PVE (%)[c]	Add[d]
				Left marker	Right marker			
Tygerhoek 2015	*QSr.sgi-1B.21*	1B	116.00	SNPmarker365	SNPmarker367_1B	9.13	3.21	-2.60
	QSr.sgi-2A	2A	27.00	SNPmarker2792	SNPmarker725_2A	8.28	2.60	2.29
	QSr.sgi-2D	2D	32.00	SNPmarker1155	SNPmarker1132_2D	15.39	3.20	-2.59
	QSr.sgi-3A	3A	26.00	SNPmarker1174_3A	SNPmarker1177_3A	13.86	3.20	2.58
	QSr.sgi-3B	3B	238.00	SNPmarker1571	SNPmarker1570_3B	6.24	3.16	2.58
	QSr.sgi-4A.1	4A	25.00	SNPmarker1866_4A	SNPmarker2305	16.19	3.19	2.57
	QSr.sgi-4A.2	4A	155.00	SNPmarker1964	SNPmarker1965	2.97	2.45	-2.21
	QSr.sgi.5A	5A	169.00	SNPmarker2202	SNPmarker2200	4.30	2.68	-2.32
	QSr.sgi.6A	6A	165.00	SNPmarker2641_6A	SNPmarker2640	16.74	3.19	2.57
	QSr.sgi.6B	6B	25.00	SNPmarker2713_6B	SNPmarker2841_6B	10.77	3.18	-2.56
	QSr.sgi.7B.1	7B	26.00	SNPmarker3471_7B	SNPmarker3320_7B	14.84	3.20	2.57
	QSr.sgi-7B.2	7B	51.00	SNPmarker3321_7B	SNPmarker3332	3.34	2.46	-2.22
	***QLr/Sr.sgi.7D.1.3*[¥]**	**7D**	**216.00**	**SNPmarker3536**	**SNPmarker3538**	**10.60**	**3.20**	**-2.58**

[a]QTLs are denoted according to McIntosh et al. (2003)

[b]LOD=Logarithm of odds, where values are the peak logarithm of odds score for the given QTL

[c]PVE=Phenotypic variance explained, where values indicate the phenotypic variation explained by the QTL

[d]ADD=Addtitive, where values indicate the additive effect of the QTL; negative value means that the allele was contributed by Popo

[e]Two close regions speculated to be the same QTL

[¥]QTL in bold controls both leaf rust and stem rust

*QTL identified from single marker analysis

Chr=Chromosome; Pos=Position; cM=Centi Morgan

Identification of QTLs conditioning both leaf rust and stem rust resistance

One putative QTL identified through ICIM method (*QLr/Sr.sgi.7D.1.3*) on chromosome 7D and derived from Popo, was found to be common between leaf rust and stem rust in the studied RILs (Tables 5.3 and 5.4). This QTL was detected on position 216 cM (long arm) with LOD 6.26 and 10.59 for leaf rust and stem rust, respectively. The percentage phenotypic variation explained by this QTL was 4.73% for leaf rust and 3.19% for stem rust. No other QTL was detected to be shared amongst the two traits.

5.5. Discussion

A genetic linkage map of the RIL population

Genomics research in general, and wheat molecular breeding in particular require accurate and detailed genetic maps. The GBS is a preferred high-throughput genotyping method owing to its high reproducibility and ability to offer a broad genome coverage with a large number of SNPs (Poland and Rife, 2012; Peterson et al., 2014; Li et al., 2015). This platform can be used to build a well saturated map. In the current study, a Popo/Kariega RIL population involving 170 RILs was used for map construction and integration. A high-density genetic map was successfully generated using SNP markers from a GBS platform. The GBS has been applied to construct high-resolution maps in maize (Ganal et al., 2011), wheat (Wang et al., 2014), rice (Arbelaez et al., 2015) and barley (Perovic et al., 2004; Wenzl et al., 2006).

In the present study, the linkage groups represented all 21 wheat chromosomes fairly evenly, with markers distributed across the A, B, and D genomes. The D genome contained the fewest loci (12.68%). This finding concurs with other hexaploid wheat maps in which fewer markers were found in the D genome, especially on chromosome 4D (Akbari et al., 2006; Prins et al., 2011; Bajgain et al., 2016; Prins et al., 2016).

Mapping of QTL conferring leaf rust resistance

The studied RILs showed transgressive segregation for leaf rust resistance. The test lines were either more resistant or susceptible than the parental cultivars Popo and Kariega. This indicated the presence of diverse APR QTLs with some additive characteristics that could be combined to develop highly resistant germplasm. In the present study, most putative QTLs were contributed by Kariega, with Popo only contributing four QTLs conferring resistance to leaf rust at the adult plant stage. The South African spring wheat cultivar Kariega has been found to consistently display moderate to high levels of APR to leaf rust in several studies, mainly due to the presence of *Lr34* (Ramburan et al., 2004; Prins et al., 2011). Hence, it was not surprising that this parental cultivar contributed the most to the resistance displayed for leaf rust in the field. Even though the D genome presented fewer markers with reduced polymorphism on the linkage map, most of the putative QTLs of additive effect identified in this study were located on the D genome.

Previously, the only known loci involved in leaf rust quantitative APR included *Lr12* on chromosome 4BL, *Lr13* on 2BS, *Lr22* (alleles a and b) on 2DS, *Lr34* on 7DS, *Lr35* on 2BS, *Lr37* on 2AS, *Lr46* on 1BL, *Lr48* on 2BS, *Lr49* on 4BL, *Lr67* on 4DL and *Lr68* on 7BL (McIntosh and Yamazaki, 1973). *Lr34/Yr18/Pm38/Sr57*, *Lr46/Yr29/Pm39/Sr58* and *Lr67/Yr46/Pm46/Sr55* are believed to be race non-specific and have pleiotropic effects to other diseases, including leaf rust, stripe rust, powdery mildew, stem rust and spot blotch (William et al., 2003; Espino et al., 2008; Herrera-Foessel et al., 2014). None of these QTLs were detected in this study. All of the putative QTLs detected in the present study to control leaf rust had not been reported before in previous studies, but some of the detected QTLs were identified at close proximity to the locations of known genes/QTLs. Therefore, it is speculated that the Popo/Kariega population potentially harbours unexploited genes for leaf rust resistance in wheat.

For example, two genomic regions designated as *QLr.sgi-7D2* (on position 257 cM) and *QLr.sgi-7D2** (on position 242.81 cM) identified on chromosome 7D through ICIM and SMA respectively, explained most of the phenotypic variation (18.37% and 14.06%). The two regions are probably one QTL, linked to SNPmarker3516_7D.

Though this QTL co-localize in the same chromosome with known APR gene *Lr34* (detected on 7DS) (Schnurbusch et al., 2004; Lillemo et al., 2008; Li et al., 2015), its position on the long arm of 7D has not been reported before. This result could mean that this is a novel region controlling leaf rust resistance.

On chromosome 5A, a putative QTL designated as *QLr.sgi-5A.1* was also detected in two environments (Tygerhoek 2014 and 2015), explaining 10.06% and 10.48% of the phenotypic variation, respectively. The positions of the QTL in the two environments is slightly different, with the QTL peaks mapping 7 cM apart but on the same linkage group. This is speculated to be the same QTL flanked by the same markers (SNPmarker2239 and SNPmarker1136). No known leaf rust resistance genes have been catalogued on chromosome 5A to date, however, several studies including Messmer et al. (2000), Singh et al. (2009), and Rosewarne et al. (2012) have reported a number of QTLs in this region. Singh et al. (2009) detected a significant QTL with additive effects on chromosome 5AS, contributing to lowering leaf rust response over years. The QTL on 5A identified in the present study is in close proximity with *QLr.cimmyt-5AL* identified by Rosewarne et al. (2012) and four QTLs identified by Kertho et al. (2015) reported to control seedling resistance to leaf rust. Further exploitation of the 5A chromosome is necessary as this region has been proven to harbour important leaf tip necrosis (LTN) genes, a trait which is phenotypically correlated with leaf rust resistance.

Mapping of QTL conferring stem rust resistance

Screening of APR to stem rust at Tygerhoek (2014) and Tygerhoek (2015) provided inconsistent QTLs. From the identified QTLs, two putative loci designated as *QSr.sgi-1B.1* and *QSr.sgi-7D.2* explained most of the phenotypic variation of 21.21% and 15.11%, respectively. These QTLs were both contributed by Kariega. Cultivar Popo contributed only 14 of the 34 identified QTLs. Most of these QTLs were detected on the B genome. This result agrees with Bajgain et al. (2016) where most of the QTLs detected from a nested association mapping analysis were from the B genome chromosomes. However, no QTL was detected in the LMPG-6/Popo population in their study. LMPG-6 is a highly susceptible spring wheat line developed by Knott (1990).

The large contribution of resistance QTLs from Kariega confirms that the parental cultivar carries some heritable and effective resistance genes/alleles, reinforcing its use in breeding for resistance to wheat rust diseases. The inconsistency in QTLs detected across the two environments, however, was possibly due to differences in weather conditions and more likely due to varied disease pressure. Further phenotypic analysis for agronomic attributes of the population used in this study is necessary to validate the identified resistance QTLs.

Environmental effect

It is evident that the environment had a significant effect on the expression of certain resistance genes/QTLs in the Popo/Kariega population. Analysis of variance (Chapter 4, Section 1.4) showed a significant effect of the environment on the response of the genotypes to both leaf rust ($P \leq 0.0001$) and stem rust ($P \leq 0.01$). In general, the ICIM method detected more QTLs with only three QTLs common between the ICIM and SMA methods. This is expected because the ICIM is a superior method of QTL detection compared to SMA. Environments possibly differed in the time of plant development when infection started, in the infection pressure, and most likely in the pathogen population and virulence spectra of the pathotypes. Thus different genes might be relevant for resistance in different environments. The loci controlling resistance might be specific to the test environment and perhaps to specific loci. Further, the vast differences in temperatures across seasons (mainly the drought experienced in the 2015 season) could have contributed to the differences in genes/QTLs expressed, hence the inconsistency within environments. The differences in inoculum load could have also contributed to the expression of certain resistance QTL that were not detected in some environments. Bagjain et al. (2016) detected some inconsistencies in QTL detection across different test environments. Hence continuous phenotyping is necessary to elucidate consistent and significant QTLs controlling resistance.

The results of this study shows that not all loci contributed to disease severity at the same level. Furthermore, the QTLs identified have additive effects in controlling leaf rust and stem rust resistance in the field. This suggests that when one or two of these

minor QTLs are present in a breeding line without other supporting resistance loci, they do not have an observable phenotypic effect under high disease pressure. This is typical of APR genes which have partial resistance. The identified additive QTLs could be exploited through marker assisted selection (MAS), gene pyramiding and other breeding strategies to transfer these QTLs into elite wheat lines.

5.6. Conclusions

Overall, the present study detected a total of 44 putative QTLs (10 for leaf rust and 34 for stem rust) with additive genetic effects in the studied RILs. One consistent genomic region designated as *QLr.sgi-5A.1* contributed by Kariega was identified on chromosome 5A controlling leaf rust at the Tygerhoek testing site during 2014 and 2015. Another QTL, *QLr/Sr.sgi-7D.1.3*, also derived from Kariega was detected under the two testing sites (Cedara 2015 and Tygerhoek 2015), controlling both leaf rust and stem rust and explaining 4.78% and 3.20% of the phenotypic variation, respectively. The identified QTLs are minor with additive characteristics of small to intermediate effects, suggesting that the individual effects of the alleles contributing to minor QTL can provide substantial disease resistance. The combinational effect of such 'undervalued' genes or gene alleles may result in reduced disease severity. Hence, the identified QTLs can possibly be explored as genetic markers to be used in marker-assisted breeding of wheat emphasising leaf rust and stem rust resistance. This study concluded that slow rusting APR to leaf rust and stem rust is complex. However, high levels of resistance can be achieved by combining multiple minor genes, additive in nature. Further work to validate the resistance loci identified in this study is necessary, not overlooking the effects of the environment. A combination of an increase in the size of the mapping population and addition of a large number of new markers, especially in the D genome may substantially improve the quality of the genetic map and resolution, assisting in fine mapping of resistance loci. Positional cloning of genes and QTLs through fine mapping within the gene-rich regions could lead to identification of more APR QTLs for better detection, mapping and estimation of gene effects. This may also lead to a development of reliable, user friendly and tightly linked or gene specific markers that can be used to accelerate the incorporation of novel resistance into elite breeding wheat lines.

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General Overview of Research Findings

Introduction and objectives of the study

Wheat (*Triticum aestivum* L.) is an important crop in South Africa next to maize in total production. It is grown mainly as a cash and food crop predominantly by commercial farmers. South Africa is the leading producer of wheat in the Southern African Development Community (SADC) region. However, South Africa's total wheat production and production area have declined progressively from 2.5 million tons and 974,000 hectares in 2002 to approximately 1.7 million tons and 500,000 hectares in 2013, in that order. The country is therefore, increasingly reliant on wheat imports to sustain domestic demand. Much of the wheat production area is being lost to other economically important and profitable crops such as canola, oat, maize and soybean, mainly due to different biotic and abiotic stress factors and socio-economic constraints affecting wheat production and productivity. Leaf rust caused by *Puccinia triticina* Eriks. and stem rust (*P*. *graminis* f. sp. *tritici*) are the most destructive foliar diseases of wheat worldwide, resulting in substantial yield losses. The recent detection of a potentially devastating wheat stem rust race Ug99 and its variants in South Africa has threatened wheat production in the country. Detection of virulent leaf rust races from Zimbabwe and Zambia with similar genetic lineages to those recently discovered in South Africa have also been alarming to the wheat industry.

Host plant resistance is the most profitable and environmentally safe strategy to control rust diseases. A continuous search for new sources of effective rust resistance genes is necessary to develop improved varieties with stable and durable rust resistance. Successful integration or pyramiding of a number of effective wheat rust resistance genes can enhance rust resistance durability. However, the limited availability of diagnostic molecular markers greatly impedes the screening and gene pyramiding processes. Pre-breeding and breeding efforts are well undertaken in many institutions around the world to address the challenges. In South Africa, the Agricultural Research Council–Small Grain Institute in collaboration with private wheat

breeding companies and Universities have invested significantly on wheat rust research to develop cultivars with effective rust resistance genes using a variety of genetic resources. Thus, this study complements the work that is being done in South Africa to search for new sources of resistance to leaf rust and stem rust. The specific objectives of this study were:

1. to assess adult plant resistance (APR) of selected wheat genotypes to leaf rust and stem rust and to develop segregating populations for resistance breeding,

2. to investigate the inheritance of adult-plant resistance in a recombinant inbred line (RIL) population of a cross between 'Popo' and 'Kariega', and to identify wheat breeding lines possessing both leaf and stem rust resistance, and

3. to undertake a genome-wide scan for identification of QTLs that significantly affect adult plant resistance to leaf rust and stem rust of a newly developed recombinant inbred line (RIL) population of wheat developed from a cross of two cultivars, Popo and Kariega, and to construct a genetic linkage map useful to locate genes/QTLs controlling other important agronomic traits in the Popo/Kariega genetic background.

Research findings in brief

Adult plant resistance of selected Kenyan wheat cultivars to leaf rust and stem rust diseases

This study evaluated eight selected Kenyan cultivars (Gem, Romany, Paka, Fahari, Kudu, Pasa, Ngiri and Popo) with known resistance to stem rust, together with local checks (Kariega, Morocco, McNair and SST88) for leaf rust and stem rust resistance at seedling stage and also across four environments. Selected diagnostic markers were used to determine the presence of known genes. All eight cultivars were crossed with two local checks (Kariega and Morocco) using a bi-parental mating design. The main findings of this chapter were as follows:

- The tested Kenyan wheat cultivars exhibited adult plant resistance not only to stem rust but also to leaf rust.
- The presence of two APR genes, i.e., *Sr2/Yr30/Lr27/Pbc1* and *Sr57/Lr34/Yr18/Pm38* was confirmed in some of the tested cultivars. Resistance gene *Lr34* was confirmed in the cultivars Gem, Fahari, Kudu, Ngiri and Kariega, while *Sr2* was present in Gem, Romany, Paka and Kudu.
- The seedling resistance gene, *Sr35,* was only detected in cultivar Popo.
- Overall, the study developed 909 $F_{6:8}$ recombinant inbred lines (RILs) as part of the nested mating design.

Adult plant resistance to leaf rust and stem rust of wheat in a newly developed recombinant inbred line population

This study evaluated a panel of 179 recombinant inbred line (RIL) population of a cross between two parental cultivars (Popo and Kariega) and three susceptible check varieties (Gariep, SST88 and Morocco). The RILs were field evaluated across four diverse environments for resistance to leaf rust and stem rust diseases. The main aim was to investigate the inheritance of adult-plant resistance in the RILs, and to identify wheat breeding lines possessing both leaf and stem rust resistance. The main outcomes were as follows:

- Analysis of variance indicated highly significant ($P < 0.001$) differences among the tested RILs for both pathogens across the testing environments.
- The broad sense heritability estimates were 0.53 and 0.77 for leaf rust and stem rust, respectively, suggesting that the use of the newly developed RILs in the genetic background of Popo/Kariega can enhance pre-breeding for rust resistance against the two diseases.
- Twenty-six RILs had average severity scores that were better than the parental varieties, and showed higher levels of resistance to both pathogens. Seeds from the best performing lines are available for public use and have been shared with two major South African breeding companies; Sensako and Pannar.

Linkage mapping and quantitative trait loci analysis of genes conferring adult plant resistance to leaf rust and stem rust of wheat

In this study, a Diversity Array Technology-sequencing (DArT-seq) methodology was used for genetic analysis and to develop a linkage map using a newly developed recombinant inbred line population derived from a cross between two spring wheat cultivars *viz.* Popo and Kariega. The RIL population was evaluated for adult plant resistance (APR) to leaf rust and stem rust under field conditions across two diverse environments. Quantitative trait loci analysis was performed using single marker analysis (SMA) and inclusive composite interval mapping (ICIM) methods with QTL IciMapping 4.0 software. The main findings of this chapter were as follows:

- A total of 44 putative QTLs (10 for leaf rust and 34 for stem rust) with additive genetic effects were detected on 14 chromosomes explaining 2.45 to 21.21% of the phenotypic variation present in the RILs, however, 42 of these QTLs were not consistent across environments.
- A consistent genomic region designated as *QLr.sgi-5A.1* was identified on chromosome 5A to control leaf rust.
- Another consistent QTL designated as *QLr/Sr.sgi-7D.1.3* was identified on chromosome 7D contributing to both leaf rust and stem rust resistance.

Implications of research findings to resistance breeding for leaf rust and stem rust of wheat in South Africa

- The developed 909 $F_{6:8}$ recombinant inbred lines (RILs) can be used as useful genetic resources for further studies and will add value in the identification and validation of a new set of APR genes that are present in the Kenyan cultivars used as parents.
- Twenty-six RILs performed better than the parental varieties, and showed higher levels of resistance to both leaf rust and stem rust. The selected RILs can be valuable genetic resources for breeding against leaf and stem rust and can be used in pre-breeding programmes aiming at developing germplasm with stable and durable resistance.
- The identified QTLs can possibly be explored as genetic markers to be used in marker-assisted breeding of wheat, emphasising leaf rust and stem rust resistance.

- Further work to validate the resistance loci identified in this study is necessary, not overlooking the effects of the environment.
- Future studies are required, focusing on larger sizes of mapping population and new markers for fine mapping of resistance gene loci. Positional cloning of genes and QTLs through fine mapping within the gene-rich regions could lead to identification of more APR QTLs for better detection, mapping and estimation of gene effects.