Pathogen variation and genetic control of *Puccinia triticina* in Zimbabwe

by

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Declaration

I, Nyashadzashe Chiuraise, declare that the thesis I herewith submit for the Doctoral Degree in Plant Pathology at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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Dedication

I dedicate this thesis to my family for nursing me with affection, love and their dedicated partnership for success in my life.

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List of Abbreviations

Abbreviation	Meaning
AEC	Average environment coordination
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive main effect and multiplicative interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
APR	Adult plant resistance
ARC-SG	Agricultural Research Council-Small Grain
ASO	Allele specific oligonucleotide
ASR	All-stage resistance
ART	Agricultural Research Trust
AUD$	Australian dollar
bp	Base pairs
BGRI	Borlaug Global Rust Initiative
BIC	Bayesian Information Criteria
CAPS	Cleaved Amplified Polymorphic Sequence
CBI	Crop Breeding Institute
$ChCl_3$	Chloroform
CIMMYT	International Maize and Wheat Improvement Centre
cm	Centimetre(s)
cM	Centimorgan
CSI	Variety superiority index
CTAB	Cetyltrimethylammonium bromide
DAPC	Discriminant Analysis of Principal Components
DArT	Diversity Arrays Technology
dCAPS	Derived Cleaved Amplified Polymorphic Sequences
df	Degrees of freedom
DNA	Deoxyribonucleic acid
E	Environment
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed Sequence Tags
EtBr	Ethidium bromide
F_{IS}	Inbreeding coefficient

Abbreviation	Meaning
f. sp.	*Forma specialis*
F_{ST}	F-statistics
G	Genotype
gDNA	genomic DNA
GGE	Genotype and genotype-environment model
GPS	Global Positioning System
GS	Genomic selection
GWAS	Genome-wide association study
GxE	Genotype-by-environment interaction
g	Relative centrifugal force
h	Hour(s)
ha	Hectare(s)
H	High (virulent)
HCL	Hydrochloric acid
H^2	Broad sense heritability
H_e	Expected heterozygosity
H_o	Observed heterozygosity
H_0	Null hypothesis
INDELL	Insertions/Deletions
I_A	Index of association
IAA	Isoamylalcohol
IPCA	Interaction Principal Component Axes
IT(s)	Infection type(s)
K	“True” number of sub-populations
kb	Kilobyte
kPa	Newtons per square metre
kV	Kilovolt
L	Litre(s) or Low (avirulent)
KASP	Kompetitive Allele Specific PCR
kg	Kilogram(s)
kg/hl	Kilogram per hectolitre
Lr	Leaf rust resistance
LRCI	Leaf rust coefficient of infection

Abbreviation	Meaning
LSD	Least significant difference
m	Metre(s)
m^2	Square metre(s)
M	Million or Molar
MAD	Marker-assisted detection
MAS	Marker-assisted selection
masl	Meters above sea level
mb	Moisture basis
MCMC	Monte Carlo Markov Chain
MCPA	2-methyl-4-chlorophenoxyacetic acid
ME	Mega-environment(s)
MET	Multi-environment trials
mg	Milligram(s)
$MgCl_2$	Magnesium chloride
min	Minute(s)
ml	Millilitre(s)
MLG(s)	Multilocus genotype(s)
mm	Millimetre(s)
mM	Millimolar
MR	Moderately resistant
ms	Mean square
MS	Moderately susceptible
NA	North America
NaCl	Sodium chloride
ng	Nanogram(s)
NJ	Neighbour-joining
nm	Nanometre
NPK	Nitrogen, Phosphorus and Potassium
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal component analysis
PCR	Polymerase chain reaction
pH	Potential Hydrogen

Abbreviation	Meaning
Pgt	*Puccinia graminis* f. sp. *tritici*
Pst	*Puccinia striiformis* f. sp. *tritici*
Pt	*Puccinia triticina*
QTL	Quantitative Trait Locus
R	Resistant
R	Pearson correlation coefficient
RAPD	Random Amplified Polymorphic DNA
RARS	Rattray Arnold Research Station
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
S	Susceptible
r/s	Revolutions per second
SA	South Africa
SADC	Southern Africa Development Community
SCAR	Sequence Characterised Amplified Region
SNP	Single Nucleotide Polymorphism
spp.	Species
Sr	Stem rust resistance gene
S	Second(s)
ss	Sum of squares
SSA	Sub-Saharan Africa
SSR	Simple Sequence Repeat
STS	Sequence-Tagged Site
T	Trace
T.	*Triticum*
TAE	Tris/Acetate/EDTA
TBE	Tris-HCl/Borate/EDTA
TE	Tris-HCl/EDTA
TEMED	N,N,N’,N’-tetramethylethylenediamine
t/ha	Tonnes per hectare
Tris	Tris (hydroxymethyl) aminomethane
UFS	University of the Free State

Abbreviation	Meaning
UK	United Kingdom
US$	United States of America dollar
USA	United States of America
UV	Ultraviolet
V	Volt
V	Volume
W	Weight
w/v	Weight per volume
Yr	Stripe (Yellow) rust resistance gene
χ^2	Chi-square
°C	Degrees Celsius
$\mu E/m^2/s$	Microeinstein
µg	Microgram(s)
µl	Microliter(s)
%	Percentage

Abstract

Genetic resistance is the most cost-effective approach to manage wheat leaf rust caused by *Puccinia triticina* Eriks. (*Pt*). However, the continuous emergence of more virulent races can deplete monogenic sources of resistance. The aim of this study was to determine the distribution, race and genetic diversity of *Pt* isolates in Zimbabwe and to characterise the sources of resistance in selected wheat accessions. In total, 104 single pustule isolates of *Pt* were established from infected wheat samples that were collected from the main wheat production regions of Zimbabwe during surveys from 2019 to 2021. Results from phenotyping a set of 46 differential and additional wheat lines revealed *Pt* race MCDS as dominant in Zimbabwe. Genotyping of 48 *Pt* isolates with 19 microsatellite markers, followed by DARwin and STRUCTURE analyses, confirmed a high genetic similarity between the Zimbabwean isolates and representative isolates of the South African *Pt* races MCDS, MCPS and MFPS. However, five isolates (19_1_2019, 24_3_2019, 5_1_2020, 20_1_2020, 23_2_2020) with genetic similarity to South African races SDDN and SCDS were detected. The detection of the five genetically distinct *Pt* isolates among the Zimbabwean isolates indicates genetic variation that could have arisen from foreign introductions.

The infection type (IT) data from screening the 39 differential lines and 72 Zimbabwean wheat accessions with nine *Pt* races were not informative in postulating the presence of any all-stage resistance genes (ASR). Forty-nine Zimbabwean varieties showed low (resistant) seedling ITs to all nine *Pt* races tested in the greenhouse and at least 53 varieties were strongly resistant with immune responses to races CFPS+*Lr20* and MFPS in the field. From these, 25 wheat lines with ASR to all *Pt* race isolates were crossed with an MCDS susceptible variety. Twenty-three varieties displayed an F_2 segregation ratio of 3:1, indicating the inheritance of a single dominant leaf rust (*Lr*) resistance gene. Molecular markers detected *Lr19* in 20 of these varieties. Five adult plant resistance genes (APR) namely *Lr27*, *Lr34*, *Lr37*, *Lr46* and *Lr68* were detected in the Zimbabwean germplasm, with *Lr46* being the most common and *Lr34* the least common.

A multi-environmental trial (MET) conducted over two seasons in Zimbabwe identified wheat varieties SC001, SC002, SC004, SC027 and SC W9101 as widely adapted with stable yields, acceptable leaf rust resistance while meeting the quality traits required in the wheat value chain. Overall, the outcomes of this study make a valuable contribution to shaping longer term strategies to control wheat leaf rust in Zimbabwe.

Keywords: *Puccinia triticina*, resistance gene postulation, genotyping.

General Introduction

Bread wheat (*Triticum aestivum* L.), is the second most consumed cereal in Zimbabwe, feeding more than 8 million (M) families daily. Wheat is grown under irrigation during the winter months of April to August by commercial and small-scale farmers on approximately 50 000 hectares (ha) mainly in the highveld and middleveld areas that annually receive more than 600 mm of rainfall. However, production is affected by the perennial occurrence of leaf rust caused by the fungus *Puccinia triticina* Eriks. (*Pt*). Under severe outbreaks, yield losses of up to 40% can occur and the disease is considered the primary reason for rejection of varieties by farmers and consequent replacement by seed companies.

Control of the wheat rusts (leaf-, stem- and stripe rust) in Zimbabwe relies mainly on a combination of genetic resistance and fungicide application. Although it is possible to minimise losses by the timely application of fungicides, it brings a huge financial liability to the farmer on top of irrigation costs that require electricity. Furthermore, overreliance on fungicides induces considerable selection pressure on the respective pathogens, which may result in fungicide tolerance or resistance. Moreover, chemical residues are considered harmful to consumers and environment. When large areas are planted with susceptible (S) or moderately susceptible (MS) varieties, the cost of repeated fungicide applications becomes economically and ecologically unsustainable. Thus, incorporating resistance genes into wheat varieties by breeders and their successful commercialisation is an environmentally friendly and more profitable way of leaf rust control. However, the ability of *Pt* races to quickly overcome major resistance genes when deployed singly in varieties, remains problematic. Therefore, selecting varieties with more complex sources of resistance with the potential to offer more durable resistance to *Pt* is of paramount importance.

Following the discovery of the wheat stem rust (*Puccinia graminis* f. sp. *tritici* Pers (*Pgt*)) variant Ug99 in the late1990s, the Borlaug Global Rust Initiative (BGRI) set up an international cereal rust monitoring system to track the spread of new more virulent rust races. Many countries have since established annual rust surveys with individual populations of the leaf-, stem- and stripe rust pathogens being examined for molecular variation and virulence. These surveillance efforts provide knowledge of the distribution, race and genetic diversity of rust isolates and highlight the potential impact of existing and emerging virulent races on wheat production. This information is fundamental in formulating and adopting appropriate national and international policies, investments and strategies in plant protection, plant breeding, seed systems and rust pathogen research. Similarly, South Africa (SA) has been conducting annual rust surveys since the 1980s and

this has helped in the early detection and implementation of preventative control measures to mitigate the impact of new rust races. In expanding the surveys to neighbouring countries, occasional studies have been conducted in Zimbabwe over the past few decades. Results from these studies indicated that new race incursions have significantly increased in SA and Zimbabwe. These studies reported phenotypically similar stem- and leaf rust races in SA and Zimbabwe, providing evidence of inoculum exchange in the Southern African region. This emphasises the importance of continued coordinated regional surveillance for proactive management of wheat rusts in these countries. Previous studies have not comprehensively examined the genetic relationship and diversity of *Pt* isolates between SA and Zimbabwe.

The genetic control of rusts has had considerable success in regions where race surveys are closely integrated with pre-breeding and breeding efforts targeting rust resistance, and with post-release management of wheat varieties. The current study evaluated *Pt* isolates and Zimbabwean commercial wheat varieties and SeedCo breeding lines, which were characterised using conventional phenotyping and genotyping. Assessing current Zimbabwean commercial wheat varieties and SeedCo breeding lines for resistance to common *Pt* races will help to determine whether any existing or possibly new *Pt* races pose a risk to commercial wheat production in Zimbabwe. This study was divided into five chapters, designed to provide a holistic picture of the status of wheat production and the control of leaf rust. Chapter 1 consists of a literature review. Chapter 2 reports on the phenotypic and genotypic characterisation of *Pt* isolates from Zimbabwe. In total, 104 single pustule *Pt* isolates were established from the samples collected in surveys from 2019 to 2021. A set of 46 differential and additional wheat lines were used to phenotypically characterise the individual isolates based on their avirulence/virulence profiles. Isolates were named according to the North American race nomenclature system. A representative set of 48 *Pt* isolates were genotyped for multilocus genotypes (MLGs) using a standard set of 19 microsatellite markers. Isolates from eight known South African *Pt* races were used as controls.

Limited information on the identity and number of leaf rust resistance (*Lr*) genes present within the current Zimbabwe commercial wheat varieties and advanced breeding lines of the SeedCo breeding programmes, is available. The lack of information makes it difficult to make informed decisions when planning new crosses and selecting advanced lines for final release considering sustainable resistance gene deployment. Chapter 3 was designed to detect and identify all-stage resistance (ASR) and adult plant resistance (APR)

genes in 72 Zimbabwean wheat varieties, which included the advanced SeedCo breeding lines and selected Zimbabwean commercial wheat varieties. Gene postulation efforts in the greenhouse included phenotyping the panel of 72 Zimbabwean wheat accessions with a representative isolate of Zimbabwean race MCDS and eight South African *Pt* races for ASR. The field responses of the 72 Zimbabwean wheat accessions to ascertain APR genes were determined in a screening nursery planted at the Corteva Agrisciences™ Research Farm near Greytown, KwaZulu-Natal during the 2021 growing season under artificial inoculation with *Pt* races CFPS+*Lr20* and MFPS. The trial was also conducted under natural infection of *Pt* race CFPS+*Lr20* on the Sensako Research Farm near Napier, Western Cape, during the 2020 and 2021 seasons under rain-fed conditions. Based on pedigree analysis a selected set of 25 Zimbabwean wheat lines, with resistance to all nine *Pt* race isolates, were crossed with the susceptible wheat variety PAN 3497 to generate F_1 seed. The resulting F_2 seedlings were phenotyped with an isolate of race MCDS to determine the F_2 segregation ratios. To identify the presence of known *Lr* resistance genes, 25 molecular markers linked to 17 *Lr* genes were used to screen the 72 Zimbabwean wheat varieties.

Before the commercial release of wheat varieties, breeders test advanced breeding lines in multi-location trials in order to identify specific genotypes adapted or stable in different environment(s), thereby achieving quick genetic gains. Frequently, genotype-by-environment (GxE) interactions complicate breeding, testing and selection of superior genotypes for grain yield. Therefore, Chapter 4 describes the field evaluation of 72 Zimbabwean bread wheat varieties for yield stability, leaf rust response and selected quality traits.

Statistical procedures such as additive main effect and multiplicative interaction (AMMI), as well as genotype and genotype-environment (GGE) model biplot analyses were used to determine the results of multi-location trials and GxE data. However, in addition to high grain yield, disease resistance and wide adaptability, an ideal wheat variety needs to maintain high end-use quality attributes required by millers, bakers and consumers. Seed from the respective wheat entries, harvested from different localities and years, was subjected to selected quality assessments.

Finally, Chapter 5 summarises the findings from all three research chapters, outlining important conclusions and recommendations. The following objectives applied to the three research chapters:

- To determine the avirulence/virulence and genotypic profiles of *Pt* rust isolates from Zimbabwe over two seasons in comparison with selected isolates of *Pt* races recently identified in SA;
- To characterise selected Zimbabwean commercial wheat varieties and SeedCo advanced breeding lines for their seedling ITs and field responses to the dominant *Pt* race(s) found in Zimbabwe, as well as selected South African races;
- To make crosses with selected leaf rust resistant (R) lines from the SeedCo breeding programme to a susceptible wheat variety to determine the F_2 segregation ratio and thereby the number of effective resistance genes involved;
- To screen selected Zimbabwean wheat varieties with molecular markers linked to known ASR and APR *Lr* genes, and;
- To evaluate selected Zimbabwean wheat varieties for yield, leaf rust response, quality traits and GxE effects in multi-environment trials (MET) over two seasons.

Chapter 1: Literature Review

1.1 Global importance of wheat

Wheat is one of the world's oldest plants to be domesticated by ancient civilisations between 8 000 and 10 000 years ago. Plants were derived from wild grasses native to the northwest arid regions of the Fertile Crescent (between Armenia and the Caspian Sea in western Asia) (Shiferaw *et al*., 2013; Erenstein *et al*., 2022). Currently, wheat is grown almost everywhere in the world and is regarded as a key grain commodity in the global economy. Wheat grain contributes 20% of calories and protein consumed globally and is consumed in 173 countries, with consumption levels exceeding 50 kg/capita/year in 102 countries (OECD-FAO, 2020). Covering an estimated 217 M ha annually with production of 750 M tonnes, wheat is among the most widely cultivated crops in the world (FAOSTAT, 2020).

Bread wheat (*T*. *aestivum* L.) is used mainly for making of a wide range of flat and leavened bread, and a range of baking products, account for over 90% of the total wheat production. Durum wheat (*T. durum* L.), which is utilised to manufacture macaroni and pasta (Pena, 2002) accounts for much of the remaining share (less than 10%). Bread wheat contains three sets of seven pairs of chromosomes giving a total of 21 pairs (AABBDD, 2n = 42), with seven pairs that belong to each of the A, B, and D genomes. The crop's complex genome gives it enormous elasticity, which accounts for its vast range of adaptations. It can thrive in a variety of settings, including warm and cold regions with little and high rainfall as well as under irrigation (Dubcovsky and Dvorak, 2007). On the other end, durum wheat is genetically different with only two sets of seven pairs of chromosomes from the A and B genomes, giving a total of 14 pairs (AABB, 2n = 28) of chromosomes resulting in entirely different flour quality characteristics (Van Poecke *et al*., 2013).

At least 120 nations, covering both emerging and developed economies, grow wheat throughout Asia, Europe, the Americas, Africa and Oceania (Xiong *et al*., 2020). From an agronomic perspective, wheat performs better in temperate environments lying between latitudes 30°N and 60°N, 27°S and 40°S and altitudes above 1200 meters above sea level (masl). Despite climatic variations, wheat is effectively produced with irrigation in hot and dry lowlands with lower altitudes (<400 masl). And also beyond latitudes boundaries up to 67°N and 45°S and near to the equator in tropical highlands of Central America, Latin America and Africa as a rain-fed crop and even (Tadesse *et al*., 2010). Wheat is grown over 150 M ha in environments where sub-zero temperatures occur during the wheat growing season due to its ability to withstand frost. Most staple crops cannot be grown under freezing environmental conditions since they are prone to frost damage. Rye, oats,

triticale, barley, canola, and a few legumes are examples of crops that can withstand little frost. As a result, wheat is one of the few physiologically and commercially viable crops that can thrive in regions with sub-zero temperatures during the crop cycle (Dixon, 2007).

Globally, Asia contributes 44% of the world's total wheat production, followed by Europe (34%) and the Americas (15%), with a modest but comparable share of between 3.4-3.5% for Oceania and Africa (Figure 1.1). Despite a 5% reduction for the Americas (down from 20%, related with the growth in maize and soybean output) and minor increases of 1-2% in each of the other areas, the relative production distribution by region has mostly stayed stable over the past few decades.

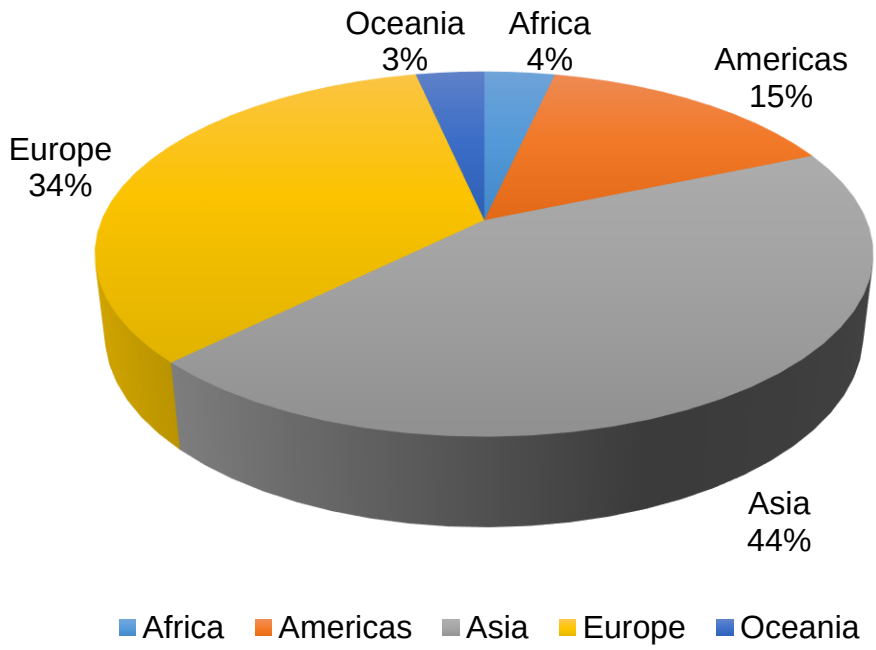

Figure 1.1: Distribution of global wheat production by region. (Prepared with data from FAOSTAT, 2020).

A handful of nations have dominated wheat production, with China, India, Russia, Ukraine, the United States of America (USA), Canada and France accounting for 53% of the global production. Similar nations have dominated wheat production since the 1960s, albeit with changing trends in different countries over time. For instance, production in both China and India more than doubled from 10+ M tonnes each in the early 1960s to each surpassing 100 M tonnes each today, becoming the top two producers of wheat in the world (OECD-FAO, 2020). The Green Revolution, which included the use of high-yielding wheat varieties, fertiliser, efficient irrigation techniques, and legislative assistance, was what led to India's development (Eliazer-Nelson *et al*., 2019).

According to FAOSTAT estimates, the world will require around 900 M tonnes of wheat by 2050 from its current production level of 760 M tonnes (FAOSTAT, 2020). This demand excludes the requirement for livestock feed and the negative effects of climate change on wheat production. To meet this demand, developing countries must increase their wheat production output by 77% (Tadesse *et al*., 2019). According to projections, Sub-Saharan Africa (SSA) would consume 76.5 M tonnes of wheat in 2030, with 49 M tonnes or around 63% of its total demand coming from imports (OECD-FAO, 2020). Therefore, SSA relies on imports of at least US$12 billion worth of grain each year to meet the gap in consumption demand thereby depleting the meagre foreign currency reserve of the respective countries.

1.2 Wheat production and consumption in Sub-Saharan Africa

Historically, wheat was not a major staple crop in SSA. Wheat and its by-products have gradually gained importance as a result of the rapid population rise, greater urbanisation and changes in dietary preferences (Sharma *et al*., 2015). The average annual wheat consumption of 30 kg/capita in SSA is still significantly lower compared to North Africa's average annual wheat consumption rate of 200 kg/capita (Shiferaw *et al*., 2013). Sub-Saharan Africa accounts for a third of the total wheat production in Africa. Over 95% of the wheat farmed in the region is produced by just seven SSA nations: Ethiopia, SA, Sudan, Kenya, Zimbabwe, Tanzania and Zambia listed in descending order (FAOSTAT, 2017). Ethiopia is the top wheat producer in SSA, producing an average of 3.6 M tonnes annually on an average area of around 1.7 M ha primarily in Arsi, Bale, Shoa, Gojam, Gondar, Wollo and Tigray regions of the country. With an average annual production of 2.2 M tonnes on an average area of 524 000 ha, SA is the second top producer with an average productivity ranging from 2.4 tonnes per hectare (t/ha) under rainfed to 7.9 t/ha under irrigated conditions (Grain SA, 2021). The major wheat-growing areas in South Africa include the Free State, Northern Cape, North West, KwaZulu-Natal, Mpumalanga, Gauteng, Limpopo, and the southern portions of the Western Cape (Swartland and Rûens). Spring wheat is the pre-dominant cultivated wheat type in SSA. However, in the Free State province, winter- and facultative wheat varieties are predominantly grown under restored soil moisture, accounting for about 20% of production in the country. Spring wheat is grown during the winter season under irrigation on the interior as well as under rainfed conditions in the Western Cape (Negassa *et al*., 2013, Grain SA, 2021).

Kenya produces an average of 375 000 tonnes annually on an estimated production area of 150 000 ha, which includes Meru Central, Nakuru, Keiyo, Trans Mara, Nyandarua, Trans

Nzoia, Uasin, Gishu and Laikipia as the most important wheat growing regions. Countries such as Eritrea, Uganda, Rwanda, Malawi, Mozambique, Lesotho, the Democratic Republic of Congo and Burundi have a minimal total annual average varying from 3 000 to 80 000 tonnes. Between 1970 and 1990, Zimbabwe reported the highest average wheat yield in SSA of 4.8 t/ha. The production trends have since changed with Zambia reporting the highest yield of 6.8 t/ha between 2010 and 2014, followed by SA (3.7 t/ha), Kenya (2.6 t/ha), Zimbabwe (2.5 t/ha) and Ethiopia (2.3 t/ha) (FAOSTAT, 2017). Consequently, Eastern and Southern Africa are the two key wheat growing regions in Africa, mainly due to the cooler winters favourable for spring wheat development.

1.3 Wheat production in Zimbabwe

European missionaries brought the cultivation of bread wheat to Zimbabwe in the late 19th century, but it only became a significant crop after the Unilateral Declaration of Independence in 1965 when grain imports were reduced, driving the country's need for self-sufficiency in the production of staple grains (Morris, 1988). The 2 000 commercial wheat producers in the country at the time met the challenge by quickly developing a profitable wheat business. According to historical data (Figure 1.2), the increase in production between 1965 and the present resulted from both an expansion of the area planted under wheat and a steady increase in yields.

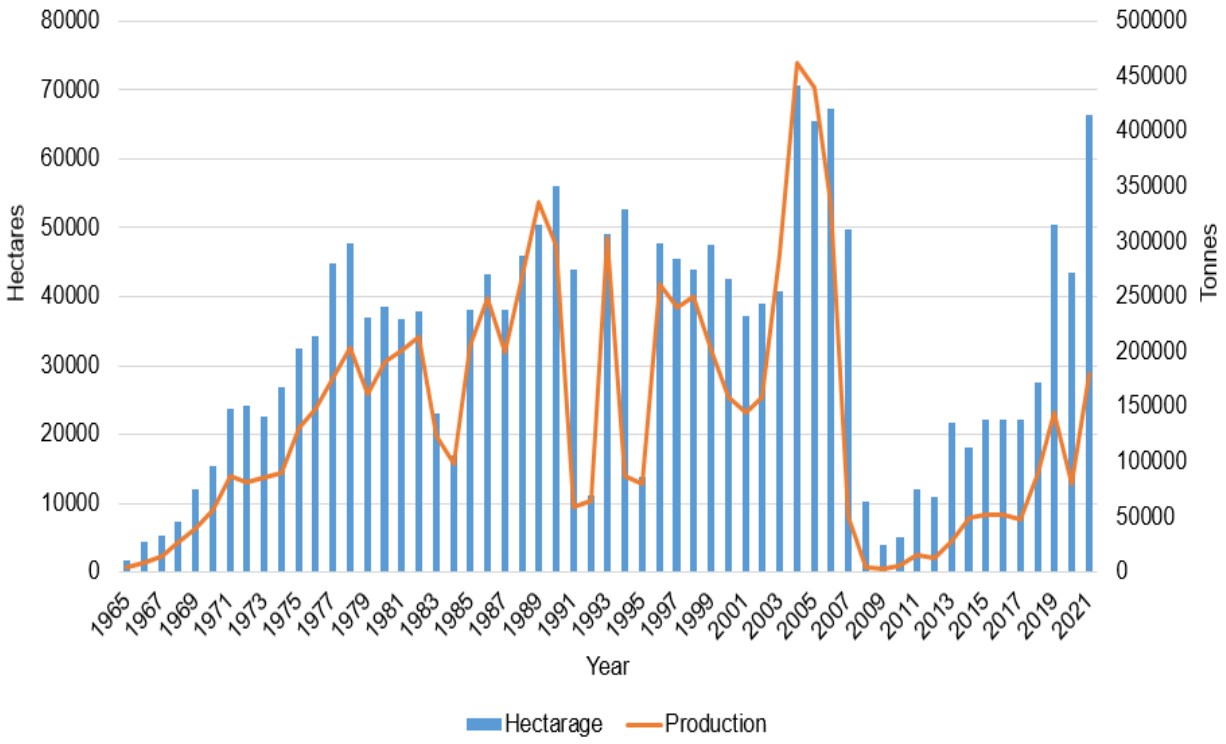

Figure 1.2: Trends in the Zimbabwean wheat cultivated area in hectares and production in tonnes from 1965 to 2021. (Data compiled from Zimstat, 2021).

The development of the domestic wheat industry was made possible by several factors. Firstly, Zimbabwe's wintertime (May to August) climate is often conducive to the production of irrigated spring wheat. Agro-ecological regions in Zimbabwe are classified into five regions based on the average annual rainfall they receive (Figure 1.3). Region I receives an average annual rainfall of above 1500 mm and region V has an annual average rainfall of less than 400 mm. Most spring wheat in Zimbabwe is grown in the highveld of regions I and IIA (>1 200 masl) and middleveld of regions II and III (800 to 1 200 masl). The winter temperatures in these regions range between 0-20°C which is conducive for spring wheat development. In the lowveld regions IV and V (<800 masl), wheat is mainly grown by small-scale farmers in irrigation schemes. Mashonaland West, Mashonaland Central, Mashonaland East and Harare provinces account for more than 80% of the wheat production in Zimbabwe. This is in contrast to many SSA countries north of Zimbabwe close to the equator where temperatures are warmer all year round and not conducive for spring wheat to thrive.

Secondly, switching to wheat production was not too difficult for commercial farmers because wheat production technologies were similar to those used for other crops previously grown in Zimbabwe. Farmers took less time to move to wheat production because a large portion of the equipment required for soil preparation, fertiliser and pesticide application and irrigation was already available.

Thirdly, government initiatives offered compelling incentives for commercial farmers to start growing wheat. Producer prices for wheat were kept higher than import parity prices throughout the second part of the 1960s, and subsidised credit schemes were implemented to encourage wheat production. Since the 1980s, there has been an inconsistent pattern in production trends due to the effect of nonaligned government policies in support of producers. However, the introduction of a government subsidy in 2019 has maintained the production area above 40 000 ha with a further increase forecasted due to improved irrigation infrastructure.

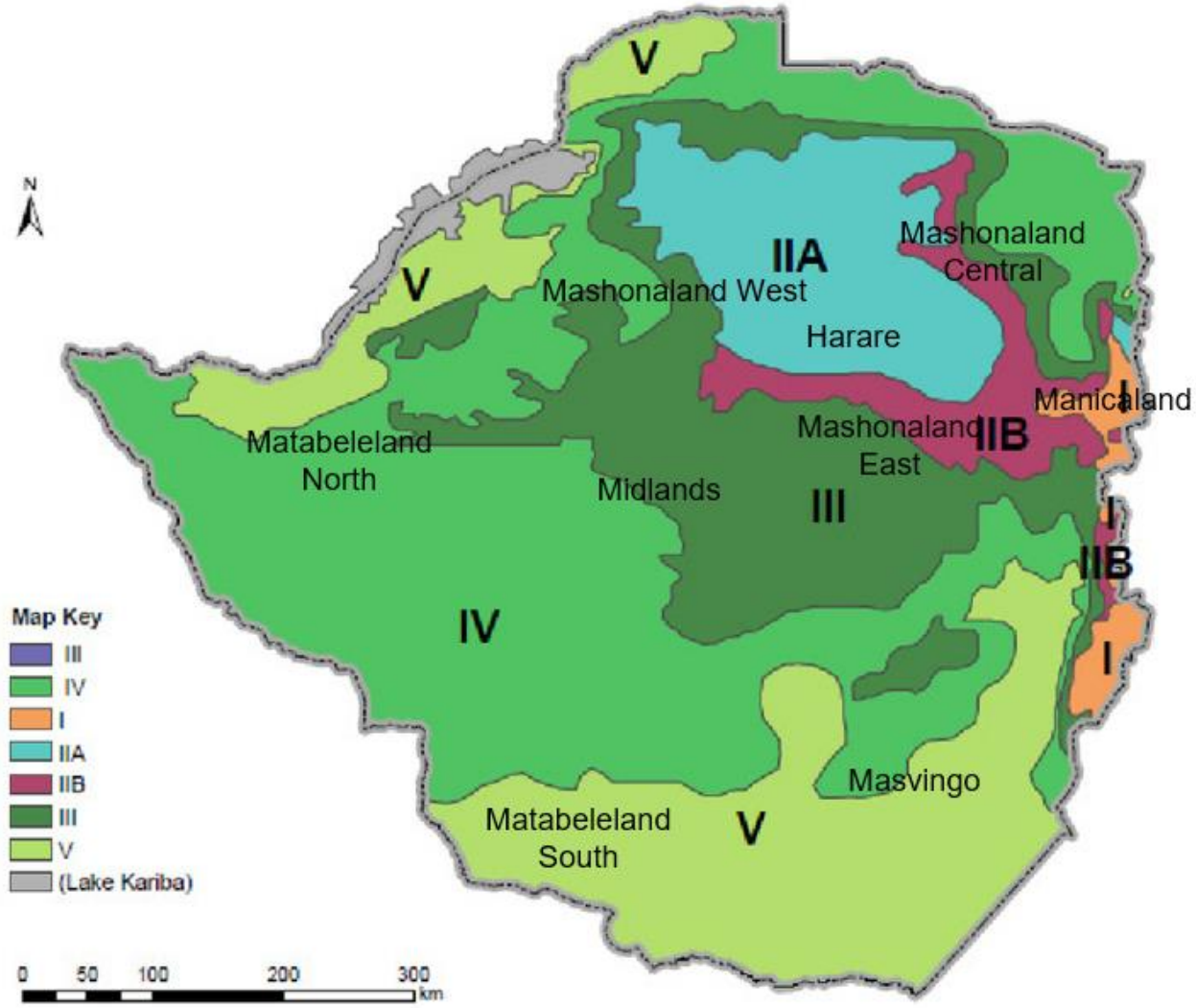

Figure 1.3: Agro-ecological areas in Zimbabwe showing the main wheat growing regions.

1.4 Challenges in wheat production

With the increase in the demand for wheat products coupled with the world population estimated to surpass nine billion people by 2050, future wheat production must increase exponentially (Weigand, 2011). In this regard, the yearly production of cereal is expected to increase by over one billion tonnes (Rosegrant and Agcaoili, 2010). However, the quest to match yield and quality in wheat is faced with several constraints. Reduction in the availability of suitable farmland, wheat production in Zimbabwe and throughout the world is continuously challenged by the effects of climate change and a wide range of unanticipated biotic and abiotic pressures. Climate change characterised by less and inconsistent rainfall patterns (drought), rising temperature (heat), or excessive rainfall (flooding) poses a significant challenge to humanity and the agriculture sector as a whole. The biotic stresses in wheat production include pests like insects (Russian wheat aphid), fungal diseases (stripe rust, stem rust, leaf rust, septoria and fusarium) and bacterial

disease (bacterial leaf streak) making agricultural productivity less predictable (Bakala *et al*., 2021; Juroszek and Tiedemann., 2013). Additionally, a loss of genetic diversity in search for superior, high perfoming varieties may be a factor in the sharp increase in pathogen emergence, which may further threaten wheat production.

1.4.1 Abiotic stresses

The most significant abiotic stressors in rain-fed ecosystems of the East African highlands and the Western Cape in South Africa include pre-harvest sprouting, drought, erosion, soil acidity, and waterlogging. For irrigated wheat in Zimbabwe, Zambia and Malawi the most important abiotic constraints are heat stress, water availability and irrigation costs from equipment installation and electricity supply. Heat stress associated with climate change during the reproductive and developmental stages of wheat cause significant yield losses, early leaf senescence, negative biochemical and physiological changes resulting in poor grain quality (Chand *et al*., 2014). Asseng *et al*. (2015) reported that a 6% reduction in worldwide wheat production is expected for every 1°C rise in temperature. In China, wheat yield reductions of up to 10% are estimated for a 1°C temperature increase during the growing season (You *et al*., 2009).

1.4.2 Biotic stresses

Fungi like *Puccinia* spp. (rusts), *Septoria* spp. (septoria leaf blotch), *Blumeria graminis* (powdery mildew) and *Fusarium* species are among the top ten most important fungal pathogens (Dean *et al*., 2012). Historical and current sources report epidemics leading to some severe yield losses in wheat caused by these pathogens. It is anticipated that new pests and diseases will emerge as a result of climate change, as evidenced by the epidemics of stem rust strain Ug99 with virulence to *Sr31* in Eastern and southern African countries (Pretorius *et al*., 2000) and stripe rust outbreaks across Central and West Asia and North Africa (Solh *et al*., 2012).

Stripe (yellow) rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* (*Pst*), a pathogen highly prevalent in temperate regions with cool day temperatures (12-20°C) combined with wet weather conditions, is the most common and devastating disease in the highlands of East Africa. Outbreaks of stripe rust following the collapse of resistance in well-known varieties like Wabe and Dashen, which possessed the *Yr9* gene from the 1B/1R translocation, have resulted in severe yield losses of up to 100% in the Ethiopian highlands (Hulluka *et al*., 1991; Badebo *et al*., 2008). Similarly, Galema (HAR 604) and Kubsa (HAR 1685) with the *Yr27* gene were also dominant bread wheat varieties in the highlands and mid-altitude areas of Ethiopia until they were wiped out by stripe rust in 2010 causing up

to 100% yield loss (Hodson, 2011). Stripe rust was reported for the first time in Zimbabwe in 2018 and race 30E142A+, which was genotypically closely related to the Kenyan race Ken12/09, was detected (Boshoff *et al*., 2019). In seedling assessments, this race was virulent to 22 of the 24 tested Zimbabwean bread wheat varieties compared to the three using an isolate of South African race 6E22A+. The race has not yet been detected in SA and could therefore pose a threat in future since there is evidence of wind-mediated rust-inoculum migration between the two countries (Visser *et al*., 2019).

Stem rust caused by *Pgt* is prevalent in low and mid-altitude areas (≤800 masl) with warmer day temperatures (20-35°C). The Ug99 race appeared in Uganda in 1998, moved from Africa to the Middle East, and evolved into several variants, all of which served to highlight the impending threat to wheat supply (Pretorius *et al*., 2000). According to early estimates, 90% of wheat varieties worldwide were sensitive to Ug99 and its variants (Singh *et al*., 2015). Three variants of race Ug99 namely TTKSF, PTKST and TTKSF+ were first detected in SA in 2000, 2009 and 2010, respectively. These three races were also reported in Zimbabwe between 2009 and 2010, with race PTKST detected in Mozambique in 2010. A *Pgt* race similar to that first detected on the variety Digalu causing a severe epidemic in Ethiopia in 2014, was detected in Germany (Olivera Firpo *et al*., 2015; 2017). Race TKTTF is dominant and regarded as a serious danger to wheat production in the East African region, even though it does not form part of the Ug99 race group (Olivera Firpo *et al*., 2015).

Leaf rust, caused by *Pt* (Figure 1.4), is the most common and widely distributed of the three wheat rust diseases (Huerta-Espino *et al*., 2011). The disease is prevalent in areas with warmer temperatures (15-25°C) accompanied by moist conditions. Infection-related yield losses are ascribed to a decline in kernel weight and the amount of grains produced per head. Although leaf rust causes varying degrees of grain losses across geographical regions, the disease has a considerable economic impact (Kolmer, 2005). Leaf rust is a problematic disease because the pathogen exhibits high genetic diversity with a constant emergence of new virulence combinations. The pathogen displays high adaptability to a wide range of climatic conditions (Kolmer, 2005; McCallum *et al*., 2016).

Figure 1.4: Flag leaf responses to *Puccinia triticina* for a resistant (SC W9101, left) and susceptible (PAN 3497, right) wheat variety following inoculation with races CFPS+*Lr20* and MFPS in a field trial.

1.5 History and distribution of *Puccinia triticina*

Puccinia triticina occurs more regularly and in more regions in the world than *Pgt* and *Pst*. The fungus is biotrophic and heteroecious, therefore requires a telial/uredinial host such as wheat, rye, triticale and an alternate (pycnial/aecial) host (*Isopyrum fumaroides* or *Thalictrum speciosissimum*) to complete its life cycle. Historians speculate that *Pt* could have originated from the Fertile Crescent region of the Middle East, where the natural range of the primary and alternate hosts overlap (D'Oliveira and Samborski, 1966). In other regions of the world like North America (NA), *Pt* was introduced with the advent of wheat cultivation in the early 17th century (Chester, 1946). However, wheat leaf rust was not regarded as a disease of economic importance as it did not affect grain quality as much as other diseases such as Fusarium head blight (Goswami and Kistler, 2004) and stem rust (Leonard and Szabo, 2005). Historical records suggested leaf rust was already present in SA over a century ago (Pretorius *et al*., 2007). The first pioneering descriptions of physiologic races of leaf rust in SA were made by Verwoerd in the 1930s (Verwoerd, 1937). Yield losses in wheat from *Pt* infections are usually the result of decreased numbers of kernels per head and lower kernel weights. *Puccinia triticina* is now recognised as an important pathogen in wheat production worldwide, causing significant yield losses over large geographical areas (Roelfs *et al*., 1992; Marasas *et al*., 2004).

Leaf rust is widely spread and is a major production constraint in Asia (central, south and southeast), North Africa, Europe, North and South America, New Zealand and Australia (Roelfs *et al*., 1992). The world's wheat growing areas were clustered into epidemiological regions based on the general direction of wind and spread of urediniospores in each cropping year. These regions include: 1) México, Canada and the USA; 2) South America and South Asia; 3) West Asia, Eastern Europe and Egypt; 4) Southern Africa; 5) Northern Africa and Western Europe; 6) the Far East; 7) South-East Asia; 8) Australia and New Zealand (Huerta-Espino *et al*., 2011). Suitable alternate hosts to enable *Pt* to complete its sexual life cycle are rarely present despite the fact that leaf rust is found virtually everywhere where wheat is grown. Thus, the *Pt* populations found in most parts of the world are clonal (Bolton *et al*., 2008). The lack of a viable alternate host and molecular genotyping data show that the sexual life cycle is a small source of genetic diversity for *Pt* in most wheat producting areas and does not contribute epidemiologically to disease transmission (Kolmer, 2005). Despite this drawback, over 70 races of the pathogen are detected annually in NA (Kolmer *et al*., 2007) where the pathogen persists through asexual reproduction from urediniospores. The urediniospores, once released can be widely dispersed by wind, insects or water and infect host plants grown several kilometres away which may result in rust epidemics across countries and even continents (Visser *et al*., 2019).

1.6 Nomenclature of *Puccinia triticina* races

In the early 1920s and 1930s, variation in the cereal rust fungi was often assessed by testing the different races (pathotypes) on wheat varieties that possess different types and numbers of resistance genes (Kolmer, 2013). Following the development of Flor's gene-for-gene theory, a better understanding of the genetics of rust resistance in wheat led to a change in the phenotypic variation assessments of isolates Consequently, several near isogenic lines of the variety Thatcher, each containing a single known *Lr* gene were developed to assess the variation in virulence of *Pt* (Carver, 2009). A system for designating virulence combinations of *Pt* isolates based on the North American system of nomenclature was accepted by the North American Wheat Leaf Rust Research Workers Committee in 1986 and later adopted by researchers worldwide (Long and Kolmer, 1989; Fetch and Jin, 2007). Isolates of the fungus are separated based on their ability to infect near-isogenic lines carrying a single *Lr* gene. The lines are also known as differential lines. The set of 16 *Lr* genes contained in standard differential lines for use in the nomenclature system are *LrB*, *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr16*, *Lr17*, *Lr18, Lr24*, *Lr26*, and *Lr30*. Additional single-gene lines may be used to supplement the standard set

of 16 differential lines (Kolmer, 2003). Host lines are grouped into four sets of four and infection types (ITs) are recorded for each line. The avirulence/virulence of isolates is determined by low (L) and high (H) ITs, respectively (Long and Kolmer, 1989). The avirulence/virulence phenotyping and naming of *Pt* isolates are described in detail in sections 2.2.3 and 2.2.4.

1.7 Host range of *Puccinia triticina*

Worldwide, the primary host of *Pt* is hexaploid bread wheat (Roelfs *et al*., 1992). *Puccinia triticina* also occurs on tetraploid durum (*T. turgidum ssp*. *durum*), domesticated emmer wheat (*T. dicoccon*), triticale (X Triticosecale) and wild emmer (*T. dicoccoides*). The secondary or alternate hosts are *T. speciosissimum* and *I. fumaroides* (Anikster *et al*., 2005; Bolton *et al*., 2008). *Puccinia triticina* is also present on *Aegilops cylindrica* (common goatgrass) in the Americas (Kolmer *et al*., 2007). Infections of *Pt* have not been noted in natural stands of wild wheat species such as *Ae. sharonensis*, *Ae. variabilis*, *Ae. bicornis, Ae. tauschii*, *Ae. longissima*, *Ae. ovata*, *T. urartu* or *T. timopheevii*. However, infections on these hosts can be obtained in artificial greenhouse inoculations with *Pt* isolates that are virulent to common wheat (Ordoñez and Kolmer, 2007).

1.8 Life cycle of *Puccinia triticina*

The leaf rust-causing pathogen *Pt* is a heteroecious, macrocyclic and biotrophic fungal species whose life cycle contains five unique spore stages and genetically diverse host species (Kolmer, 2013). Urediniospores produced on wheat hosts are dikaryotic that can re-infect the primary host under suitable environmental conditions (20-25°C and free moisture on the leaf surface) (Figure 1.5A). As the host plant develops, the uredinial infections mature to produce dikaryotic two celled teliospores in the asexual cycle. The teliospores are brown-black with thick, smooth walls (Figure 1.5B). In Mediterranean climatic regions, teliospores allow the rust to withstand the dry and hot summers to infect the alternate hosts in the autumn to complete the sexual life cycle (Figure 1.5C-D). On the surface of the wheat leaf, the teliospores germinate to become haploid basidiospores. The basidiospores infect the alternative host and produce haploid pycnial spores in the pycnial structures after being spread by wind, water or insects (Carver, 2009).

Subsequently, the pycniospores are dispersed by insects to other pycnial infections whereby sexual reproduction between two genetically distinct cells (opposite mating types) occurs, resulting in plasmogamy (Kolmer, 2013). At the final stage, the aecial cups release millions of aeciospores that will be dispersed by wind to infect wheat plants nearby. The sexual stage of *Pt* mediates the exchange of genetic material between physiologic races

and possibly genetically different populations. Due to the unavailability of alternate hosts in most wheat growing regions of the world, sexual reproduction of *Pt* provides a minimal contribution as the direct inoculum source of the pathogen to wheat species. Sexual reproduction of *Pt* on the alternate host *T. speciosissimum* and other *Thalictrum* spp. was sporadically reported in Portugal, Italy, Spain and the USA (Liu *et al*., 2014). However, molecular marker data in population studies indicate that in worldwide populations the reproduction of urediniospores is predominantly clonal, with little if any evidence of sexual recombination (Ordoñez *et al*., 2010; Kolmer *et al*., 2011). Infections on the alternate host *I. fumaroides* appear to be confined to Siberia (Chester, 1946). Figure 1.5 summarises the asexual and sexual life cycle of *Pt*.

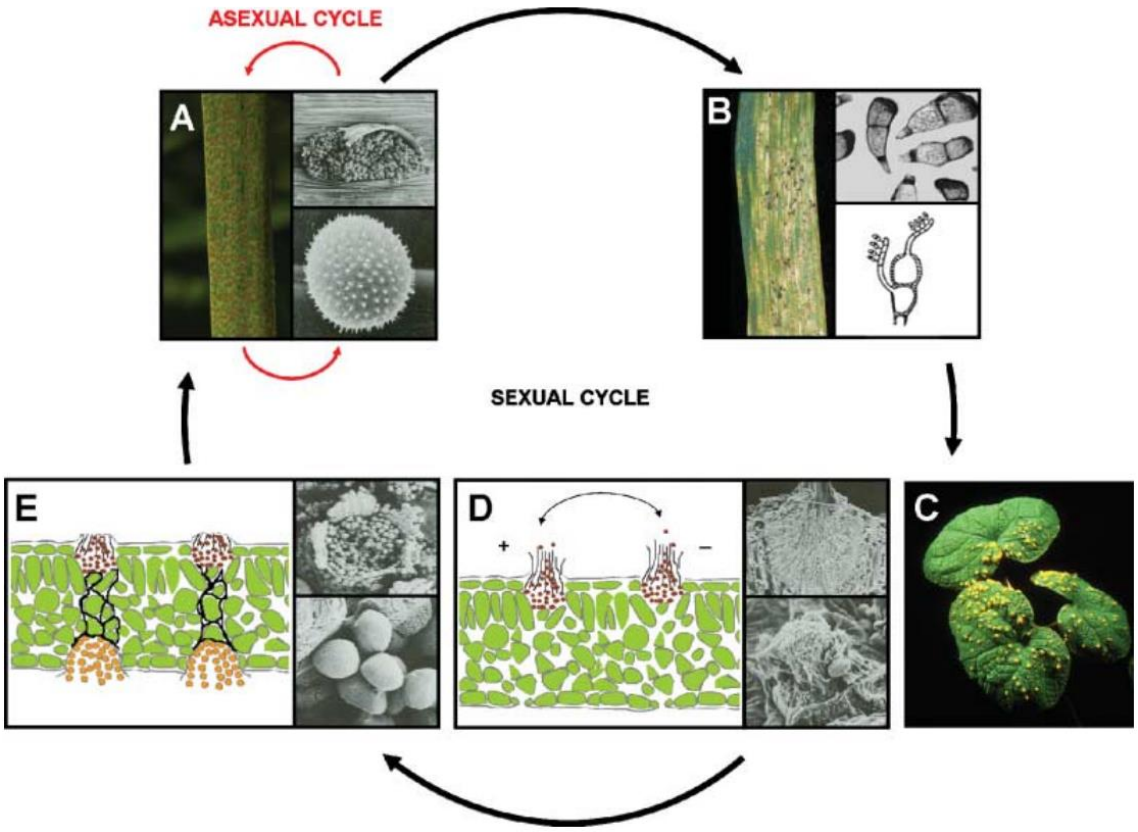

Figure 1.5: Summary of the life cycle of *Puccinia triticina*. (**A**) Uredinia on wheat leaf surface containing single cell dikaryotic urediniospores produced by urediniospores or aeciospores; (**B**) Telia that typically form at the bottom of the leaf epidermis towards the end of the growing season, are the size of uredinia, black and erupt when fully mature; (**C**) Pycnia, released by basidiospores on *Thalictrum*, appear as yellow-orange pustules on top of leaf surfaces. (**D**) Cross-section of *Thalictrum* with pycnia; (**E**) Cross-section of *Thalictrum* with pycnia and aecia. Image adapted from Bolton *et al*. (2008).

1.9 Economic importance of leaf rust

The economic significance of leaf rust was highlighted when outbreaks on the wheat variety Thatcher occurred in 1938, severely damaging wheat fields across NA. Since then, leaf rust has been considered a threat in wheat growing areas of the USA, former Union of Soviet Socialist Republics, and China (Chester, 1946). Modern wheat varieties continue to be affected by *Pt* and the breakdown of resistance is the main reason for numerous new wheat varieties to be rejected by producers soon after their introduction and widespread adoption in agriculture. The International Maize and Wheat Improvement Centre (CIMMYT) has anticipated a 1:27 ratio of cost-to-benefit analysis for the development of leaf rust resistant varieties (Simmonds and Rajaram, 1988).

Leaf rust outbreaks affect the green leaf area of plants which results in yield losses mainly by reducing the number and weight of wheat kernels (Huerta-Espino *et al*., 2011). In the USA, wheat yield losses due to leaf rust were estimated at US$350 M between the 2000 and 2004 crop seasons. In México yield losses include US$32 M from 2000 to 2003 and US$40 M from 2008 to 2009. In South America (Argentina, Brazil, Chile, Paraguay, and Uruguay) between 1996 and 2003, the yield loss amounted to US$172 M. Leaf rust infection causes an average annual yield loss of about 3 M tonnes in China, while 40% yield losses estimated at US$86 M were experienced in Pakistan during a severe leaf rust epidemic during 1978 season (Bolton *et al*., 2008). In Australia, the annual potential yield losses due to leaf rust are estimated at AUD$197 M when susceptible varieties are grown whereas the use of resistant varieties are estimated to minimise the loss to about AUD$12 M (Murray and Brennan, 2009). In Egypt, grain yield losses due to leaf rust infection on some susceptible wheat varieties reached an average estimate of 23% annually, depending on the severity of the disease and the crop growth stage at the first infection (Omara *et al*., 2021). When susceptible wheat varieties are grown in rust-prone areas, wheat leaf rust is one of the most significant diseases in most Ethiopian wheat-growing regions, where yield losses can reach upto 75% (Gadisa, 2019; Habtamu *et al*., 2020). Studies conducted in SA indicate that yield losses caused by leaf rust can reach up to 35%. Coupled with chemical control inputs, management of the disease cost farmers millions of rands annually (Boshoff, 2001; Terefe *et al*., 2014).

1.10 Leaf rust in Zimbabwe

Leaf rust occurs annually in higher incidence compared to other diseases like stem- and stripe rust in all wheat growing regions of Zimbabwe. However, limited information on the history of leaf rust in Zimbabwe is available. Therefore, it can only be assumed that *Pt* was

introduced with wheat cultivation in the late 19th century but was not considered an important disease. Leaf rust has however become one of the major threats to successful wheat production in Zimbabwe with only a few recent *ad hoc* attempts to monitor the occurrence and pathogenicity of the leaf rust population in the country (Pretorius and Purchase, 1990; Mukoyi *et al*., 2011; Mutari *et al*., 2012; Pretorius *et al*., 2015). In contrast, in neighbouring SA, surveys to monitor the virulence of *Pt* have been conducted annually since the early 1980s (Pretorius and Le Roux, 1988). Regular surveys in SA have resulted in the detection of two new *Pt* races between 1988 and 2011 (Pretorius *et al*., 2020), four new races between 2012 and 2016 (Boshoff *et al*., 2018) and three new races between 2017 and 2020 (Terefe *et al*., 2022).

The few surveys conducted in Zimbabwe from the 1980s to date have detected only a few races including SCDS, TCPS, MCDS and FBPT (Pretorius and Purchase, 1990; Pretorius *et al*., 2015). Leaf rust in Zimbabwe can cause up to 40% yield losses when susceptible varieties are grown, amounting to a potential loss of up to US$20 M annually (based on US$300/ton price; Zimstat, 2021). In the past varieties such as SC Stallion, SC Shine, SC Shangwa, SC Shorty, Insiza, Ruya, Sengwa and Kana were rejected by commercial farmers due to their susceptibility to leaf rust. Therefore, surveys to determine the diversity and distribution of *Pt* in Zimbabwe are vital to deal with the evolution of new more virulent races of the pathogen. Findings from such annual surveys will assist in the timely detection of new races and are in support of an early warning system to timeously report on their impact on the response of commercial varieties and advanced breeding lines. This information is considered valuable to wheat breeders in the identification and deployment of effective resistance sources against the most virulent *Pt* races.

1.11 Management and control of *Puccinia triticina*

The rapid spore dispersal and constant evolution of new physiologic races complicate the control of leaf rust, necessitating an understanding of all the interrelated epidemic components (Singh *et al*., 2005). Therefore, management and control of wheat leaf rust involves an integrated approach with diverse strategies such as pathogen monitoring, genetic resistance and cultural, biological and chemical control.

1.11.1 Pathogen monitoring

Regular annual surveys confirm the predominance of the major existing races and enable the early detection of new emerging *Pt* races. The information gained from these surveys should guide investments in research and development, crop protection and application of breeding techniques. Therefore, coordinating regional and global surveillance

programmes is essential to inform management plans (Park *et al*., 2009; Terefe *et al*., 2022). A notable example and model for monitoring significant infections is the Global Cereal Rust monitoring system, which was developed in response to the appearance of Ug99 (Hodson, 2011). Critical information and suggestions for research on cereal rust pathogens and plant breeding has been provided by this system.

1.11.2 Cultural control

Existing sources of resistance are significantly enhanced by adopting a wide range of cultural practices. Consequently, an integrated approach of variety selection, planting time, removal of volunteer cereals and related grasses may contribute to effective control of leaf rust. The development of an epidemic is often directly correlated to the date of disease onset (Ellis *et al*., 2014). Some researchers suggested that the use of plant growth regulators and appropriate use of fertilisers help to control leaf rust disease in cereals (Qin *et al*., 2020). Similarly, managing the time, frequency, and volume of irrigation can reduce leaf rust incidences.

Other studies suggest that using early maturing varieties allows the plants to mature before leaf rust infections can seriously affect the harvest (Knott, 1988). Genetic diversity of the pathogen population can also be limited with crop rotation with non-host crops by reducing the number of urediniospores produced and accumulated during the season. Destruction of any volunteer plants that promote the survival of primary inoculum can also help to prevent losses resulting from severe epidemics by preventing early season infection and spread (Kolmer *et al*., 2007). Deep ploughing or clean tillage, heavy grazing and spraying with non-selective herbicides also help to eliminate the ‘green bridge’ and reduce survival and availability of early season inoculum (Roelfs *et al*., 1992).

1.11.3 Biological control

The *in vitro* and *in vivo* experiments conducted by Levy *et al.* (1988) revealed that bacterial strains of *Pseudomonas putida* can produce several types of antibiotics, siderophores and slight quantities of hydrogen cyanide which suppress *Pt* growth. The efficacy of various biocontrol agents against wheat leaf rust was examined both individually and in combinations. Biological agents like *Verticillium lecanii*, *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae* and *Cladosporium cladosporioides* were evaluated as control agents in experiments against the leaf rust pathogen under field and laboratory conditions. According to the findings, *B. bassiana, V. lecanii* and *P. fumosoroseus* displayed the most encouraging outcomes for limiting pustule size (Sheroze *et al*., 2002).

1.11.4 Chemical control

Application of fungicides is a commonly used approach to avoid yield losses caused by leaf rust. A crucial component of fungicide treatments is guarding the flag leaf from disease up until the kernels are completely formed. Modern technologies are applied to develop effective fungicides for the control of leaf rust and other important fungal pathogens (De Wolf *et al*., 2012). Examples include strobilurin (azoxystrobin, picoxystrobin, pyraclostrobin and trifloxystrobin) and triazole fungicides (tebuconazole, prothioconazole, metconazole and propiconazole), as well as formulations with a combination of both. Over time, the continued application of fungicides exerts a significant selection pressure on the pathogens, which may result in fungicide resistance or tolerance. For example, fungicide tolerance has been detected in *B*. *graminis* (powdery mildew of cereals), *Fusarium* spp. and *Septoria* spp. (Becher and Wirsel, 2012; Cools *et al*., 2013). The resistance to azoles is initiated by mutations of the sterol 14-demethylase P450 (*CYP51*) gene and resistance to strobilurins is due to mutations in the cytochrome *b* gene (Cools *et al*., 2013). The application of fungicides also brings a financial liability to the farmer that also reduces profit margins (Lopez *et al*., 2015). Moreover, chemical residues may be harmful which is of concern to consumers. In order to prevent crop losses, reduce fungicide load in the environment, and minimise fungicide residue in grain ingested by livestock and people, much effort has been dedicated to the development of varieties carrying genetic resistance to *Pt*.

1.11.5 Genetic resistance to *Puccinia triticina*

Breeding for resistance achieved by the introgression of *Lr* genes when developing new varieties is the most cost-effective and environmentally friendly approach to control the disease. Furthermore, this approach immensely benefits poorly resourced farmers across the world (Simmonds and Rajaram, 1988). In the early 1900s, Biffen was the first to explain the genetic basis of wheat rust resistance (Biffen, 1905). From this important breakthrough, global wheat breeding programmes now consider the discovery of genetic variation for rust resistance an essential strategy. Leaf rust resistance is based on two types used in plant breeding programmes namely ASR and APR (Stubbs *et al*., 1986).

1.11.5.1 All-stage resistance

All-stage resistance, also known as seedling, race-specific or monogenic (major gene) resistance, is conferred by a single resistance gene (Stubbs *et al*., 1986; Roelfs *et al*., 1992). This type of resistance is often only effective against specific races of a pathogen and can break down easily with the appearance of new races, often referred to as a boom-

and-bust cycle (Knott, 1988). All-stage resistance is expressed at the seedling stage and remains effective throughout all developmental stages of the plant (Lagudah, 2011). Among the approximately 80 known *Lr* genes identified and characterised, the majority are ASR genes (Kolmer *et al*., 2018).

1.11.5.2 Adult plant resistance

Some APR sources are known to be race non-specific and provide slow rusting resistance controlled by polygenic (often minor gene(s)). These sources of resistance are considered durable with resistance conferred by quantitative trait loci (QTL) or multiple genes with each gene providing a partial increase in resistance (McIntosh *et al.*, 1995; Singh *et al*., 2016). This type of resistance is best expressed at the adult plant stages and the resistance is therefore often observed in the field. Some APR genes are effective against a wide spectrum of pathogen races and are considered key sources in achieving durable resistance (Sallam *et al*., 2016). Therefore, wheat breeders and pathologists have always emphasised the introgression of APR genes as an integral trait to increase the level of field resistance. From the *Lr* genes catalogued, at least 14 confer APR, the most prominent ones include *Lr12*, *Lr13*, *Lr22*, *Lr34*, *Lr35*, *Lr37*, *Lr46*, *Lr48*, *Lr49*, *Lr67*, *Lr68*, *Lr75*, and *Lr77* (Lin and Chen 2009; McIntosh *et al*., 2017). Some of these genes like *Lr12*, *Lr13*, *Lr22b* and *Lr48* are known to be race-specific and are associated with hypersensitive cell death (McIntosh *et al*., 2017). Only a few designated wheat leaf rust APR genes confer confirmed partial resistance to multiple pathogens or races of a particular pathogen, and these include *Lr34*, *Lr46*, and *Lr67* (Lagudah, 2011).

1.11.6 Durable rust resistance

When a variety has been widely grown for a significant period of time and over a large geographic region while withstanding disease pressure, it is deemed to possess durable resistance (Huerta-Espino *et al*., 2011). Leaf rust resistance is frequently associated with genes or alleles that confer durable resistance to multiple pathogens. Examples include *Lr34/Yr18/Sr57* (Ellis *et al*., 2014), *Lr46/Yr29/Pm39* (Kolmer *et al*., 2015), and *Lr67/Yr46/Sr55/Pm46/Ltn-3* (Herrera-Foessel *et al*., 2014). When ASR and APR genes are successfully combined (gene pyramiding), they may complement each other resulting in resistance responses different from individual genes. Kloppers and Pretorius (1997) observed improved resistance in selected wheat varieties carrying a combination of *Lr34* and *Lr13* even in the presence of *Pt* races possessing virulence to *Lr13*. Kolmer *et al*. (2007) also reported on durable resistance from a combination of the resistance genes *Lr16*, *Lr23*, and *Lr34* in NA. Likewise, Vanzetti *et al*. (2011) found that ASR genes such as

Lr16, *Lr19*, *Lr21*, *Lr25*, *Lr29*, *Lr41* and *Lr47* contributed to durable resistance when combined with APR genes such as *Lr34*, *Lr27*/*Sr2*, and *Lr46* in Argentinian wheat. 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). Additionally, interaction between two or more APR genes can offer durable resistance that is almost immune, helping in significantly reducing production costs (Singh *et al*., 2016).

1.11.7 Sources of leaf rust resistance

The wild relatives of wheat contain numerous genes conferring resistance to rust pathogens that can be exploited as novel sources of resistance for use in wheat breeding (Kolmer, 2013). Cultivated and wild species belonging to the secondary gene pool of wheat, such as perennial wheat grasses (*Thinopyrum* spp.) and rye (*Secale cereale*), are not common hosts of wheat rust pathogens. Such derived resistance genes are frequently broad-spectrum and effective over an extended period of time because most pathogens reportedly are less likely to infect and cause disease in species that differ from their normal hosts (Bettgenhaeuser *et al*., 2014). To date, from the more than 80 *Lr* genes that have been identified and characterised in bread, durum and diploid wheat species more than 50% were derived from wild relatives (McIntosh *et al*., 1995; 2017). Examples include *Lr9* (*Ae. umbellulata*); *Lr19*, *Lr24*, and *Lr29* (*Ae. elongatum*); *Lr37* (*Ae. ventricosa*); *Lr38* (*T. intermedium*); *Lr28*, *Lr35*, *Lr36*, *Lr51*, and *Lr66* (*Ae. speltoides*); *Lr21*, *Lr22a*, *Lr32*, *Lr39, Lr41, Lr42*, and *Lr43* (*Ae. tauschii*); *Lr57* (*Ae. geniculata*); *Lr58* (*Ae*. *triuncialis*); *Lr59* (*Ae. peregrina*); *Lr14a* and *Lr61* (*T*. *turgidum*); *Lr62* (*Ae. neglecta*); *Lr63* (*T. monococcum*), and *Lr53* and *Lr64* (*T. dicoccoides*); (McIntosh *et al*., 2017; MASWHEAT, 2022). Gene postulation and phenotypic analysis is often used by researchers to introgress resistance sources into new varieties, however these methods are laborious and time-consuming. Molecular markers closely linked to resistance genes have been developed to facilitate expeditious pyramiding of these genes into elite backgrounds, making it more efficient, cost-effective and less time-consuming.

1.12 Application of molecular markers for resistance breeding

Over the last few decades, several efficient molecular markers for *Lr* genes have been developed. The majority of molecular markers closely linked to *Lr* genes were developed based on the polymerase chain reaction (PCR) technique and can be applied relatively easily by wheat researchers. Molecular markers are used for two purposes in resistance breeding: (1) to monitor the introgression of designated resistance genes or QTL into elite wheat genotypes (i.e., marker-assisted selection, MAS) and (2) to identify resistance

genes in varieties and breeding lines where the genetic background is unknown (i.e., marker-assisted detection, MAD). One of the key benefits of molecular markers is that they are highly heritable and can be applied at the seedling stage, thus plant breeders can select resistant plants without phenotypic screening. Additionally, molecular markers have been extensively utilised to characterise *Pt* populations, this resulted in an improvement in our understanding of the origin, phylogeny, and spread of *Pt* races.

The first generation of molecular markers developed at the commencement of MAS in the late 1980s were based on Restriction Fragment Length Polymorphism (RFLP), a time-consuming and high-cost technology. The introduction of the PCR technology brought in a number of cost-effective and quicker second-generation molecular markers such as Sequence-Tagged Site (STS), derived markers such as Sequence Characterised Amplified Region (SCAR), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Diversity Arrays Technology (DArT), Expressed Sequence Tags (EST) and Single Nucleotide Polymorphism (SNP). However, different marker classes have unique drawbacks including insufficient genome density for fine mapping (SSR and DArT markers), limited informativeness (DArT markers in their original version) and low throughput (SSR markers) (Zhang *et al*., 2019). In the past decade, arrays like the Illumina 90K have been developed, making it feasible to effectively employ SNP markers. (Maccaferri *et al*., 2022; Van Poecke *et al*., 2013). Since thousands of samples must be collected for fine mapping, the availability of SNP markers in the wheat genome and their compatibility with high-throughput genotyping techniques like Kompetitive Allele Specific PCR (KASP) makes them the perfect choice.

The unparalleled degree of marker density and fine mapping was achieved thanks to the deployment of the SNP array technology and genotyping-by-sequencing (Van Poecke *et al*., 2013). A few arrays such as the Illumina iSelect wheat 9K, 90K and the Affymetrix 35K arrays were quickly embraced by wheat researchers (Sun *et al*., 2020; Maccaferri *et al*., 2022). This opened the door for the collection of mapping data necessary to produce newer, extremely detailed consensus and reference maps. Across the whole genome, these maps had a density of 1 to 10 markers per cM. The amount of data generated by these technologies is immense, thereby presenting data management constraints to the platforms. The urgent need to address the collapsing genetic diversity in cultivated wheat varieties as a result of hybridisation, domestication (that resulted in targeted selection) and inbreeding (Winfield *et al*., 2016; Allen *et al*., 2017) have also allowed these platforms to avail their databases to the public to facilitate breeders and other researchers to make

strides in developing wheat varieties that will meet future global wheat demands. A timeline progression of molecular marker techniques and their advantages and disadvantages is summarised in Table 1.1.

Table 1.1: Timeline progression in molecular marker technologies with their advantages and disadvantages.

Marker type	Platform technology	Time interval	Time assay	Marker numerosity	Advantages and disadvantages / Multiplex ability
Hybridisation-based markers					
Restriction Fragment Length Polymorphism (RFLP)	Enzyme restriction + probe hybridisation + electrophoresis + southern blot + radioactive /chemiluminescent detection.	’70 - '90	Several days	Hundreds	Time-consuming and costly. Mainly codominant. Multiplex (yes, 2-3 assay).
Polymerase chain reaction (PCR) - based markers					
Simple PCR from Expressed Sequence Tags (EST)	PCR + horizontal electrophoresis.	’80 – present	24 h	Hundreds, custom design.	Allows detection of insertions/deletions (INDEL). Multiplex (no) and codominant.
Cleaved Amplified Polymorphic Sequences (CAPS)	PCR + enzymatic restriction + electrophoresis.	’80 – present	24 h	Hundreds, custom design.	Allows detection of single nucleotide substitutions or INDEL. Multiplex (no) and codominant.
Allele Specific Oligonucleotide PCR (ASO)	PCR + electrophoresis.	’80 – present	24 h	Hundreds	Allows detection of single nucleotide substitutions or INDEL. Multiplex (no) and codominant.
Random Amplified Polymorphic DNA (RAPD)	PCR +electrophoresis.	’80 - ‘90	24 h	Hundreds	Multiplex (yes), robustness drawback and dominant.
Amplified Fragment Length Polymorphisms (AFLP)	PCR + vertical electrophoresis (polyacrylamide gel fragment analysis).	’80 - ‘90	24 h	Hundreds	Multiplex (yes). Robust and dominant.
Simple Sequence Repeat (SSR)	PCR + vertical electrophoresis.	’80 – present	24 h	Thousands	Multiplex (yes, 3-6 assay). Robust and codominant.
Real-Time hydrolysis probes (TaqMan)	SNP + fluorescent detection.	’90 – present	24 h	Thousands, custom design.	Multiplex (no). Robust, codominant and custom design.
Kompetitive Allele Specific PCR (KASP)	SNP + fluorescent detection.	2000 - present	24 h	Thousands, custom design.	Multiplex (no). Robust, codominant and custom design.
Single Nucleotide Polymorphism arrays (SNP arrays)	DNA microarrays. Illumina and Affymetrix technology.	2000 - present	<24 h	Highly flexible based on discovery panel.	High Multiplex of 10K-50K-90K-280K-660K-800K. Robust and codominant. Suitable for whole genome analysis (GWAS, GS).
Targeted resequencing	Illumina Next Generation massive sequencing.	2010 - present	<24 h	Highly flexible based on discovery panel.	Multiplex of 1K-10K. Robust and codominant. Suitable for (GWAS, GS).

Table adapted from Maccaferri *et al*. (2022).

1.13 Bread wheat yield and quality parameters

Before the commercial release of wheat varieties, breeders evaluate them as advanced breeding lines in multi-location trials to find certain genotypes that are suited or stable to diverse environment(s), resulting in rapid genetic gains (Crespo-Herrera *et al*., 2016). Frequently, GxE complicates breeding, testing and selection of superior genotypes for grain yield (Yan and Kang, 2003). Breeders analyse MET and GxE data using a range of statistical techniques, including AMMI and GGE-biplots analysis. The AMMI analysis can also be used to determine the stability of the genotypes across locations using the principal component analysis (PCA) scores and variety superiority index (CSI) (Crossa, 1990). However, in addition to having high grain yield, disease resistance and wide adaptability, an ideal wheat variety needs to maintain the required high end-use quality attributes.

The wheat value chain in the agri-food industry consists of many stakeholders from the farmer to the millers, bakers, retailers to most importantly the end-user, the consumer. This means that an ideal wheat variety can have different attributes depending on preferences of each of these stakeholders. According to the farmer, a variety with the lowest input requirements, best grain yield and grading outputs and sold at a premium on the market would be ideal. Millers on the other end will classify the varieties according to the flour output together with the energy requirements for extracting it (Igrejas *et al*., 2020). Rheological dough characteristics including mixing time and water absorption, as well as the ability to produce multiple products from the flour of a particular variety, is relevant to bakers. Retailers may also consider shelf life as a key attribute for various wheat products.

The consumer needs for an ideal variety are subjective parameters that are often challenging to analyse. At the same time, the wheat breeder might select a variety with good disease resistance, high yield and wide adaptation. These varieties may be accepted by the farmer, valued by the miller and desired by the baker, and should produce products with favourable taste to the consumer. In most cases, the possibility of a uniform response from all the stakeholders is highly unlikely. Probably, for a particular variety, these various perceptions can range from positive to negative.

In this regard, wheat grain attributes play an integral role in determining grain quality characteristics. Three components of wheat grain namely proteins (7-18%), lipids (1.5-2%) and carbohydrates (60-75%) are considered key in defining grain quality, while other minor components include vitamins and minerals (Wieser, 2007). Grain protein content, and carbohydrates, especially starch and arabinoxylans (the main component of wheat grain fibre), have notable influence on three grain characteristics closely linked to the technical

qualities of wheat grain required for a wide range of baking products. These are grain hardness or texture, the gluten viscoelastic and starch properties, which are associated with milling, processing and end-use quality. Flour or semolina yellow colour are other complementary parameters that sometimes have great importance when grain quality is evaluated (Mastrangelo and Cattivelli, 2021).

1.13.1 Grain hardness

The degree of adhesion between the protein matrix and starch granules inside the wheat endosperm determines the hardness or texture of the grain. Wheat grain hardness has been used to classify bread wheat for ages, being the differentiator in the global commerce of wheat grain (Pasha *et al*., 2010). Based on grain hardness, different types or grades of bread wheat exists namely soft, hard or very hard. Grain hardness is closely related with the botanical classification of wheat like tetraploid wheat (subspecies of *T. turgidum* including durum wheat) which exhibits very hard texture. The *T. aestivum* group (hexaploid wheat including bread wheat) displays a texture that varies from soft to hard. Grain hardness or texture is the single important parameter that determines enduse and industrial utilisation of wheat (Laidig, 2017). Grain hardness affects several traits related to wheat milling including flour yield, loaf volume, particle size distribution of the flour and semolina, energy requirement and percentage of starch damage (which strongly affects the dough water absorption linked with end-use quality). Based on the differences in hardness, hard common wheat is preferred for bread-making in temperate regions of the world like Europe and NA where hard wheat varieties are adapted and commonly grown. The very hard durum wheat is preferred for pasta while soft common wheat is preferred for cookies and pastries (Mastrangelo and Cattivelli, 2021). Due to the warmer tropical climate in most parts of SSA except for the Western Cape region of SA, spring wheat (soft to intermediate in hardness) is the most widely grown. Therefore, some industries import hard wheat to blend with soft wheat to improve the quality of the bread (Issarny *et al.,* 2017).

1.13.2 Protein and gluten content

Grain protein content in wheat varieties usually ranges from 7-18%. Around 80% is constituted by the protein components that form gluten. Gluten is the protein that forms a continuous viscoelastic network when wheat flour is mechanically mixed with water during processing (Wieser, 2007). The wheat dough’s distinctive qualities, which enable processing into a wide range of baking items such as bread, biscuits, cakes, noodles and pasta are imparted by this continuous protein network. When baking bread, gluten gives the dough its viscoelasticity, allowing the trapping of carbon dioxide generated by the yeast during leavening. Gluten also

gives dough the cohesion it needs to extrude and develop into a desired shape in making pasta (Chaudhary *et al*., 2016). Flour with strong extensibility is needed to function effectively in sheeting operations when manufacturing products such as cookies, flatbread and noodles. This highlights the commercial impact of the grain protein content to various stakeholders involved in the wheat value chain. For example, in northern European countries such as France, Germany and the United Kingdom (UK), farmers are paid a premium price based on the grain protein content (Laidig, 2017). Their millers and bakers require a minimum of 12.8% or even higher protein content in wheat flour. Achieving this protein content under high yield scenarios requires application of high rates of fertiliser after anthesis for high build-up of storage protein (Xue, 2016). Despite significant efforts by breeders, the negative correlation between yield and grain protein content is difficult to bridge (Mosleth, 2015).

1.13.3 Starch content

About 70% of the total dry mass of wheat grain is constituted by starch. It is composed of two polymers based on D-glucose residues, namely amylopectin with α-(1,4) and α-(1,6) linkage residues representing 65-78%, and one linear polymer formed by α-(1,4) residues (amylose) that represents 22-35% (Shevkani *et al*., 2017). The polymers are synthesised in the amyloplast by two distinct synthetic routes. The changes in the amylose/ amylopectin ratio have been the cornerstone of research to identify varieties with modification of the enzymes leading to the generation of novel starches (Igrejas *et al*., 2020). The interaction between the two polymers may impact how starch behaves physically and chemically, including its ability to gelatinise, paste and gel.

1.14 Conclusions

Globally, wheat leaf rust is a devastating disease and growing resistant varieties is a key strategy to manage and control the disease. However, the continuous evolution of the pathogen poses a major and continuous threat to wheat production. Development of new resistant wheat varieties require knowledge of pathogen distribution, diversity and sources of genetic resistance to a particular new or emerging race. In Zimbabwe, there is a knowledge gap required to control wheat leaf rust. There is therefore a need to have regular annual surveys of all wheat rust pathogens to generate data that creates awareness among local farmers, and regional and global researchers. The knowledge of sources of resistance in the germplasm pool is also critical to deploy these genes efficiently in case of introduction or emergence of new more virulent races. Gene pyramiding of ASR and APR genes in a single variety has proved to be a handy strategy in potentially providing durable resistance. The adoption of MAS is an important tool in modern wheat breeding programmes as it reduces

the time and improves the efficiency of gene deployment in the development of new varieties. Above all, wheat varieties need to be evaluated for parameters such as yield, wide adaptation, grain protein, gluten and starch content to meet the need for the whole wheat value chain that includes farmers, millers, bakers and consumers.

1.15 Chapter 1 references

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Chapter 2: Phenotypic and genotypic characterisation of *Puccinia triticina* isolates from Zimbabwe

2.1 Introduction

Genetic resistance is the most cost-effective approach to manage leaf rust of bread wheat (*T. aestivum* L.), caused by the fungus *Pt*. However, the frequent emergence of new rust races that overcome resistance sources deployed in commercial cultivars remains problematic. New races may develop locally through genetic mutations occurring in isolates of existing races or can be introduced into a specific region through windborne urediniospore dispersal or accidentally through contaminated clothing (Terefe *et al*., 2014a). It is thus important to constantly monitor changes in the local *Pt* population through annual surveys to determine the frequencies of existing races and identify new races. Surveillance studies are also vital to determine the impact new races could have on commercial wheat cultivars and advanced breeding lines, and to identify effective resistance sources (Boshoff *et al*., 2018). This knowledge is critical to breed wheat cultivars with effective and sustainable sources of resistance to *Pt*.

Spring wheat requires cool temperatures (15-22°C) to produce an economically viable yield, thus in Zimbabwe it is mainly grown under irrigation during winter (May to August). Since winter cropping requires irrigation, it implies a significant initial capital investment in dam and water reservoir construction, the drilling of boreholes, purchasing of water main-lines, and a reliable electricity supply. Given this significant investment, wheat is mainly produced by medium and large-scale commercial farmers. However, small-scale farmers under smallholder irrigation schemes and on wetlands also produce wheat for household use (Anseeuw *et al.*, 2011). Zimbabwean wheat production area trends since 1980 have shown an inconsistent pattern ranging from a high of 70 500 ha in 2004 to a low of 4 000 ha in 2009. However, a steady increase in hectarage for wheat production has been experienced in the past decade (2010-2021; Figure 2.1). The introduction of a government subsidy from 2019 has maintained the production area above 40 000 ha with a further increase forecasted due to improved irrigation infrastructure.

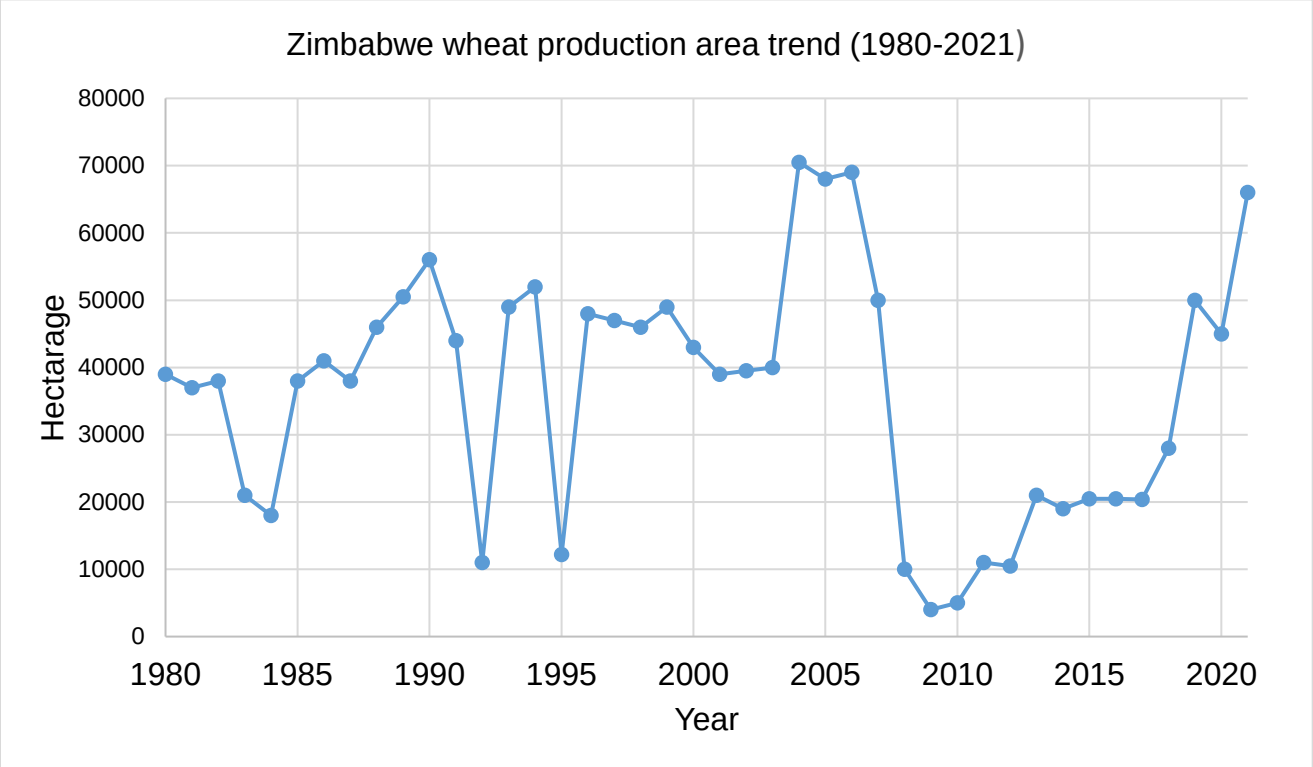

Figure 2.1: Zimbabwe wheat production area (ha) trends from 1980 to 2021. (Data compiled from Zimstat, 2021).

Over the past decade, many wheat cultivars have been released by both the national Crop Breeding Institute (CBI) programme and the private sector (SeedCo, Corteva Agriscience™ and Klein Karoo). However, only a few cultivars were widely adopted and grown by farmers (Table 2.1).

Wheat production in Zimbabwe is frequently affected by leaf rust due to the moist and hot environmental conditions at the beginning of the summer season. The disease occurs regularly in all growing regions. However, there have been only a few *ad hoc* studies to monitor the occurrence and pathogenicity of the leaf rust pathogen (*Pt*) in the country (Mukoyi *et al*., 2011; Mutari *et al*., 2012; Pretorius *et al*., 2015). In neighbouring SA, virulence of *Pt* has been monitored annually since the early 1980s (Pretorius and Le Roux, 1988). Although the two countries share a border, it is crossed daily and therefore poses a high-risk of human, as well as windborne urediniospore dispersal between the two countries. There were no previous studies on the genetic relationships between the Zimbabwean and South African *Pt* populations to confirm the previously described phenotypic similarities.

In this study a survey, which involves the collection of *Pt* infected wheat samples from the main wheat production regions of Zimbabwe, was conducted. Using *Pt* isolates from SA as controls, isolates from Zimbabwe were phenotypically identified based on their

avirulence/virulence profiles on a set of standard and additional differential lines. These isolates were also genotyped using microsatellite markers to determine the genetic relationships amongst *Pt* isolates from the two countries.

Table 2.1: Popular wheat cultivars grown in Zimbabwe for the period 2010 to 2021.

Season	Dominant cultivars grown
2010	SC Nduna, SC Shield, SC Shine, PAN 3402, Runde
2011	SC Nduna, SC Shield, SC Shine, PAN 3402, Runde
2012	SC Nduna, SC Shield, SC Shine, PAN 3402, Runde
2013	SC Nduna, SC Shield, PAN 3402, Runde
2014	SC Nduna, SC Shield, PAN 3402, Runde
2015	SC Nduna, SC Shield, PAN 3402, Runde
2016	SC Nduna, SC Shield, PAN 3402
2017	SC Nduna, SC Select, PAN 3402
2018	SC Nduna, SC Select, PAN 3402, PAN 3494
2019	SC Nduna, SC Select, Peregrine, PAN 3494
2020	SC Nduna, SC Select, Peregrine, SC Serena, PAN 3494, SC Sahai
2021	SC Nduna, SC Select, Peregrine, Dande, SC Serena, PAN 3494, SC Sahai

Data compiled from Department of Research and Specialist Services (DR&SS), Ministry of Agriculture, Mechanisation and Irrigation Development, Harare, Zimbabwe.

Thus, the aim of this study was to determine the diversity and distribution of *Pt* races in Zimbabwe during the 2019 to 2021 wheat production seasons. The objectives were to phenotype Zimbabwean *Pt* isolates using differential and additional wheat lines to identify the races that were present. These isolates were then used in a microsatellite marker study to determine the genetic relationships between the Zimbabwean isolates and selected South African *Pt* races.

2.2 Materials and methods

2.2.1 Surveillance studies from 2019 to 2021

Wheat leaf samples infected with *Pt* were collected in Zimbabwe during the 2019 and 2020 winter and spring months, as well as the 2021 summer months from commercial wheat fields and breeding nurseries. These irrigation wheat production areas are in the highveld (>1 200 masl), middleveld (800 to 1 200 masl) and lowveld (<800 masl). The surveys were conducted in seven major wheat growing provinces namely Harare, Mashonaland East, Mashonaland West, Mashonaland Central, Midlands, Manicaland and Masvingo (Figure 2.2).

Field isolates of *Pt* were collected through sampling of infected leaves from individual wheat cultivars in either commercial planting or trial plots. Leaves were placed in a paper envelope labelled with the locality, wheat variety, leaf rust incidence in the commercial field or nursery and date of collection. For each locality the Global Positioning System (GPS) coordinates were noted (Appendix 2.1). At least 2-3 leaves were sampled per plot of each sampled variety. The envelopes were left open to air-dry at room temperature for 48 h before sealing. The samples were sent via courier to the University of Free State (UFS) rust laboratory, Bloemfontein, SA. Shipment and handling of *Pt* isolates used in this study were conducted following permit authorisation and in accordance with the phytosanitary conditions set by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA (Appendix 2.2).

Upon arrival, urediniospores from each sample were collected into a size 00 gelatine capsule using an air vacuum attached to a spore collector, Vacuubrand® pump (model MZ2, Thermo Fischer Scientific, UK), as described by Pretorius *et al*. (2019). Individual capsules were placed in Cryo.STM tubes, numbered and stored at -80°C for later use.

2.2.2 Establishment of single pustule isolates

Urediniospores representative of each field isolate stored at -80°C were retrieved and heat shocked at 46°C for 6 min in a water bath and then suspended in 300 µl Soltrol® 130 isoparaffinic oil (Chevron Phillips, Borger, Texas, USA). For each *Pt* isolate, at least 40 seeds of the universally susceptible wheat variety Morocco were planted in a 10 cm diameter pot filled with Mikskaar Professional Potting Soil 70® (Hygrotech, Pretoria, SA). The Morocco seedlings were drenched with 50 ml 0.03% (w/v) maleic hydrazide Reagent Plus® (Sigma-Aldrich, St. Louis, Missouri, USA) solution per 10 cm pot upon coleoptile emergence to retard plant growth and enhance sporulation (Knott, 1989). Seedlings were fertilized once with 2.5 mg/L Multifeed-Classic water-soluble fertilizer [Effekto®, NPK Analysis 19:8:16 (43)] prior to inoculation.

Figure 2.2: Different localities where *Puccinia triticina* (*Pt*) infected wheat leaf samples were collected in Zimbabwe.

The suspended urediniospores were sprayed onto seven-day old Morocco seedlings using a vacuum pressure pump set at 25 kPa (Vacuubrand pump-model MZ2) attached to an inoculation device (Pretorius *et al*., 2019). Inoculations were carried out in an enclosed booth, which was cleaned through automated spraying of hot water (±65°C) for 60 s between isolates. After inoculation, the seedlings were dried in a Conviron growth cabinet fitted with fluorescent growth tubes (200 µE/m^2/s light) at 25°C for about 1 hour. Inoculated seedlings were then incubated overnight (±18 h) in a dew simulation chamber at 19°C (±1°C). Dew was formed by vapour released from two water baths at ±40°C fixed at the bottom of the chamber. From the dew chamber, the seedlings were dried again in the Conviron growth cabinets for at least 2 h as described above before transferring them to the greenhouse. Each pot was placed in a separate compartment in the greenhouse to prevent cross-contamination between isolates. After 12 days, two single pustules were collected from each field isolate using a

vacuum connected spore collector. Single pustule isolates were multiplied in a second round of inoculation of Morocco seedlings as described above. After 10 to14 days, urediniospores were collected in mass by tapping rust inoculated seedlings, representative of each single pustule isolate, over a piece of A_4 paper, and then transferring the spores to gelatine capsules which were stored at -80°C. In total, 104 single pustule isolates were included in this study (Appendix 2.1).

2.2.3 Avirulence/virulence phenotyping of single pustule isolates

The seedling ITs of the 104 single pustule *Pt* isolates were determined on a standard set of differential wheat lines carrying *Lr* genes RL6003 (*Lr1*), RL6016 (*Lr2a*), RL6019 (*Lr2b*), RL6047 (*Lr2c*), RL6002 (*Lr3a*), RL6042 (*Lr3bg*), RL6007 (*Lr3ka*), RL6010 (*Lr9*), RL6004 (*Lr10*), RL6053 (*Lr11*), RL6013 (*Lr14a*), RL6052 (*Lr15*), RL6005 (*Lr16*), RL6008 (*Lr17a*), Harrier (*Lr17b*), RL6009 (*Lr18*), Agatha (*Lr19*), Thew (*Lr20*), Gaza (*Lr23*), Agent (*Lr24*), RL6078 (*Lr26*), Gatcher (*Lr27+31*), CS_2A/2M (*Lr28*), RL6049 (*Lr30*), RL6086 (*Lr32*), Trident (*Lr37+*) and RL6051 (*LrB*) (Table 2.2; Appendix 2.3). The differential lines were planted in clusters (5 to 8 seeds per entry) following a circular pattern in 10 cm diameter pots filled with Mikskaar potting soil. Five pots were placed per 30 cm diameter plastic tray to allow watering from below. After planting, seeds were germinated at 25°C in a growth chamber for three days before placement under lights in a rust-free greenhouse cubicle at 18-25°C. Seedlings were fertilized with 100 ml per tray of a 2.5 mg/L Multifeed-Classic water-soluble fertilizer [Effekto®, NPK Analysis 19:8:16 (43)].

Seven-day old seedlings of the differential lines having a fully developed primary leaf were inoculated with each isolate as previously described (2.2.2). Representative isolates of South African *Pt* races MCDS (UFS isolate UVPt21_75) and FBPT (UVPt22_1), detected for the first time in the 2010 wheat season in SA (Terefe *et al*., 2014a; 2014b), were included as controls in all race typing experiments.

The seedling ITs of South African *Pt* races MCDS and FBPT, as well as isolate Pt1_1_2019 as a representative of all the Zimbabwean isolates, were determined on 12 additional differential lines, namely RL6043 (*Lr21*), RL6084 (*Lr25*), RL6080 (*Lr29*), RL6057 (*Lr33*), E84081 (*Lr36*), RL6097 (*Lr38*), RL6147 (*Lr44*), RL6144 (*Lr45*), KS90H450 (*Lr47*), KS96WGRC36 (*Lr50*), R05 (*Lr51*) and RL6017 (*Lr52*) (Table 2.2), in a repeated experiment.

Designated adult plant *Lr* genes can further differentiate between *Pt* isolates. The virulence of Zimbabwe isolate Pt1_1_2019 was therefore determined on flag leaves of wheat lines RL6011 (*Lr12*), CT263 (*Lr13*), RL6044 (*Lr22a*), Thatcher (*Lr22b*), RL6058 (*Lr34*, non-race

specific slow rusting gene), RL6082 (*Lr35*) and RL6081 (*Lr37*) (Table 2.2). Seed of these lines was planted in plastic cones (4×4×10 cm) filled with vermiculate and incubated at ±6°C for three weeks in a cold room. Seedlings were transplanted into 2 L pots filled with sterilized soil and grown in a greenhouse at ±21°C. Plants were fertilized once a week with 2.5 mg/L Multifeed-Classic water-soluble fertilizer [Effekto®, NPK Analysis 19:8:16 (43)] (50 ml/pot). Two pots with three plants each per rust isolate and differential line combination, were inoculated between flag leaf and head emergence (Zadoks growth stage 41 to 53; Zadoks *et al*., 1974) with urediniospores of Zimbabwean isolate Pt1_1_2019. Representative isolates of South African *Pt* races MCDS (UVPt21_75) and FBPT (UVPt22_1) were included as controls. Urediniospores (±1 mg/ml) were suspended in distilled water containing 0.03% (v/v) Tween 20® and applied to mainly the flag leaves using a compressed air sprayer as described previously for stripe rust (Pretorius *et al*., 2007). Plants were incubated in the dark in a dew chamber at ±19°C for 18 h as previously described above. After the dew cycle, plants were dried at 20°C before moving them to a greenhouse cubicle set at an 18-25°C night/day schedule. Leaf rust flag leaf response types were recorded 12 days after inoculation for each of the lines.

Table 2.2: List of differential lines, their University of the Free State (UFS) seed source and leaf rust resistance (*Lr*) gene(s) used to characterise Zimbabwean single pustule isolates of *Puccinia triticina* (*Pt*).

Entry	UFS source	*Lr* gene	Entry	UFS source	*Lr* gene
RL6003[1]	Bainsvlei_09	*1*	CS_2A/2M[1]	Bainsvlei_07	*28*
RL6016[1]	Bainsvlei_05	*2a*	Gatcher[1]	Bainsvlei_09	*27+31*
RL6047[1]	Bainsvlei_05	*2c*	RL6086[1]	Bainsvlei_07	*32*
RL6002[1]	Bainsvlei_05	*3a*	Trident[1]	Bainsvlei_15	*37*
RL6010[1]	Bainsvlei_16	*9*	RL6043[2]	Bainsvlei_10	*21*
RL6005[1]	Bainsvlei_05	*16*	RL6084[2]	Bainsvlei_07	*25*
Agent[1]	Bainsvlei_12	*24*	RL6080[2]	Bainsvlei_11	*29*
RL6078[1]	Bainsvlei_05	*26*	RL6057[2]	Bainsvlei_10	*33*
RL6007[1]	Bainsvlei_11	*3ka*	E84081[2]	Bainsvlei_07	*36*
RL6053[1]	Bainsvlei_05	*11*	RL6097[2]	Bainsvlei_07	*38*
RL6008[1]	Bainsvlei_09	*17a*	RL6147[2]	Bainsvlei_07	*44*
RL6049[1]	Bainsvlei_07	*30*	RL6144[2]	Bainsvlei_07	*45*
RL6051[1]	Bainsvlei_16	*B*	KS90H450[2]	Bainsvlei_07	*47*
RL6004[1]	Bainsvlei_05	*10*	KS96WGRC36[2]	Bainsvlei_07	*50*
RL6013[1]	Greenhouse_17	*14a*	R05[2]	Bainsvlei_07	*51*
RL6009[1]	Bainsvlei_11	*18*	RL6017[2]	Bainsvlei_07	*52*
RL6019[1]	Bainsvlei_05	*2b*	RL6011[3]	Bainsvlei_11	*12*
RL6042[1]	Bainsvlei_16	*3bg*	CT263[3]	Bainsvlei_14	*13*
RL6052[1]	Bainsvlei_11	*15*	RL6044[3]	Bainsvlei_11	*22a*
Harrier[1]	Bainsvlei_15	*17b*	Thatcher[3]	Bainsvlei_10	*22b*
Agatha[1]	Bainsvlei_15	*19*	RL6058[3]	Bainsvlei_10	*34*
Thew[1]	Bainsvlei_15	*20*	RL6082[3]	Bainsvlei_10	*35*
Gaza[1]	Bainsvlei_10	*23*	RL6081[3]	Bainsvlei_07	*37*

[1] Standard differential lines used to phenotype 104 Zimbabwean *Puccinia triticina* isolates; [2,3] Additional differential lines used to compare seedling infection types (ITs) and adult plant response types for *Puccinia triticina* race MCDS (isolate UVPt21_75) and FBPT (isolate UVPt22_1) from South Africa and isolate Pt1_1_2019 from Zimbabwe, respectively.

2.2.4 Naming of *Puccinia triticina* isolates

Twelve days after inoculation, leaf rust seedling ITs were assessed on primary leaves of the primary and additional differential lines using a 0 to 4 scale (McIntosh *et al*., 1995). The same scale was applied 14 days after inoculation of the flag leaves to assess ITs of the differential lines carrying APR sources to *Pt*. According the scale, rating “0” = immune, “;” = fleck, “C” = excessive chlorosis, or “N” = excessive necrosis, “1” = minute uredinia, “2” = small to medium sized uredinia, “3” = large uredinia sometimes encircled by slim chlorosis, “4” = large uredinia without any chlorosis, “X” = random spreading of variable-sized uredinia on a single leaf, “Y” = ordered spreading of different sized uredinia with larger uredinia at the leaf tip and “Z” = ordered spreading of different sized uredinia with larger uredinia at the leaf base. Infection types “0” to “2+” and combinations thereof were considered avirulent or low (L) for a particular *Lr* gene and ITs 3 to 4 as virulent or high (H). Using the North American system of nomenclature for *Pt* races (Kolmer *et al*., 2007), the 16 standard differential lines were grouped into four sets of four genes each. Once seedling ITs were determined, a corresponding *Pt* letter code was assigned to the four-letter IT pattern. For example, an unknown *Pt* isolate can be identified as race MCDS when the set 1 IT pattern corresponds to the letter M (HLLH), set 2 to C (LLLH), set 3 to D (LLHL) and set 4 to S (HHHL) (Table 2.3).

2.2.5 Selection and collection of isolates for genotyping

After successful multiplication of field isolates in the greenhouse, scissors and tweezers were used to collect leaf material containing at least two *Pt* pustules for each isolate on a 1 cm leaf piece. The collection equipment was sterilized with 70% (v/v) ethanol between each sample to prevent contamination. The samples were placed in 2 ml Eppendorf tubes and kept on ice for the duration of the sampling before being stored in a freezer at -80°C. Forty Zimbabwean *Pt* isolates, representative of the 2019 to 2021 seasons, collected from different localities, were selected for genotyping (Appendix 2.4). The collected leaf material was freeze-dried before deoxyribonucleic acid (DNA) extraction.

Table 2.3: Grouping of 16 standard differential lines, each carrying a unique *Puccinia triticina* (*Pt*) resistance (*Lr*) gene, into four sets and the applied letter codes according to the potential outcome of seedling infection types (ITs) per set.

Differential set	***Pt* resistance genes**			
Set1	*Lr1*	*Lr2a*	*Lr2c*	*Lr3*
Set2	*Lr9*	*Lr16*	*Lr24*	*Lr26*
Set3	*L3ka*	*Lr11*	*Lr17a*	*Lr30*
Set4	*LrB*	*Lr10*	*Lr14a*	*Lr18*
Letter Code[1]	**Outcome of ITs**			
B	L	L	L	L
C	L	L	L	H
D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

[1] *Pt* code consists of the designation for set 1 followed by sets 2, 3 and 4.

2.2.6 Deoxyribonucleic acid extraction

Genomic DNA was extracted from freeze-dried inoculated leaf material using a modified cetyltrimethylammonium bromide (CTAB) method according to Saghai-Maroof *et al.* (1984) as described by Visser *et al*. (2009). Two sterile 5 mm stainless steel balls were added to each 2 ml Eppendorf tube containing freeze-dried leaf samples. The samples were ground to a fine powder using Qiagen's TissueLyser (Haan, Germany) for 1 min at 30 r/s. To each sample, 750 µl CTAB extraction buffer (100 mM Tris (hydroxymethyl) aminomethane hydrochloride [Tris-HCl] pH 8.0, 20 mM ethylenediaminetetraacetic acid (EDTA) pH 8.0, 1.4 M NaCl, 2% (w/v) CTAB) was added and the samples mixed until the dried tissue was completely liquefied. Samples were incubated for 1 h at 65°C and inverted every 15 minutes. A volume of 500 µl chloroform ($ChCl_3$)/isoamylalcohol (IAA) (24:1 v/v) was added to each sample. After mixing, the samples were centrifuged for 10 min at 12 000 *g* at 4°C. The supernatant of each sample was transferred to a new 1.5 ml Eppendorf tube containing 500 µl isopropanol and mixed, whereafter the DNA was precipitated for 20 min at room temperature, followed by centrifugation for 10 min at 12 000 *g* at 4°C. After discarding the supernatant, the DNA pellet was washed once with 500 µl ice-cold 70% (v/v) ethanol for 20 min at room temperature. After centrifugation for 5 min at 12 000 *g* at 4°C the supernatant was discarded, and the DNA pellet air-dried for 1 h at room temperature. The pellet was finally dissolved overnight at 4°C in 200 µl TE buffer (10 mM Tris-HCI, 1 mM EDTA pH 8.0).

RNA was removed from the extracted DNA by adding 2 µl RNase A (10 mg/ml) to each sample and incubating the tubes for 2 h at 37°C. To remove the RNase A, 20 µl of 7.5 M ammonium acetate and 200 µl $ChCl_3$/IAA (24:1 v/v) were added to each sample and mixed, followed by centrifugation for 10 min at 12 000 *g* at 4°C. The supernatant was transferred to a new 1.5 ml Eppendorf tube and the DNA precipitated overnight at -20°C with 500 µl ice-cold 100% (v/v) ethanol. After centrifugation for 15 min at 12 000 *g* at 4°C, the pellet was washed once with 500 µl ice-cold 70% (v/v) ethanol, air-dried and finally dissolved in 50 µl TE buffer at 37°C.

2.2.7 Deoxyribonucleic acid quantification and dilution

Genomic DNA was quantified with the NanoDrop™ 2000 spectrophotometer (Thermo Scientific™, Massachusetts, USA). Optical density (OD) readings at 230, 260 and 280 nm were used to determine both the concentration and purity (OD_{260}/OD_{280} and OD_{260}/OD_{230}) of each sample. Thereafter, the DNA samples were diluted to a working concentration of 10 ng/µl using water.

2.2.8 Agarose gel electrophoresis

DNA samples were separated on a 0.8% (w/v) agarose gel prepared in 0.5x TAE (20 mM Tris-acetate, 0.5 mM EDTA pH 8.0) containing 10 µg/ml ethidium bromide (EtBr) (Sambrook *et al*., 1989) to determine their quality. After adding loading buffer (15% (w/v) Ficoll, 2.5 mg/ml Orange G) to the samples in a 2.5 to 1 ratio, the samples were loaded on the gel, as well as a 1 kb DNA ladder (Promega, Madison, WI, USA). The DNA was resolved at 100 V for 20 min in 0.5x TAE buffer. Gels were visualized and photographed using the Gel Doc™ EZ Imager (Bio-Rad Laboratories, Hercules, California, USA).

2.2.9 Microsatellite marker analysis of leaf rust isolates

Nineteen described microsatellite primer combinations (Table 2.4; Szabo and Kolmer, 2007; Wang *et al*., 2010) were used to genotype forty Zimbabwean *Pt* isolates (Appendix 2.4) and eight South African *Pt* race control isolates (Table 2.5). Among the eight controls, races MCDS (3SA146) and FBPT (3SA147) were included on the basis that they were previously detected in both Zimbabwe and SA (Terefe *et al*., 2014a; 2014b). While races CFPS (3SA10) and CFPS+*Lr20* (3SA248) (Boshoff *et al*., 2018), and MCPS (3SA127) and MFPS (3SA100) (Terefe *et al*., 2021) are newly described races in SA, races SCDS (3SA137, detected in 2011; Terefe *et al*., 2011) and SDDN (3SA144, detected in 2005; Pretorius and Bender, 2010) represented two of the older South African races.

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Table 2.4: Nineteen described microsatellite markers, their primer sequences, annealing temperatures (°C), size of the amplicon in base pairs (bp) and repeat length, used in the current study.

Locus	Forward primer (5'-3')	Reverse primer (5'-3')	°C	bp	Repeat
Szabo and Kolmer (2007)					
PtSSR50	CATCGGAATGGTCTGTCTCC	CCAAATGCTATGAGTGGAAAA	62	360	2
PtSSR55	AGCTTACGGTCCTCAATCG	AGTGAAAGGGGCTGGGAGT	62	302	2
PtSSR61	CGAACTGGTACAACGCACTG	CGCAAAAAGGCTGATCTCTG	62	300	2
PtSSR92	CCAAGGAACAGTCCACCAAG	GAGTCGGGTAAGCCATCTGA	62	240	2
PtSSR152	CTCCGTTCCTCTTTCTGTCG	CCATCGCAACCAACAAACA	62	380	3
PtSSR158	GACGACTTCGTCACTGCTGA	GAGGAGAAGCCGTTCTGTTG	62	230	3
PtSSR161	ACTGCCTCCTGTGCCTTCT	TAGTCCGAGGGTGACGAAGT	62	210	2
PtSSR164	GTGGAAGTGAGCGGAAGAAG	GGAGATGGGCAGATGAGGTA	62	215	2
PtSSR184	GGTCTGGCGAATCTTTCCTT	CATTTTTAGTTGTGAGCCCTTG	62	370	2
PtSSR186	GCCACGAGAAATACATAGAAATAAAA	GGTTGTTGATGGGCTTGAGT	58	340	3
Wang *et al*. (2010)					
PtSSR0083	ATGGATTTGGAGACCAGTCG	GTTGAAAGATCTGGGGGTGA	60	250	2
PtSSR0189	TCTCAACCAAAAATCAATCTACG	CTTCCACGAAGACGAAGCAC	56	110	2
PtSSR0243	CTCACTCGCTCGCTTGTTCT	GACGAAAAGATCGGGTTTGA	58	200	2
PtSSR0481	CCACAATCCTCCGTTCTGAT	CGAAAGCAAAACACATGAGG	60	190	3
PtSSR0639	TCTCCGCCTACCAACACTG	AAAGGAGGGAGAGGGGAGG	60	200	3
PtSSR0801	CAATGGTAGTGGCAAGCAAA	GCACCTCTCACGCTCTTAGC	60	200	2
PtSSR5594	CGGACCAAACACAAAGGAAA	CCCTGCGTTTAACACCTTGT	62	210	3
PtSSR6386	AATGAGGTGACTCGGATGGA	GAAGAAGGCGAAGTTGTTGC	58	190	3
PtSSR6542	TGTGATCTCGCCCGTACATA	TGGGAATGATGGACACACAC	60	140	2

Table 2.5: List of South African *Puccinia triticina* (*Pt*) control races used in the current study.

ARC-SG[1]	UFS[2]	NA[3]	Year	Reference
3SA144	UVPt19	SDDN	2005	Pretorius and Bender (2010)
3SA137	UVPt12	SCDS	2009	Terefe *et al*. (2011)
3SA146	UVPt21	MCDS	2010	Terefe *et al*. (2014a)
3SA147	UVPt22	FBPT	2010	Terefe *et al*. (2014b)
3SA248	UVPt26	CFPS+*Lr20*	2016	Boshoff *et al*. (2018)
3SA10	UVPt27	CFPS	2016	Boshoff *et al*. (2018)
3SA127	UVPt29	MCPS	2019	Terefe *et al*. (2021)
3SA100	UVPt30	MFPS	2020	Terefe *et al*. (2021)

[1] Agricultural Research Council-Small Grain race notation; [2] University of the Free State race notation; [3] North American race notations.

Each 10 µl PCR reaction contained 10 ng DNA, 1 µM of each primer set and a 1x concentration of KapaTaq Ready-mix (Sigma-Aldrich, USA). The amplification regime was 95°C for 3 min, followed by 40 cycles of 94°C for 30 s, varying annealing temperatures (Table 2.4) for 30 s and 72°C for 1 minute. A final 3 min extension step at 72°C was included. Successful amplification was confirmed on a 1.5% (w/v) agarose gel.

Microsatellite amplicons were finally resolved on the QIAxcel Advanced system (QIAGEN, Germany) using a QX DNA High Resolution cartridge and the OM800 separation method (5 kV injection voltage, 10 s injection time, 6 kV separation voltage, 920 s separation time). The size of each amplicon was determined using the QX 50-800 bp v2.0 DNA size and QX 15 bp/1 kb alignment markers according to the suppliers' instructions, and finally hand-edited according to the repeat length of each microsatellite locus. A data matrix based on allele size was prepared for each dikaryotic individual.

2.2.10 Genetic analysis of the Zimbabwean *Puccinia triticina* field isolates

The suitability of the microsatellite markers to genetically describe the selected Zimbabwean *Pt* field isolates and South African control races was first tested. Parameters included the gene diversity, number of alleles per locus, allelic richness, the observed (H_o) and expected (H_e) heterozygosity, and the *F*-statistics parameter (F_{ST}) and inbreeding coefficient (F_{IS}) (Weir and Cockerham, 1984) for each locus individually, as well as for all loci combined, using GENEPOP web version 4.0 (Raymond and Rousset, 1995; Rousset, 2008) and FSTAT web version 2.9.4 (Goudet, 2003). Linkage disequilibrium across all microsatellite loci was calculated with the index of association (I_A), and also with a measure corrected for the number of loci, rBarD, using MULTILOCUS version 1.3 (Agapow and Burt, 2001).

To evaluate the extent of clonal reproduction, the presence of identical MLGs within the isolates, was recorded. MLGsim version 2.0 using 10 000 simulations (Stenberg *et al*., 2003)

was used to assess which individuals belonged to the different MLGs. Clonal MLGs containing more than one isolate or race, were reduced to a single representative individual for further analysis. A new allelic data set was thus created containing data for just these MLGs.

The genetic relationships between the forty Zimbabwean *Pt* field isolates and South African control races, were described using the unweighted neighbour-joining (NJ) cluster analysis within DARwin version 5.0.158 (Perrier and Jacquemoud-Collet, 2006). The minimal amount of valid data per marker was set at 70%, and 30 000 bootstraps were used to create the unrooted phylogram.

The genetic structure of the field isolates and control races was tested by grouping individual isolates into clusters using both the Bayesian-model based STRUCTURE and multivariate Discriminant Analysis of Principal Components (DAPC) analyses. Ten separate runs with a burnin duration of 10,000, 10,000 Monte Carlo Markov Chain (MCMC) repeats after burnin, and 10 iterations each run were done in STRUCTURE version 2.3.4 (Pritchard *et al*., 2000). The output data was analysed with CLUMPAK (Kopelman *et al*., 2015), and the *ad hoc* ΔK statistic (Evanno *et al*., 2005) was used to calculate the optimal number of clusters (*K*). This optimal *K* value was then used to re-run the analysis in STRUCTURE using a burnin period of 100 000, 100 000 MCMC repetitions after burnin and the determined optimal number of clusters.

The Bayesian Information Criterion (BIC) value provided the appropriate number of clusters for the DAPC analysis (Jombart *et al*., 2010) carried out with ADEGENET in the R environment.Clustering of individuals and the preparation of graphs were done within the ADEGENET webserver (Available from https://github.com/thibautjombart/adegenet).

2.2.11 Sources of genetic variation between clusters of Zimbabwean *Puccinia triticina* isolates and South African races

The significance of the proposed DAPC genetic structure was tested with Analysis of Molecular Variance (AMOVA; Excoffier *et al*., 1992) within ARLEQUIN version 3.5.1.2 (Schneider *et al*., 2010) at the 0.05 significance level. The significance level of both the AMOVA components and the F-statistics parameter F_{ST} were tested using a total of 16 000 permutations. The interpretation of an F_{ST} value above 0.15 was that there was a highly significant genetic discrimination between clusters (Wright, 1951). The pairwise comparison between clusters as revealed by STRUCTURE analysis was also tested with AMOVA within ARLEQUIN version 3.5.1.2 at the 0.05 significance level.

2.2.12 Correlation between phenotypes and genotypes of tested *Puccinia triticina* isolates

The avirulence/virulence profiles of the Zimbabwean *Pt* field isolates and eight South African *Pt* control race MLGs against 27 wheat differential lines were used to prepare a binary data matrix where avirulence was scored as 0, virulence as 1 and missing data as 9 (Appendix 2.6). Using this data set, a dendrogram was constructed using the unweighted NJ cluster analysis within DARwin version 5.0.158 (Perrier and Jacquemoud-Collet, 2006). Possible correlation between the MLG phenotypic dendrogram and genotypic phylogram was calculated using the Mantel test within GenAIEX 6.5 (Peakall and Smouse, 2006; 2012) using the respective distance matrices.

2.3 Results

2.3.1 Prevalence of *Puccinia triticina* in commercial fields and trial plots during 2019 to 2021 in Zimbabwe

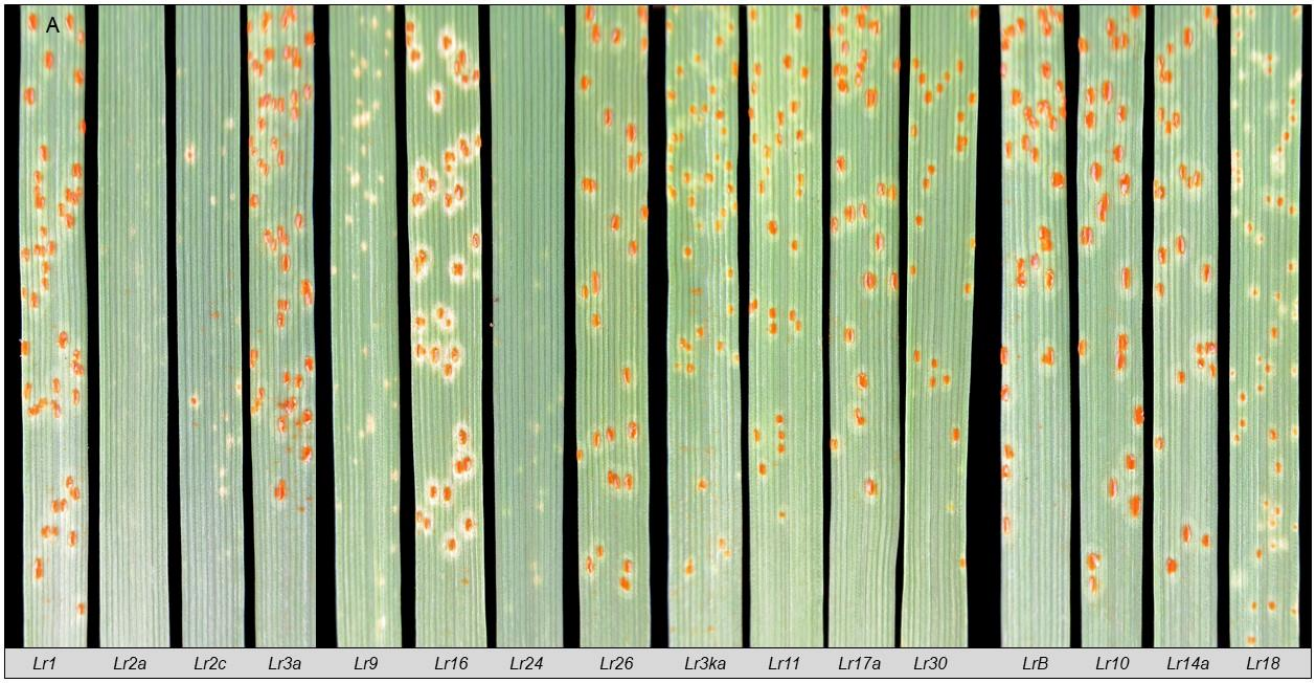
The distribution and severity of leaf rust observed from 2019 to 2021 was generally similar in the lowveld, middleveld and highveld regions of Zimbabwe. In all seasons, the disease occurred with high incidence and severity on susceptible wheat cultivars in breeding plots and commercial fields that were not sprayed with fungicides. However, the lowveld sites of Chiredzi, Save Valley and Chisumbanje had higher incidence levels in 2019 and 2020 compared to other sites. Commercial cultivars SC Nduna and Peregrine were the most widely grown cultivars during 2019 and 2020. Fewer samples were collected in 2021 as maize was the preferred summer crop due to potentially higher yields per unit area. The summer-adapted cultivar SC Sahai was the most grown cultivar during the 2020/21 summer season. High incidences of *Pt,* up to 100%, were observed wherever wheat cultivars SC Nduna, Peregrine and SC Sahai were grown (Appendix 2.1).

2.3.2 Phenotypic analysis of *Puccinia triticina* isolates

The ITs for the control races MCDS (isolate UVPt21_75) and FBPT (UVPt22_1) and 104 Zimbabwean isolates recorded on the standard and additional differentials are presented in Appendix 2.3. The IT responses of the Zimbabwean isolates were generally similar for all the *Lr* genes with only slight deviations observed. With no differences observed in the avirulence/virulence profiles, only one *Pt* race was identified from all isolates collected from 2019 to 2021 in Zimbabwe. The identified race matched the ITs recorded for South African race MCDS (isolate UVPt21_75) which was used as the first control, with avirulence/virulence formula: *Lr2a, Lr2b, Lr2c, Lr3ka, Lr9, Lr11, Lr16, Lr18, Lr19, Lr23, Lr24, Lr28, Lr30*, *Lr32/Lr1, Lr3a, Lr3bg, Lr10, Lr14a, Lr15*, *Lr17a, Lr17b, Lr20, Lr26, Lr27+31* (not shown)*, Lr37, LrB*

(Figure 2.3). The isolates differed from race FBPT (UVPt22_1) (avirulence/virulence formula: *Lr1, Lr2a, Lr2b, Lr9, Lr11, Lr15, Lr16, Lr17b, Lr19, Lr20, Lr23, Lr24, Lr26, Lr27+31, Lr28, Lr32, Lr37/Lr2c, Lr3a, Lr3bg, Lr3ka, Lr10, Lr14a, Lr17a, Lr18*, *Lr30, LrB*).

The adult plant ITs showed that APR genes *Lr22a* (IT = ;1 to 1+), *Lr34* (Z3- to 3=, typical low slow rusting response for this gene) and *Lr35* (1 to ;1+) are effective against the South African MCDS isolate and Zimbabwean isolate Pt1_1_2019 that also typed as MCDS (Table 2.6; Figure 2.4). Both MCDS isolates were virulent to *Lr12* (IT = 3+), *Lr13* (3C), *Lr22b* (4), and *Lr37* (3+ to 33+) (Table 2.6; Figure 2.4). The APR genes *Lr12*, *Lr13*, *Lr22a*, *Lr34*, *Lr35* and *Lr37* were effective against the South African isolate of *Pt* race FBPT (Table 2.6) confirming that race MCDS is more virulent at adult stage than race FBPT.



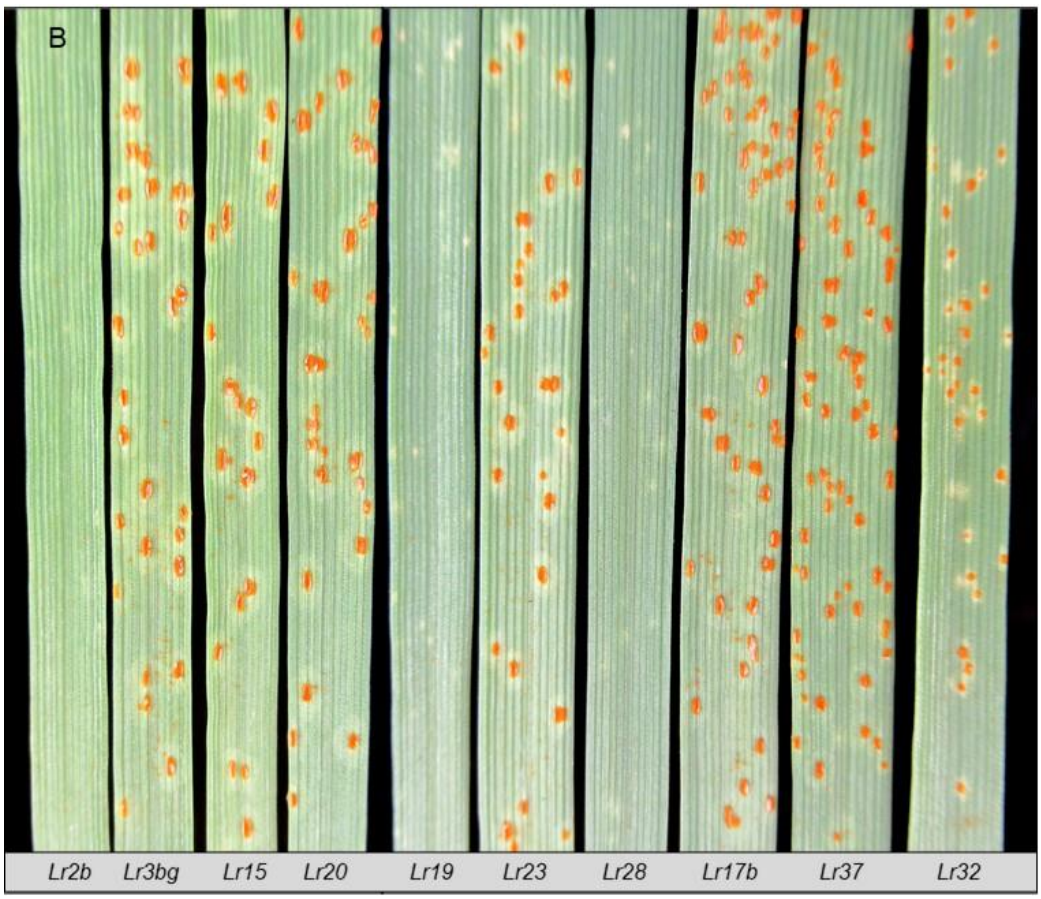


Figure 2.3: Typical seedling infection types (ITs) observed for Zimbabwean *Puccinia triticina* (*Pt*) isolate Pt1_1_2019 on 26 differential lines listed in Table 2.2 carrying designated *Lr* genes.

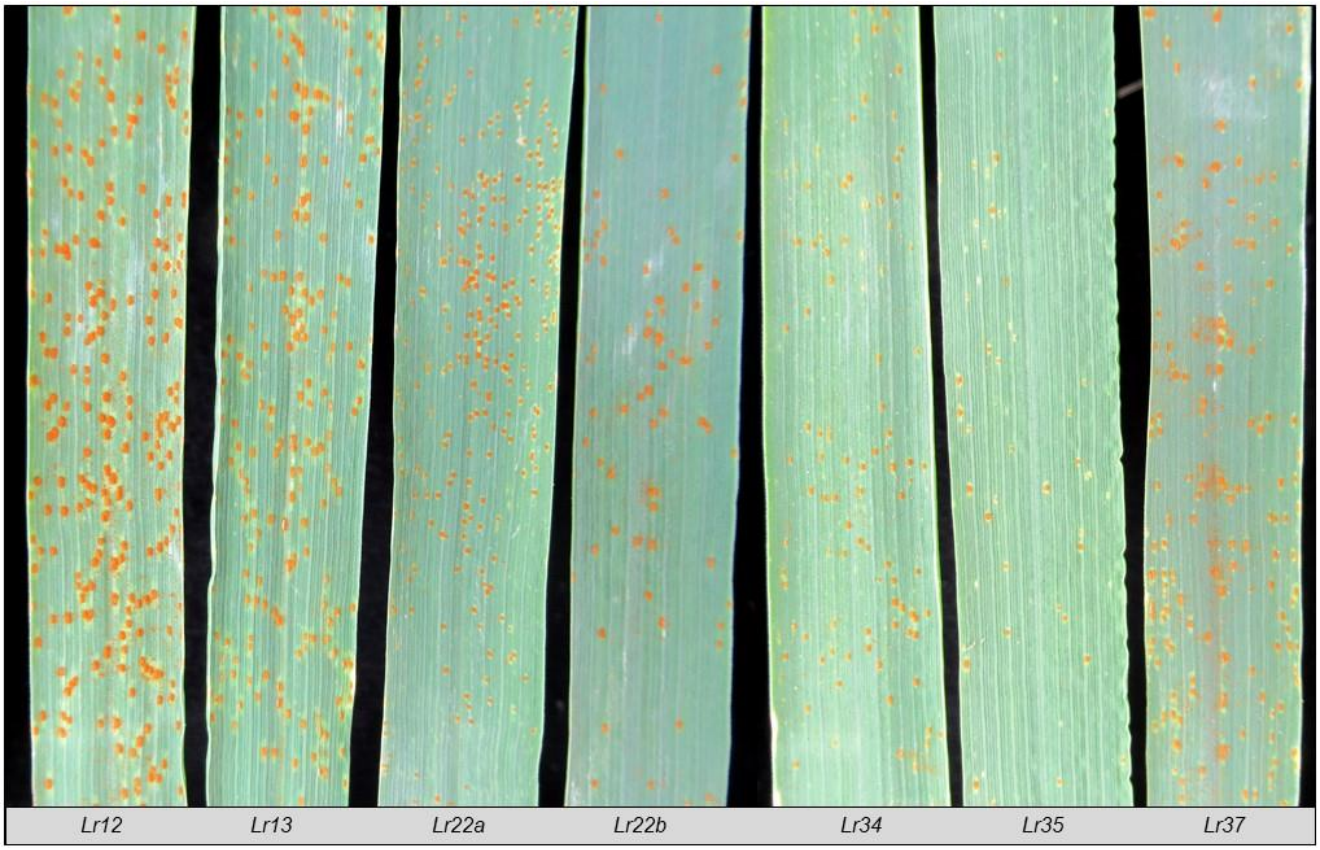


Figure 2.4: Flag leaf infection types (ITs) observed for Zimbabwean *Puccinia triticina* (*Pt*) isolate Pt1_1_2019 on the susceptible control Thatcher (*Lr22b*) and differential lines listed in Table 2.2 carrying designated adult plant *Lr* genes.

Table 2.6: Seedling- and adult plant infection types (ITs) recorded for *Puccinia triticina* (*Pt*) isolates of races MCDS and FBPT from South Africa (SA) and Zimbabwe (ZIM) on additional differential lines containing leaf rust resistance (*Lr*) genes.

	***Pt* isolate and ITs**		
***Lr* gene**	**MCDS UVPt21_75 (SA)**	**FBPT UVPt22_1 (SA)**	**MCDS Pt1_1_2019 (ZIM)**
21[1]	;1+	;1	;1+
25[1]	;1N	;1	;
29[1]	;	;1-	;
33[1]	2++C	2++C	2++C
36[1]	;1N	;1-	;1=
38[1]	;	;	;
44[1]	;	;1CN	;
45[1]	;1=	;1=	;1=
47[1]	;	;	;
50[1]	;1+X	;1+X	;1+X
51[1]	;1N	;1-	;1=
52[1]	;	;1-	;
12[2]	3+	;	3+
13[2]	3C	;	3C
22a[2]	;1	0;	1+
22b[2]	4	3+	4
34[2]	Z3-	3-	3=
35[2]	1	0;	;1+
37[2]	3+	0;	33+

[1] Seedling ITs were recorded 12 days following inoculation of the primary leaves; [2] Adult plant infection types were recorded 14 days following inoculation of the flag leaves.

2.3.3 Genotypic analysis of Zimbabwean *Puccinia triticina* isolates

Seventeen of the 19 microsatellite markers used in this study were polymorphic with only microsatellite markers PtSSR161 and PtSSR0481 being monomorphic (Table 2.7). Microsatellite markers PtSSR158, PtSSR152, PtSSR92, PtSSR5594, PtSSR186, PtSSR50, PtSSR0243, PtSSR6386 and PtSSR184 had the highest number of alleles (3 each), while an average of 2.37 was found for all 19 markers. Microsatellite markers PtSSR184 and PtSSR0243 had the highest gene diversity level of 0.68 and 0.65 respectively compared to the 0.36 average for all 19 markers. The averaged F_{ST} value of 0.13 ($P < 0.01$) indicated a low level of genetic differentiation within the population, while the negative F_{IS} of -0.31 indicated an excess of heterozygotes, a clear indication of clonality. Together with this, the average H_o of 0.47 that was higher than the H_e of 0.35 under the Hardy-Weinberg equilibrium, confirmed that the *Pt* population reproduced asexually (Table 2.7). This was further supported by the linkage disequilibrium parameters of I_A (3.57; $P < 0.001$) and rBarD (0.24; $P < 0.01$) that both differed significantly from 0.

The genotypic analysis of the Zimbabwean field isolates collected in 2019 and 2020 strongly supported the phenotypic data with most isolates grouping with the South African control race MCDS in cluster C of the DARwin NJ phylogram (Figure 2.5). The cluster analysis revealed three distinct genetic clusters. Cluster A was composed of three South African *Pt* control races FBPT (3SA147), CFPS (3SA10) and CFPS+*Lr20* (3SA248) that were genetically distinct from the other isolates. Cluster C consisted of 35 Zimbabwean isolates and three South African *Pt* control races MCDS (3SA146), MCPS (3SA127) and MFPS (3SA100). South African race isolates MCPS and MFPS were closely related to race isolate MCDS sharing 95% and 92% genetic similarity respectively (Appendix 2.5). Similarly, these two races were closely related to the Zimbabwean isolates with 23 isolates sharing 100% and 97% genetic similarity to MCPS and MFPS, respectively (Appendix 2.5). Five Zimbabwean MCDS isolates (5_1_2020, 20_1_2020, 23_2_2020, 19_1_2019, 24_3_2019) sharing genetic similarities varying from 70-80% with South African control race isolates SDDN (3SA144) and SCDS (3SA137), formed cluster B. Since they grouped outside cluster C containing all the other MCDS isolates and controls, these five isolates were retyped using the standard set of differentials as previously described and their MCDS phenotype was reconfirmed.

Among the forty Zimbabwean isolates and eight South African control races, 22 single and two clonal MLGs were identified (Table 2.8). The 24 MLGs were represented by either a single Zimbabwean isolate or a single South African control race isolate, except for MLG11, which included 23 Zimbabwean isolates and one South African race isolate and MLG15 consisting

of two Zimbabwean isolates. Since control race MCPS (3SA127) formed part of MLG11, it, together with Zimbabwean isolate 1_1_2019, were selected to represent MLG11, while isolate 6_2_2020 was selected to represent MLG15. Further analyses were done using the allelic data representing these 25 MLG isolates.

When the NJ phylogram was redrawn using only the MLG data, cluster A consisted again of the three South African race isolates FBPT (3SA147), CFPS (3SA10) and CFPS+*Lr20* (3SA248) (Figure 2.6). Race FBPT was the most genetically distinct only sharing 42% genetic similarity with the Zimbabwean isolates and control race MCDS. In contrast, the other two race isolates shared at least 55% genetic similarity with the Zimbabwean isolates and race MCDS. Cluster C contained twelve Zimbabwean isolates and three SA *Pt* control race isolates MCDS, MFPS and MCPS. Cluster B contained five Zimbabwean isolates (5_1_2020, 20_1_2020, 23_2_2020, 19_1_2019, 24_3_2019) sharing genetic similarity with two older South African race isolates, SDDN (3SA144) and SCDS (3SA137).

Table 2.7: Average genetic diversity of the representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African *Pt* control races using 19 microsatellite markers.

						Heterozygosity	
Marker	**Gene diversity**	**Number of alleles**	**Allelic richness**	F_{ST}	F_{IS}	H_o	H_e
Number of MLGs[1]	24						
PtSSR161	0	1	1.00	0.00	0.00	0.00	0.00
PtSSR164	0.22	2	1.59	0.28	-0.57	0.33	0.21
PtSSR158	0.39	3	1.90	0.16	0.10	0.35	0.39
PtSSR152	0.56	3	2.27	0.01	-0.78	0.98	0.55
PtSSR92	0.47	3	2.03	-0.02	0.66	0.17	0.49
PtSSR0481	0	1	1.00	0.00	0.00	0.00	0.00
PtSSR0083	0.5	2	1.99	0.00	-0.70	0.83	0.49
PtSSR5594	0.61	3	2.59	0.00	-0.66	1.00	0.60
PtSSR186	0.28	3	1.87	0.33	-0.29	0.33	0.26
PtSSR50	0.53	3	2.4	0.09	-0.23	0.64	0.53
PtSSR6542	0.17	2	1.33	0.4	-1.00	0.33	0.17
PtSSR0189	0.27	2	1.57	0.23	-0.66	0.44	0.27
PtSSR0639	0.27	2	1.55	0.09	-0.29	0.33	0.26
PtSSR0243	0.65	3	2.69	-0.01	-0.34	0.87	0.65
PtSSR6386	0.58	3	2.41	-0.01	-0.61	0.92	0.57
PtSSR61	0.26	2	1.44	0.05	1.00	0.00	0.23
PtSSR0801	0.17	2	1.34	0.4	-1.00	0.33	0.17
PtSSR55	0.22	2	1.62	0.42	-0.57	0.33	0.21
PtSSR184	0.68	3	2.97	0.05	-0.01	0.67	0.66
Average per locus	0.36	2.37	1.87				
Average F_{ST}				0.13			
Average F_{IS}					-0.31		
Average heterozygosity						0.47	0.35

[1] Multilocus genotypes.

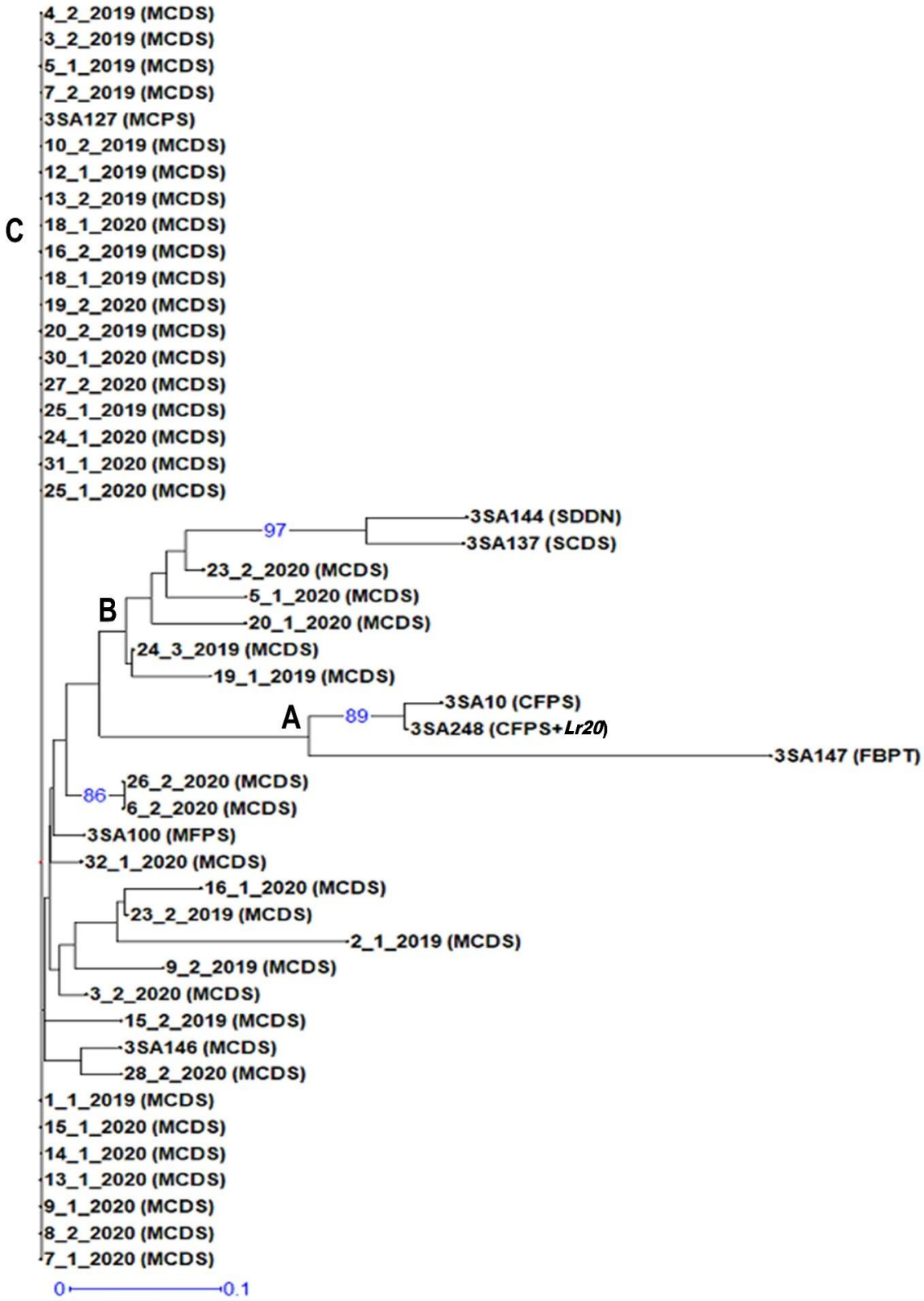


Figure 2.5: Genetic comparison of 40 representative *Puccinia triticina* (*Pt*) isolates from Zimbabwe with representative isolates of eight South African *Pt* races using an unrooted neighbour-joining (NJ) phylogram based on allelic data of 19 microsatellite markers. The North American race notations of *Pt* races are indicated in brackets.

Table 2.8: Number of multilocus genotypes (MLGs) and their significance level (*P*) identified in representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African *Pt* races.

MLG	**No.**	**Isolate(s)**	***P***
MLG1	1	9_2_2019	*
MLG2	1	16_1_2020	*
MLG3	1	5_1_2020	*
MLG4	1	20_1_2020	*
MLG5	1	24_3_2019	*
MLG6	1	19_1_2019	*
MLG7	1	23_2_2020	*
MLG8	1	3SA137	*
MLG9	1	3SA144	*
MLG10	1	3SA146	*
MLG11	24	1_1_2019; 3_2_2019; 4_2_2019; 5_1_2019; 7_2_2019; 10_2_2019; 12_1_2019; 13_2_2019; 16_2_2019; 20_2_2019; 25_1_2019; 7_1_2020; 8_2_2020; 9_1_2020; 13_1_2020; 14_1_2020; 15_1_2020; 18_1_2020; 19_2_2020; 24_1_2020; 25_1_2020; 27_2_2020; 31_1_2020; 3SA127	**
MLG12	1	30_1_2020	*
MLG13	1	28_2_2020	*
MLG14	1	3SA100	*
MLG15	2	6_2_2020; 26_2_2020	ns[3]
MLG16	1	18_1_2019	*
MLG17	1	3_2_2020	*
MLG18	1	2_1_2019	*
MLG19	1	23_2_2019	*
MLG20	1	32_1_2020	*
MLG21	1	3SA147	*
MLG22	1	3SA10	*
MLG23	1	3SA248	*
MLG24	1	15_2_2019	*
Clonal richness			
Number of clones (G)	24		
Number of individuals (N)	48		
Genotypic diversity (R)[1]	0.49		
Clonal diversity index (Pd)[2]	0.5		
Single MLGs	22		
Clonal MLGs	2		

** (*P*<0.01); * (*P*<0.05); [1] Genotypic diversity (R) = (G-1)/(N-1); [2] Clonal diversity index (Pd) = (G/N); [3] ns = not significant.

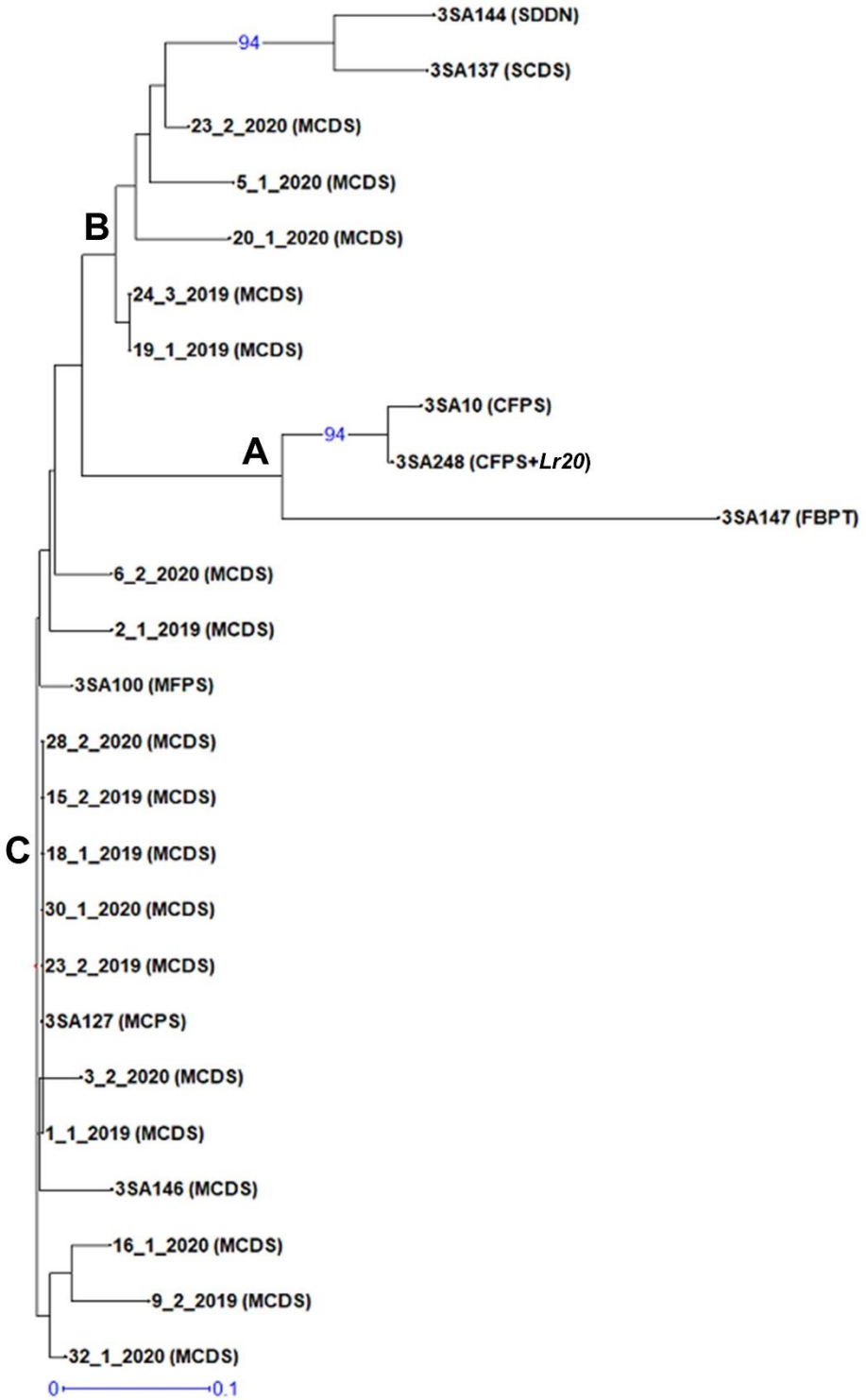


Figure 2.6: Genetic comparison of 17 representative *Puccinia triticina* (*Pt*) isolates from Zimbabwe with representative isolates of eight South African *Pt* races using an unrooted neighbour-joining (NJ) tree based on multilocus genotype allelic data of 19 microsatellite markers. The North American race notations of *Pt* races are indicated in brackets.

2.3.4 Subdivision of Zimbabwean *Puccinia triticina* isolates and South African race isolates into clusters

STRUCTURE analysis divided the 25 MLGs, representing 17 Zimbabwean isolates and eight South African *Pt* races, into three optimal clusters as calculated by the *ad hoc* ΔK statistic. While the ΔK value indicated an optimum of $K = 2$ (Figure 2.7), the systematic increase in K from one to five indicated the effective subdivision of all isolates at $K = 3$ with minimum admixture being evident. Significant admixture within individuals became evident in some isolates at $K = 4$ and further (Figure 2.8).

The Zimbabwean *Pt* population was therefore described in terms of $K = 3$ using STRUCTURE (Figure 2.9). Cluster A contained the same three South African control race isolates from the DARwin phylogram (Figure 2.6), with CFPS and CFPS+*Lr20* having trace amounts of admixture with cluster C. Cluster C contained 14 of the 17 representative Zimbabwean isolates and three SA *Pt* control race isolates, namely MCPS, MFPS and MCDS from cluster C in the phylogram (Figure 2.6). Races MCPS and MFPS had no admixture, but MCDS had trace amounts of admixture with clusters A and B. Two Zimbabwean MCDS isolates (24_3_2019 and 19_1_2019) in cluster C, shared significant admixture with cluster B isolates. Since these two isolates grouped in cluster B in the DARwin phylogram, it is clear that they share genetic similarity to isolates in both clusters. Cluster B contained three Zimbabwean isolates namely 23_2_2020, 20_1_2020 and 5_1_2020 that deviated from the majority of Zimbabwean MCDS isolates since they clustered together with two SA *Pt* races SDDN and SCDS. Isolates 20_1_2020 and 5_1_2020 were admixed for both clusters A and C, whereas isolate 23_2_2020 had significant admixture with cluster C isolates. The two SA *Pt* race isolates SDDN and SCDS shared no admixture with the Zimbabwean isolates in cluster A and C.

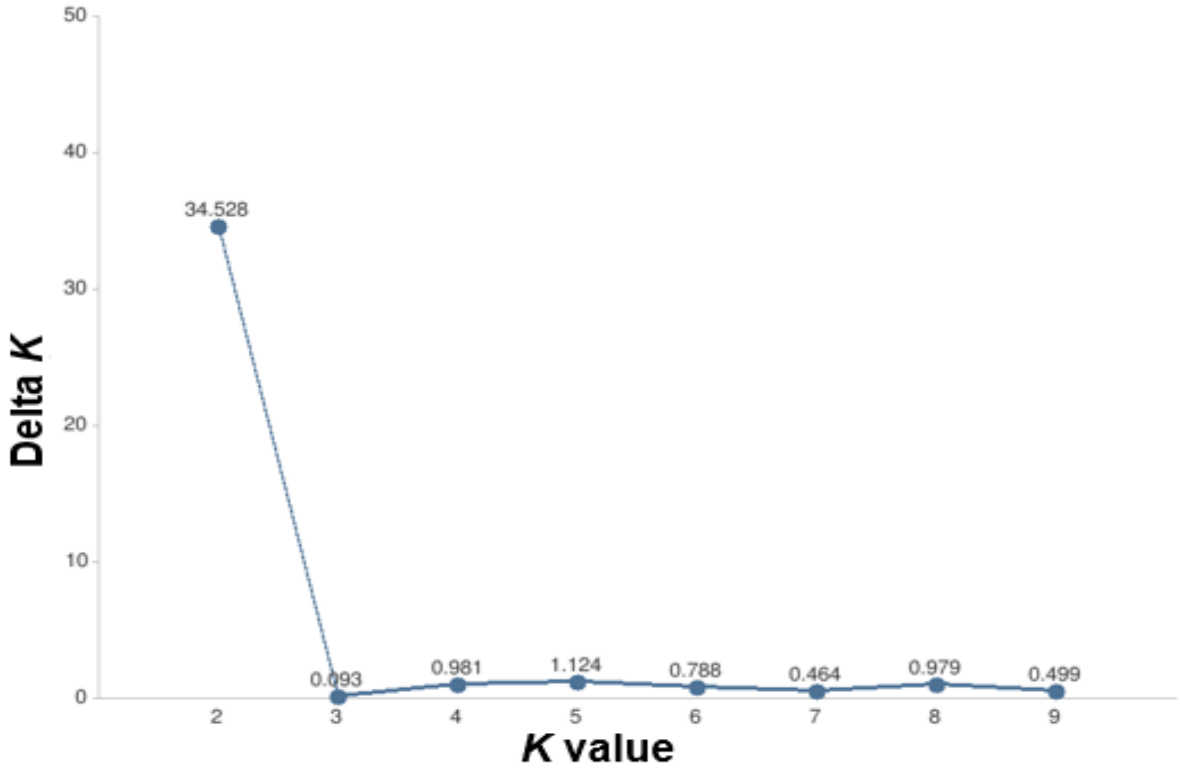


Figure 2.7: Determining the optimal number of clusters (*K*) within the Zimbabwean *Puccinia triticina* (*Pt*) population and eight South African *Pt* control race isolates using the *ad hoc* ΔK statistic as calculated using the multilocus genotype data within STRUCTURE version 2.3.4 (Pritchard *et al*., 2000).

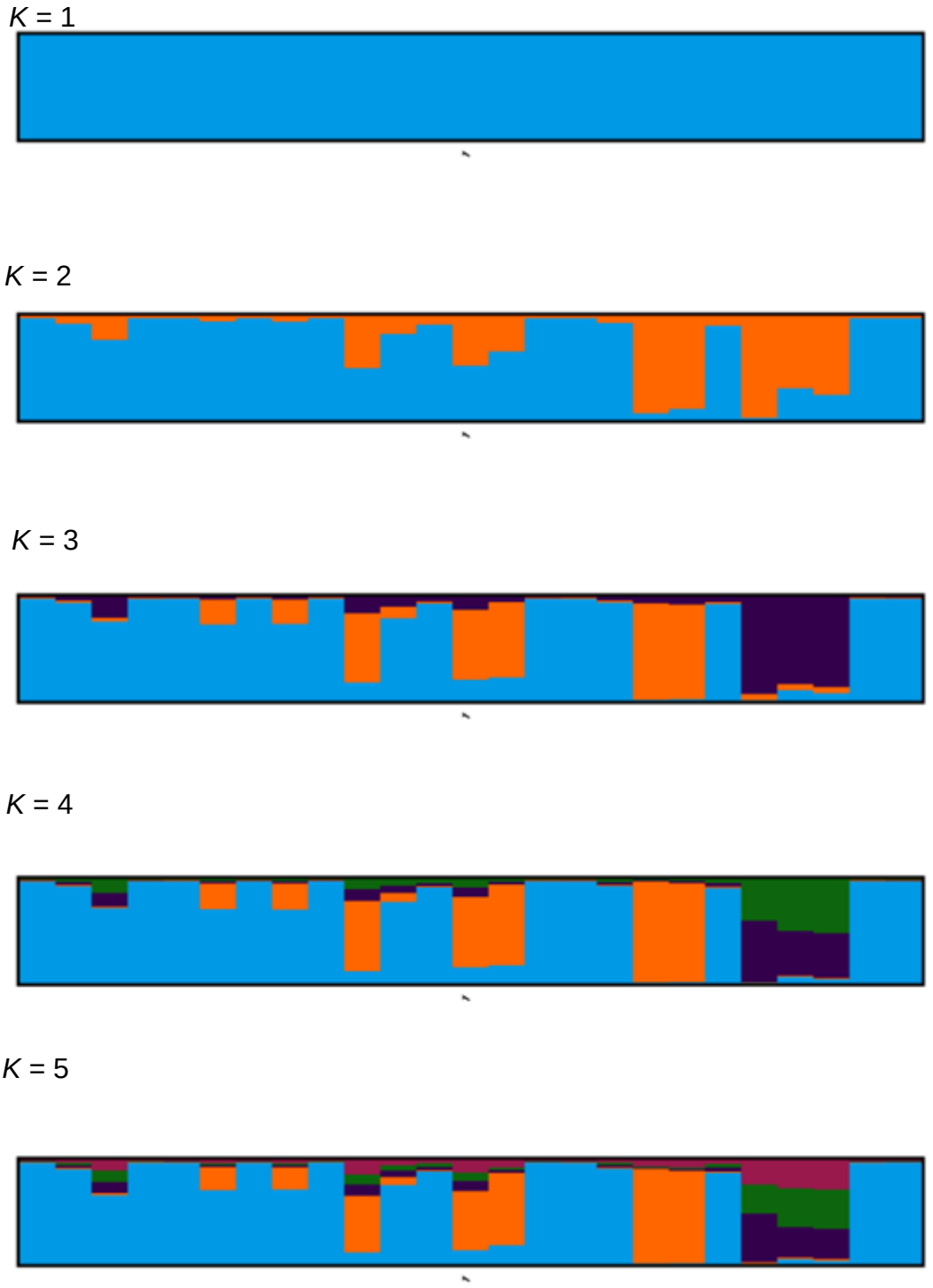


Figure 2.8: Subdivision of *Puccinia triticina* (*Pt*) multilocus genotypes (MLGs) comprising of 17 representative Zimbabwean *Pt* isolates and eight South African *Pt* control race isolates into clusters according to STRUCTURE for *K* = 1 to *K* = 5.

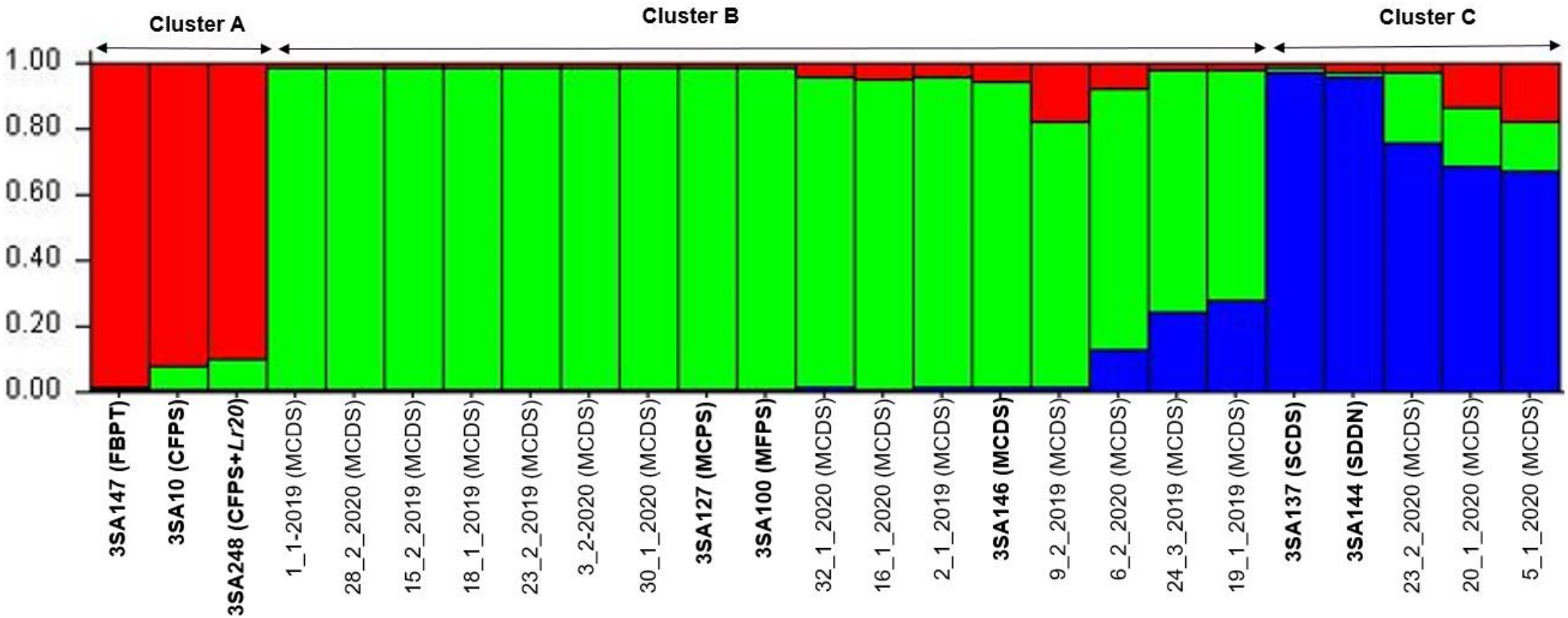


Figure 2.9: Subdivision of 17 representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African *Pt* control race isolates into three clusters using the multilocus genotype (MLG) data as calculated with the *ad hoc* ΔK statistic (Evanno *et al*., 2005) using STRUCTURE version 2.3.4 (Pritchard *et al*., 2000). Clusters are indicated in different colours. South African *Pt* control race isolates are labelled in bold.

The BIC curve generated with the DAPC analysis indicated that the optimal number of clusters for the Zimbabwean *Pt* isolates and South African controls are three as indicated by the characteristic “elbow” in the descending graph (Figure 2.10A). The scatter plot of the 25 MLG isolates divided these isolates into 3 clusters (Figure 2.10B) that were identical to the NJ phylogram (Figure 2.6). According to the scatter plot, cluster B and cluster C are closely interlinked between quadrant 2 and 3, suggesting close genetic similarity between the isolates, while the isolates in cluster A was genetically distinct.

2.3.5 Sources of genetic variation between clusters of Zimbabwean *Puccinia triticina* isolates and South African races

The division of the Zimbabwean *Pt* isolates and South African *Pt* races into three clusters was highly significant as indicated by an F_{ST} value of 0.65 (P<0.01; Table 2.9). The AMOVA determined that most of the variation could be attributed to variation among clusters (45.62%) and less among individual populations within clusters (34.35%) and between individuals within clusters (20.03%).

2.3.6 Pairwise comparison between Zimbabwean *Puccinia triticina* population clusters

A pairwise comparison between the three genetic clusters based on the STRUCTURE analysis showed moderate to highly significant differentiation between the three clusters. An F_{ST} value of 0.68 (P<0.01) indicated a highly significant discrimination between clusters A and C (Table 2.10). Moderate but still significant F_{ST} values of 0.5 (P<0.01) and 0.45 (P<0.01) were found between clusters A and B, and clusters B and C respectively.

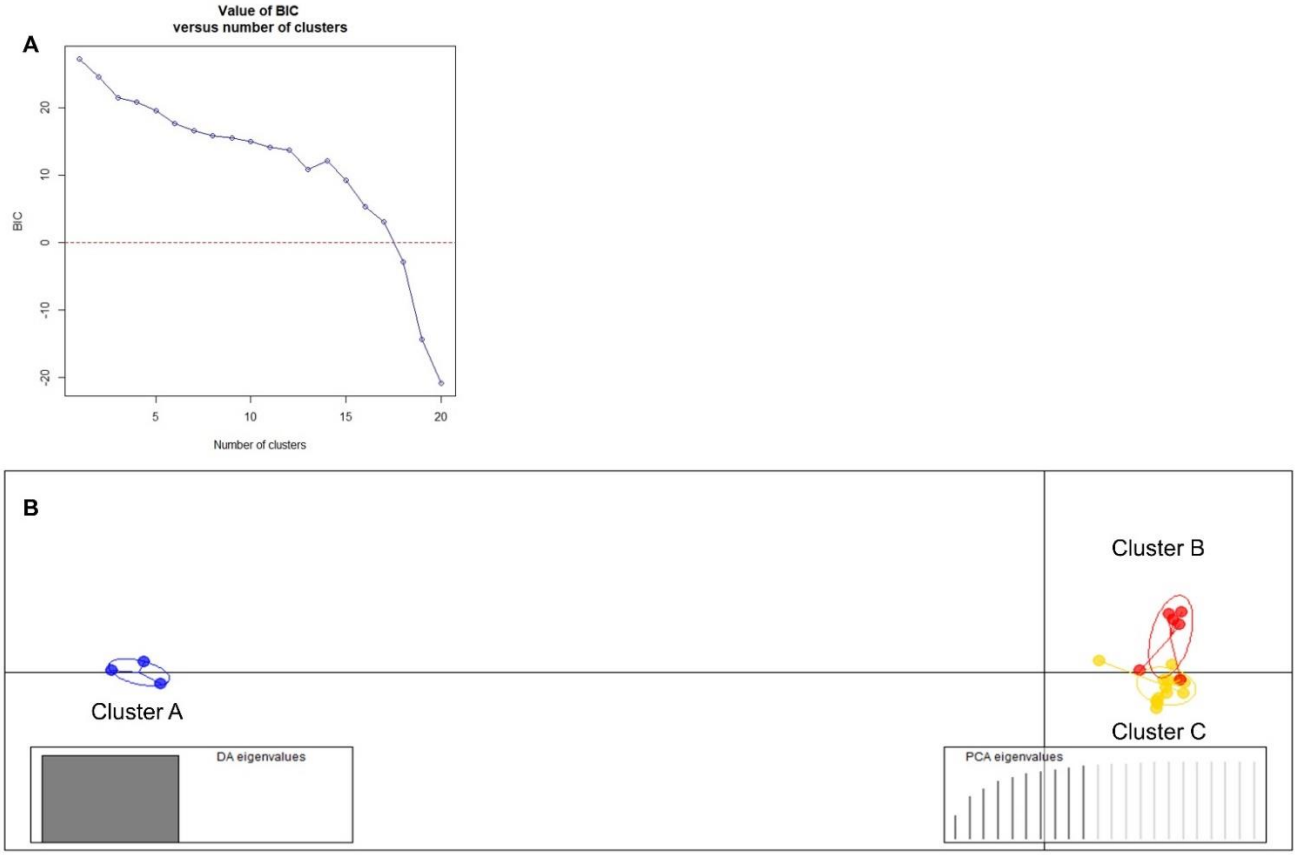


Figure 2.10: Subdivision of 17 representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African *Pt* controls into three clusters according to the multilocus genotype (MLG) data as calculated by discriminant analysis of principal components (DAPC) (Jombart *et al*., 2010). A. Bayesian information criteria (BIC) graph supports three optimal clusters. B. Scatterplot of the 25 MLG isolates divided into 3 clusters. The cluster designations corresponded to the genetic groups indicated in the unweighted neighbour-joining DARwin tree.

Table 2.9: AMOVA design explaining sources of variation between clusters of Zimbabwean *Puccinia triticina* (*Pt*) isolates and South African *Pt* race controls.

AMOVA design and results (average over 19 loci):			
Source of variation	**Sum of Squares**	**Variance components**	**Percentage variation**
Among clusters	37.84	3.27	45.62
Among groups within clusters	10.17	6.36	34.62
Individuals within clusters	34.64	1.68	20.03
Total	82.64	4.78	
Average *F*-statistics over all loci			
Fixation Indices			
F_{ST}:	0.65		
P value:	0.00		

Table 2.10: Pairwise comparison between clusters of Zimbabwean *Puccinia triticina* (*Pt*) isolates and South African control races as calculated by STRUCTURE.

	Cluster A	**Cluster B**	**Cluster C**
Cluster A	–	0.5	0.68
Cluster B	0.01	–	0.45
Cluster C	<0.01	<0.01	–

Pairwise F_{ST} values displayed in the upper and their significance values in the lower diagonal.

2.3.7 Correlation between the phenotypes and genotypes of Zimbabwean *Puccinia triticina* isolates

An unrooted neighbour-joining dendrogram based on the phenotypic profiles of the 25 MLGs, revealed two clusters (Figure 2.11). Cluster B contained all the Zimbabwean *Pt* isolates and South African control race isolate MCDS, whereas cluster A contained the other seven South African *Pt* race isolates. In contrast, the phylogram based on the genotypic data of the 25 MLGs revealed three clusters (Figure 2.6).

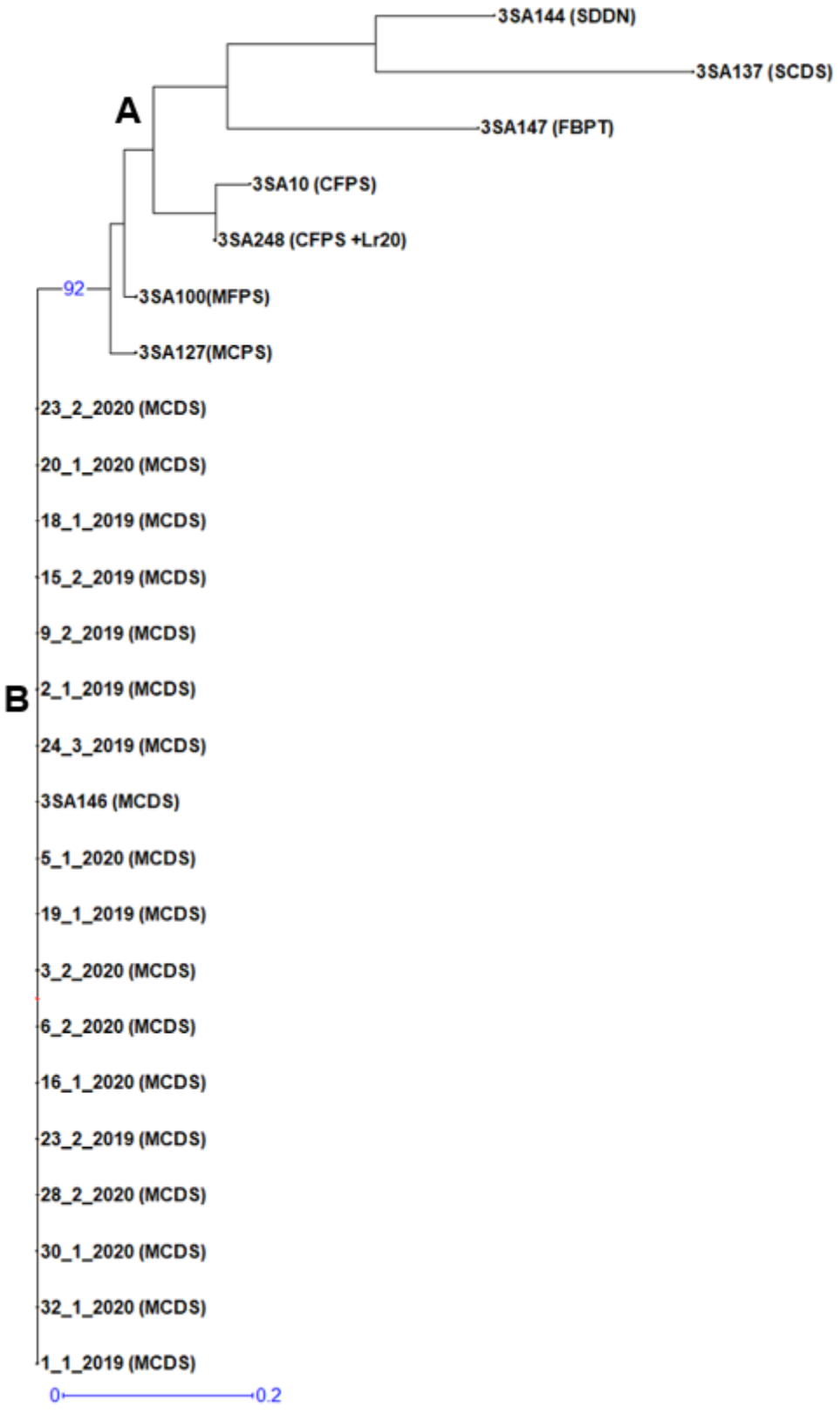


Figure 2.11: An unrooted neighbour-joining tree of representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and South African control race isolates based on their phenotypic profiles to leaf rust resistance genes in 27 differential lines.

Calculation of the correlation between the phenotypic and genotypic distance matrixes of the 25 MLGs revealed a strong and significant positive correlation of 0.77 (P<0.05) (Figure 2.12).

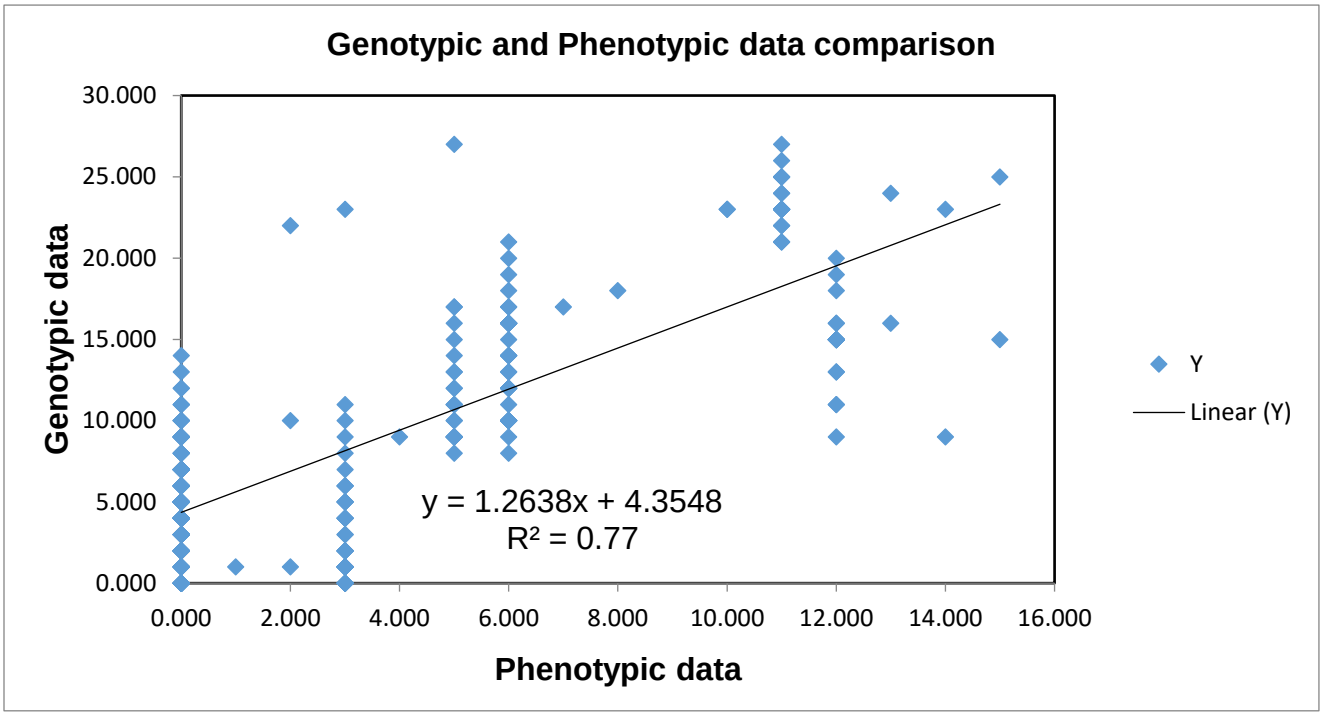


Figure 2.12: Correlation between the phenotypes and genotypes of the 25 multilocus genotypes done according to the Mantel test using their respective phenotypic and genotypic distance matrices.

2.4 Discussion

In Zimbabwe, spring wheat is mainly grown during the winter season under irrigation. Low rainfall between the 2017 to 2019 summer seasons resulted in low water reserves for wheat irrigation in the winter season. This, coupled with frequent power cuts, forced most commercial farmers to reduce their wheat hectarage. Furthermore, the land used for growing wheat during the winter seasons is far less than that of maize during the rain-fed summer season. For example, wheat was grown on 50 000 ha during the 2019 winter season compared to 1.4 M ha of maize in the summer season. The trend was the same in 2020 (62 000 ha compared to 1.5 M) and 2021 (66 435 ha compared to 1.9 M) (Zimstat, 2021). So, currently wheat in Zimbabwe is grown on a small area compared to its potential. However, the incidence of leaf rust was moderate to high despite limited planting and the use of fungicides, signifying that wheat leaf rust is a disease of economic importance in Zimbabwe.

Commercial wheat farming in Zimbabwe started in the 1960s but very little and inconsistent information is available on the occurrence and genetic diversity of *Pt* in the country due to the absence of regular annual surveys. In contrast, annual surveys have been conducted in SA

since the early 1980s (Pretorius *et al*., 1987). Boshoff *et al.* (2018) reported that 20 *Pt* races were characterized between 1984 and 2010 with an additional four races detected between 2012 and 2016. Nine races were also characterized from commercial wheat fields and rust trap nurseries across the major wheat growing regions of SA between 2017 and 2020 (Terefe *et al*., 2021; 2022). These included *Pt* races, which emerged either through stepwise mutation of local races or through exotic introductions from other countries. These studies have identified some *Pt* races that were phenotypically and genotypically similar to South African races being found in neighbouring countries providing evidence of regular inoculum exchange in the Southern African Development Community (SADC) region (Terefe *et al.*, 2014a). For example, *Pt* race MCDS (3SA146) was detected in Zimbabwe and Zambia during 2011 and 2012, races FBPT (3SA147) and SCDS (3SA137) were also confirmed in Zimbabwe and Malawi in 2012 (Pretorius *et al*., 2015). Therefore, the present study reports on the genetic relationship between the Zimbabwean and South African *Pt* populations using both phenotypic and microsatellite marker data. Summarised, the *Pt* isolates collected in Zimbabwe demonstrated a close genetic similarity with selected isolates from South African *Pt* races, thus reflecting a recent shared ancestry.

The phenotypic analysis carried out on the Zimbabwean isolates based on their avirulence/virulence profiles applying the North American nomenclature system (Long and Kolmer, 1989; Kolmer *et al.*, 2007) identified all the isolates as race MCDS. Microsatellite analysis revealed a close genetic relationship between the Zimbabwean isolates and those representative of the South African control races MCPS, MFPS and MCDS. A recent study on the diversity of *Pt* on wheat in SA from collections between 2017 and 2020 showed that among the nine races found, race MCDS was common with an average frequency of 18% (Terefe *et al*., 2021). The phenotypic data of races MCPS (2017) and MFPS (2020) revealed similar virulence profiles to the existing Zimbabwean race MCDS. Race MFPS differs in virulence from MCDS on *Lr3ka*, *Lr24* and *Lr30*, while race MCPS differed from MCDS on *Lr3ka* and *Lr30*. All the Zimbabwean *Pt* isolates and South African control races, except FBPT (3SA147) and SDDN (3SA144), were virulent to the ASR gene *Lr26*. This gene is present on the 1B/1R wheat-rye translocation which is common in wheat lines developed by the CIMMYT based in México (Liu and Chen, 2012). CIMMYT has been the main source of wheat germplasm since the beginning of the Zimbabwean wheat breeding programme in the 1980s (Ephraim Havazvide and Tegwe Soko, personal communication). This implies that this gene is likely to be present in the Zimbabwean wheat germplasm since then and that commercial cultivars currently being grown in Zimbabwe may contain the *Lr26* gene. In addition, the variety turnover in Zimbabwe has been very low and dominated by only a few cultivars. For

example, SC Nduna has been widely grown since 2010 with only a few other cultivars being introduced (Table 2.1). From 2019 to 2021, two new cultivars, SC Select and Peregrine, were also widely adopted and grown. These cultivars were developed from crosses with lines developed by CIMMYT suggesting that they may also have the *Lr26* gene. This narrow genetic background of cultivars, as well as the relatively small area planted with wheat, could have contributed to the dominance of *Pt* isolates that typed to race MCDS during the 2019 to 2021 surveys. Furthermore, all Zimbabwean *Pt* isolates that typed to race MCDS were virulent to Trident in the seedling analyses and reference isolate Pt1_1_2019 was confirmed high on RL6081 containing the APR gene *Lr37*. The *Lr37* gene is present in a small translocation from *T*. *ventricosum* Ces. (*Ae. ventricosa* Tausch) and is tightly linked with resistance genes *Yr17* and *Sr38* (Błaszczyk *et al*., 2004). In a previous study on the analysis of rust resistance within Zimbabwean wheat germplasm, Mutari *et al*. (2018) reported the presence of *Lr37* in 11% of the selected wheat lines. These findings could also contribute to the dominance of MCDS in the current Zimbabwean *Pt* population and the absence of race FBPT which was previously identified.

The main wheat growing areas are in the highveld, middleveld and lowveld areas of Harare, Mashonaland west, Mashonaland east, Mashonaland central, Manicaland and Masvingo Province which account for 95% of the area planted. These provinces are directly adjacent to each other with no significant geographical barriers between them. The geographic continuity of these regions likely contributed to the lack of genetic diversity in the Zimbabwean *Pt* population as urediniospores could be widely dispersed across the provinces with no geographic barriers such as mountain ranges, desert or sea that could result in restricted movement of urediniospores. Kolmer (2015) reported that the seven provinces of China which accounted for 74% of wheat production in the country had low genetic diversity within the *Pt* population because of their continuous geographic nature. Population size also influences gene diversity through gene drift, in which a large population will more likely cause potential new mutations compared to smaller populations (McDonald and Linde, 2002). This might aid in explaining the unique case of the dominance of race MCDS in Zimbabwe.

The *Pt* population in Zimbabwe had many characteristics of a clonally reproducing population (Halkett *et al*., 2005). There were high levels of H_o; (0.47) relative to the H_e; (0.35) under Hardy Weinberg equilibrium, a negative F_{IS} value and a significant correlation between virulence and molecular genotypes ($R^2 = 0.77$). Other worldwide populations of *Pt* also have these characteristics where clonal reproduction is found (Kolmer, 2015). A clonal population develops due to a lack of a compatible alternate host to complete the sexual life cycle (Balloux *et al*., 2003). Since wheat is mainly grown in winter in Zimbabwe, this may explain the high

clonality of race MCDS observed in the *Pt* population as there is a lack of a suitable alternative host during the summer season. However, the genotypic analysis based on allelic data revealed five isolates (5_1_2020, 20-1_2020, 23_2_2020, 19_1_19, 24_3_19) that shared the best genetic similarity to South African races SDDN and SCDS. This represents a genetic shift in the Zimbabwean *Pt* population. This scenario can be explained by possible asexual recombination within the Zimbabwean *Pt* population. This means that although low in frequency, *Pt* surviving during the summer on volunteer wheat plants and summer breeding populations are the likely sources of rust spores to winter planted wheat. These small populations can gradually increase the probability of those individuals with favourable mutations for virulence to survive, hence increasing genetic diversity (Liu *et al*., 2014).

The genotypic similarity of the above mentioned five Zimbabwean *Pt* isolates to race SDDN (virulent on *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr14a*, *Lr15, Lr17, Lr24, Lr28* and *LrB*) and SCDS (virulent on *Lr1, Lr10, Lr14a, Lr15, Lr17, Lr20, Lr23, Lr26* and *LrB*), which represent some prominent differences in virulence to *Pt* isolates that typed as race MCDS, might also suggest that these isolates represent new introductions into the Zimbabwean *Pt* population. Race SCDS has been previously reported in Malawi (Pretorius *et al*., 2015). The genetic relatedness of isolates from the different countries indicates the possible dispersal of fungal spores across the SADC region by wind, human activities, or other mechanisms. For instance, *Pt* race MCDS was detected in SA in 2010, in Zimbabwe in 2011 and 2012, and in Zambia in 2012. Similarly, *Pt* race FBPT was detected in SA and Zimbabwe in 2010 and 2012, respectively (Terefe *et al*., 2014a). Three variants of stem rust caused by *Pgt* race Ug99, namely TTKSF, PTKST and TTKSF+ were first detected in SA in 2000, 2009 and 2010, respectively. These three races were also reported in Zimbabwe between 2009 and 2010, with race PTKST being detected in Mozambique in 2010 (Mukoyi *et al.*, 2011). Kolmer *et al*. (2019) also pointed out that the widespread occurrence of MLGs across different regions and decades in time indicated that *Pt* has the potential for long distance migration, which has likely occurred in the past and more recent years. Visser *et al.* (2019) demonstrated using simulation studies that wind dispersal was the main source of introductions of *Pgt* from southern Africa into Australia, suggesting the likelihood of undetected incursions that could have occurred thereafter.

Another possibility for the low genetic variation within the Zimbabwean *Pt* population is the slow increase in the adoption of new cultivars. Cook *et al*. (2021) reported that the introduction of new cultivars with different resistance genes to manage fungal diseases creates selection pressure for the evolution of isolates with expanded virulence profiles. Mutations are one mechanism that can generate new alleles which are considered the basic sources of genetic variation in rust fungi (Liu *et al*., 2018). Ordoñez and Kolmer (2009) found that mutation was

important in *Pt* populations, leading to both microsatellite allele and single virulence differences among isolates. Mutations are estimated to occur in rust fungi at a rate of about 1.6 per 100 000 to 200 000 spores (Park and Clark, 2020). A single pustule of *Pt* can produce around 2000 urediniospores per day for two weeks or more, thereby increasing the chances of mutations causing genetic variation. The development of new races through stepwise mutation has previously been reported for the South African *Pt* population with the speculation that races CFPS (3SA10) and CFPS+*Lr20* (3SA248) could have developed locally from CCPS (3SA145) (Boshoff *et al*., 2018). Therefore, with a recent increase in hectarage and the expected release of new cultivars, we predict the evolution of more races of *Pt* in Zimbabwe. The emergence of new races in Zimbabwe also poses a threat to wheat production in SA as spore migration has previously been suggested between the two neighbouring countries (Terefe *et al*., 2014a; Pretorius *et al*., 2015).

It can also be speculated that the five MCDS *Pt* isolates (5_1_2020, 20_1_2020, 23_2_2020, 19_1_2019 and 24_3_2019) differing in their genotypic profiles within the Zimbabwean *Pt* population arose as a result of somatic hybridisation. Somatic hybridisation involves the fusion of dikaryotic vegetative hyphae, nuclear exchange, and possibly exchange of whole chromosomes between nuclei or parasexuality via the fusion of the two haploid nuclei, followed by mitotic crossing over and vegetative haploidisation giving rise to new more virulent pathotype (Park and Wellings, 2012). In Australia, Park *et al*. (1999) demonstrated the evidence for somatic hybridization when *Pt* pathotype (pt) 64-(6),(7),(10),11 was first detected in 1990. Pathogenicity on wheat and triticale genotypes indicated that this pathotype was similar to two closely related pathotypes, but differed from them in at least three virulence features, indicating that it had not arisen by a simple mutational event. This new pathotype combined several pathogenic features which, prior to its detection, were known only in two different groups of pathotypes, strongly suggesting that it had arisen via somatic hybridisation between isolates from each group. Another case is that of the emergence of *Pgt* isolate Ug99. Li *et al*. (2019) reported that the original Ug99 isolate collected in Uganda shares one haploid nucleus genotype with an Australian *Pgt* isolate of pathotype 21-0 with no recombination or chromosome reassortment. This indicated that Ug99 arose by somatic hybridisation and nuclear exchange between an African member of the *Pgt* 21 lineage and an unknown isolate. Thus, nuclear exchanges between dikaryotic fungi can contribute to the emergence of new virulent phenotypes with significant epidemiological impacts. In the case of Zimbabwean *Pt* isolates, the genetic variation could be a result of somatic hybridisation, although this is not supported when considering the avirulence/virulence profiles of the isolates typed in the current study.

In conclusion, the findings of this study reveals that the current Zimbabwean *Pt* population is clonal as shown by the total dominance of isolates that typed to race MCDS. Although there was no evidence of change in virulence phenotypes between Zimbabwean isolates based on the set of differentials used in the current study, we observed different genotypic profiles which may indicate a low frequency of isolates that may represent a shift in virulence to resistance gene(s) that we did not account. These genetic variations could have arisen due to either asexual recombination, single step mutations, somatic hybridisation or through new foreign introductions. Considering the virulence of *Pt* isolates of race MCDS to ASR gene *Lr26* and the adult plant gene *Lr37* previously reported to be present in the wheat lines used in the public and private sector breeding programs, we predict that it will remain a prevalent and threatening race compared to other races such as FBPT that was previously detected in Zimbabwe. It is therefore important to broaden the genetic base by introducing new cultivars with a different combination of ASR and APR *Lr* genes. This study therefore supports the importance of annual rust surveys in Zimbabwe and its neighbouring countries (Malawi, Zambia, Mozambique and SA) in order to monitor and timeously detect the presence of new more virulent races that could threaten wheat production in the region.

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Chapter 3: Identification of leaf rust resistance genes in Zimbabwean bread wheat varieties

3.1 Introduction

Leaf rust, caused by the biotrophic fungus *Pt*, is one of the most important foliar diseases of bread wheat (*T*. *aestivum* L.) in Zimbabwe and worldwide. This fungus is adapted to a wide range of environments and co-exists with wheat wherever it is grown (Winzeler *et al*., 2000). The pathogen can cause significant yield losses, reaching 40% in severe epidemics, depending on the crop developmental stage at infection and the susceptibility of the cultivars (Dakouri *et al*., 2013).

Disease resistance has been the most effective method of controlling wheat leaf rust because it constitutes an environmentally friendly and cost-effective long-term strategy for minimising yield losses (Pink, 2002). But at the same time, *Pt* has the ability to evolve through single step mutations which can give rise to new races with broader virulence, making rust resistance breeding a never-ending battle (Huerta-Espino, 1992). Race monitoring is used extensively in many pathosystems and continues to provide timely information about the structure of pathogen populations relevant to breeding programmes and resistance gene deployment (Nocente *et al*., 2007). Resistance to wheat leaf rust is mainly divided into vertical and horizontal resistance (Yuan, 1998). Vertical resistance, also known as race-specific or ASR, is often controlled by a single major gene and expressed throughout all plant growth stages. This type of resistance is not durable and often loses effectiveness after a few years of deployment (Wang *et al*., 2015). On the other hand, horizontal resistance, also known as non-race specific resistance and which mostly includes specific sources of APR, is often controlled by minor genes mainly expressed during the reproductive stages of plant development. This type of resistance is associated with slow disease development in the field, smaller pustule size and reduces urediniospore production (McCallum *et al*., 2016). This kind of leaf rust resistance is considered more durable with some sources that have remained effective for several decades (Das *et al*., 1992; Wang *et al*., 2015). Besides being more durable, some minor resistance genes in wheat have a pleiotropic effect on multiple diseases (Li *et al*., 2014). Therefore, to reduce the risk of continuous boom-and-bust cycles, effective resistance genes need to be combined against the prevailing *Pt* race(s) to ensure longer lasting resistance.

Before gene pyramiding is carried out, it is important to identify effective and genetically different sources of resistance. Many researchers have frequently utilised gene postulation to confirm the presence of the probable ASR gene(s) in a group of wheat genotypes (Hysing *et al*., 2006; Mebrate *et al*., 2008).

In this method, a host variety is evaluated against a collection of *Pt* isolates carrying different avirulence/virulence profiles based on phenotypic expressions in the form of seedling ITs (Kolmer, 2003). Low ITs or resistant responses occur only when a wheat variety has a gene which conditions resistance to *Pt* races with the corresponding avirulence gene. Although ASR genes can be postulated at the seedling stage based on the gene-for-gene strategy (Flor, 1971), there are obvious limitations due to the inability to identify multiple *Lr* genes when present in a single variety. A further constraint in these studies might be a lack of access to well characterised rust isolates that varies in their avirulence/virulence profiles, as well as appropriate facilities and equipment to conduct the rust assessments.

All-stage resistance genes are considered of lesser use when deployed singly in high-risk wheat production regions but remain useful when deployed in combination with other resistance genes. Although gene stacks are considered to be more durable (Roelfs, 1988), the introgression of different resistance genes is difficult to monitor by traditional phenotypic analysis because the selection of genotypes carrying combinations of two or more genes is often prevented by the lack of races with virulence matching the corresponding ASR gene(s) (Prasad *et al*., 2020). Molecular markers closely linked to resistance genes have been developed to facilitate expeditious pyramiding into elite backgrounds, making it more cost-effective and less time-consuming. Moreover, detection of genes by molecular markers is not affected by the environment and can be done during all plant growth stages (Nocente *et al*., 2007). This approach overcomes some of the problems associated with traditional gene postulation, such as gene interactions and plant stage of gene expression. To date, at least 80 *Lr* genes have been identified and mapped in the wheat genome (McIntosh *et al*., 2020; Kumar *et al*., 2022). Most of them are ASR genes that confer hypersensitive reactions and interact with the pathogen in a gene-for-gene manner. Reports indicate about 11 APR genes that are effective against leaf rust, namely *Lr12*, *Lr13*, *Lr22a*, *Lr34*, *Lr35*, *Lr37*, *Lr46*, *Lr48*, *Lr49*, *Lr67* and *Lr68*. Among these APR genes, only four, *Lr34* (Dyck, 1987), *Lr46* (Singh *et al*., 1998), *Lr67* and *Lr68* (Herrera-Foessel *et al*., 2011), are considered slow rusting resistance genes (Li *et al*., 2014). Therefore, stacking *Lr* genes into a single variety is considered a useful strategy as the combined effects of these genes give the variety a wider base of disease resistance. In theory, this strategy will extend the period of effectiveness (Roelfs *et al*., 1992; Kolmer *et al*., 2007).

There is not much information available in Zimbabwe on the *Lr* genes present in the released wheat varieties. Similarly, we lack information on the identity and number of *Lr* genes present in advanced breeding lines of the SeedCo breeding programmes. The lack of information makes it difficult to make informed decisions when planning new crosses, as well as when

considering advanced lines for final release with regards to sustainable gene deployment. This study was therefore designed to identify ASR and APR genes in 72 Zimbabwean wheat entries that include advanced breeding lines from SeedCo as well as selected commercial varieties. Gene postulation efforts included screening with multiple *Pt* races, pedigree analysis via F_2 segregation ratios and molecular marker genotyping to effectively improve the accuracy of *Lr* gene identification.

3.2 Materials and methods

3.2.1 Plant material

A set of 72 Zimbabwean wheat varieties (Appendix 3.1) used in the study consisted of 13 current commercial varieties (six from SeedCo and seven from other companies) and 59 SeedCo advanced breeding lines. These lines were selected from a F_7 nursery, naturally infected with leaf rust (unknown race) during the 2019 season, where they expressed moderate to high levels of field resistance. In addition, their grain yield performance, relative to commercial checks currently grown in Zimbabwe, was considered in the selection. The 65 SeedCo varieties were bred from diverse backgrounds with some varieties sharing common parentage. Pedigree info is considered proprietary in Zimbabwe and SeedCo lines were therefore classified into four groups based on shared parentage and previous genotyping results for stem rust, caused by *Pgt*, resistance genes (Soko, 2018; Appendix 3.1). Varieties used in this study were therefore developed from the preceding stem rust resistance project. Group 1 consisted of varieties with both *Sr2* and *Sr25*/*Lr19* genes in their parentage, group 2 had *Sr2* only, group 3 had *Sr25* only and group 4 had parentage that was not genotyped but with known pedigrees. Group 5 consisted of seven non SeedCo commercial varieties with unknown pedigrees. A total of 27 standard and 12 additional differential lines with known *Lr* genes were used to confirm the seedling responses to eight South African *Pt* pathotypes and a single representative Zimbabwean *Pt* isolate. The 72 Zimbabwean wheat varieties and susceptible check PAN 3497 were also screened for their leaf rust field response in trials planted near Napier, SA during the 2020 and 2021 seasons, as well as in a rust screening nursery planted near Greytown during the 2021 season.

3.2.2 *Puccinia triticina* races

To postulate *Lr* genes in the Zimbabwean wheat varieties, eight South African *Pt* pathotypes and a single representative Zimbabwean isolate (Pt1_1_2019) that typed as *Pt* race MCDS (Table 3.1) were used to inoculate 27 standard differentials and 12 additional lines carrying resistance genes *Lr1*, *2a*, *2b*, *2c*, *3a*, *3bg*, *3ka*, *B*, *Lr9*, *10*, *11*, *14a*, *15*, *16*, *17a*, *17b*, *18*, *19*, *20*, *21*, *23*, *24*, *25*, *26*, *28*, *29*, *30*, *27+31*, *32*, *33*, *36*, *37*, *38*, *44*, *45*, *47*, *50*, *51* and *Lr52* (Table 2.2, Chapter 2). This was done to confirm the avirulence/virulence profiles of the

representative *Pt* race isolates, which were used to postulate known ASR genes occurring in the selected Zimbabwean advanced breeding lines and selected commercial varieties. Isolates of the eight South African *Pt* pathotypes were sourced from the culture collection of the Division of Plant Pathology, UFS, SA. Fresh inoculum was obtained by multiplying urediniospores on the universal susceptible variety Morocco. The avirulence/virulence phenotyping procedure described in section 2.2.3 was followed in replicated experiments.

Table 3.1: List of South African *Puccinia triticina* control races used in the current study.

ARC-SG[1]	**UFS[2]**	**NA[3]**	**Year**	**Reference**
3SA140	UVPt13	SFDS	1987	Pretorius (1988)
3SA144	UVPt19	SDDN	2005	Pretorius and Bender (2010)
3SA145	UVPt20	CCPS	2009	Terefe *et al*. (2011)
3SA146	UVPt21	MCDS	2010	Terefe *et al*. (2014a)
3SA147	UVPt22	FBPT	2010	Terefe *et al*. (2014b)
3SA115	UVPt25	CBPS	2012	Boshoff *et al*. (2018)
3SA248	UVPt26	CFPS+*Lr20*	2016	Boshoff *et al*. (2018)
3SA100	UVPt30	MFPS	2020	Terefe *et al*. (2021)
ZIM	Pt1_1_2019	MCDS	2021	

[1] Agricultural Research Council-Small Grain race notation; [2] University of the Free State race notation; [3] North American race notations.

3.2.3 Seedling infection type studies

Five to seven seeds from each of the 72 Zimbabwean wheat accessions were planted in 10 cm diameter plastic pots in clumps of six accessions per pot. A representative isolate (Pt1_1_2019) of the dominant Zimbabwean *Pt* race MCDS (Section 2.2.3) and representative isolates of eight South African control races were used to inoculate the entries and to determine their seedling ITs. With the primary leaf fully exposed, approximately seven to eight days after planting, replicated sets containing the 72 accessions were spray-inoculated with freshly harvested urediniospores of each *Pt* race (concentration ±1 mg/ml spore suspension in Soltrol® isoparaffinic oil), dried off, incubated in the dew chamber overnight and returned to the greenhouse as described in section 2.2.2. Ten to twelve days after inoculation, primary leaf ITs were recorded according to the 0 to 4 seedling ITs scale (Table 3.2).

Table 3.2: Major seedling infection type (IT) classes used for *Puccinia triticina* (*Pt*) ratings (McIntosh *et al*., 1995).

IT[1]	Host Response	Symptoms
0	Immune	No visible uredinia
;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredinia with necrosis
2	Resistant to moderately resistant	Small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis
3	Moderately resistant to moderately susceptible	Medium sized uredinia with or without chlorosis
4	Susceptible	Large uredinia without chlorosis
X	Variable	Heterogeneous, similarly distributed over the leaves
Y	Variable	Variable size with larger uredinia towards the tip
Z	Variable	Variable size with larger uredinia towards the leaf base

[1] Variations are indicated by the use of - (less than average for the class) and + (more) as well as C and N to indicate the presence of more than usual chlorosis and necrosis. Heterogeneity on a leaf not adequately described with X, Y or Z may be written as a sequence, for example 12-. A comma indicates heterogeneity between plants in a single test, for example 1+, X.

3.2.4 Field testing

The field responses of the 72 Zimbabwean wheat accessions were determined in a screening nursery planted at the Corteva Agrisciences™ Research Farm near Greytown, KwaZulu-Natal during the 2021 growing season. The trial was set up in the second week of June by planting the wheat entries in observation rows that were 80 cm apart and 50 cm inter-row spacing. To ensure optimum plant development, 400 kg of basal fertilizer was applied at sowing and 400 kg of ammonium nitrate (34.5% N) as a top-dressing fertiliser was used in two splits. The first application was done at four weeks and second application at eight weeks after crop emergence, regular irrigation was applied. Early infection was initiated by spraying urediniospores of races CFPS+*Lr20* and MFPS (±3 mg/ml spore suspension in Soltrol® isoparaffinic oil) onto wheat rows planted with PAN 3497 using an ultralow-volume sprayer (ULVA; Micron Group, Bromyard, England). No further inoculations were applied after intitial indications of successful infection. The field response for the wheat accessions included severity ratings (% flag leaf area infected) using the modified Cobb scale (Peterson *et al*., 1948), combined with host infection response types recorded on the flag leaves for each entry using the response types R = resistant, MR = moderately resistant, MS = moderately susceptible and S = susceptible (Table 3.6) (Roelfs *et al*., 1992).

The 72 accessions were also planted under rainfed conditions on the Sensako Research Farm near Napier, Western Cape, during the 2020 and 2021 seasons. The trial was planted annually during the first week of June with trial entries planted in 1 m observation rows. The Sensako procedure was followed for the application of fertiliser and pest management and no artificial inoculations were applied. Leaf samples obtained from trial entries were utilised to confirm the *Pt* race dominated by natural infection.

3.2.5 Inheritance of resistance to *Puccinia triticina* race MCDS

3.2.5.1 Development of study populations

A selected set of 25 Zimbabwean wheat lines with ASR to all nine *Pt* race isolates (3.2.2) were crossed with MCDS susceptible wheat PAN 3497 to generate F_1 seeds. These lines represented the four SeedCo genetic groups based on their pedigrees and stem rust resistance genes (Appendix 3.1). At flowering a single ear from each of the selected Zimbabwean lines was crossed with a single ear of PAN 3497 pre-emasculated and covered with glycine crossing bags. Following drying off, the F_1 seeds were hand threshed. Ten seeds of each combination were planted in 2x 1.8 L capacity pots filled with steam-sterilized soil. The plants received weekly applications of 0.2% (w/v) Multifeed-Classic water-soluble fertiliser [Effekto®, NPK analysis 19:8:16 (43), 2.5 g/L] and daily irrigation with reverse osmosis-purified water. At flowering the ears from each pot were covered with pollination bags

(CCR Stitchworx Products, Potchefstroom, SA) to prevent cross-pollination. Upon ripening, the F_2 seeds were bulked from the 10 plants per cross and hand threshed. A summary of the steps followed is shown in Figure 3.1.

3.2.5.2 Determining F_2 segregation ratios

For each crossing combination, 300 F_2 seeds were planted, 25 seeds per pot, in 12 x10 cm diameter pots filled with Mikskaar potting soil. Six pots were placed per 30 cm diameter plastic tray to allow watering from below. The parents used in each crossing combination were planted in replicate as controls and added as a seventh pot to each tray. After planting, seeds were germinated at 25°C in a growth chamber for three days before placement under natural light in a rust-free greenhouse cubicle at 18-25°C (night/day cycle). Seedlings were fertilised twice with 100 ml of a 2.5 mg/L Multifeed-Classic water-soluble fertilizer (3.2.5.1) per tray, once prior and once post inoculation.

Seven-day old seedlings, with a fully developed primary leaf, were inoculated with freshly collected urediniospores from *Pt* race MCDS (ZIM isolate Pt1_1_2019). The seedling ITs were recorded 12 days after inoculation for each of the parents and used to determine the number of R (SC-line, IT = ;) and S (PAN 3497, IT = 3+) seedlings per crossing combination. The objective was to note the ITs of both parental lines and their F_2 progenies and to test the hypothesis of segregation (ratio of 3 R and 1 S) of single dominant genes for leaf rust resistance.

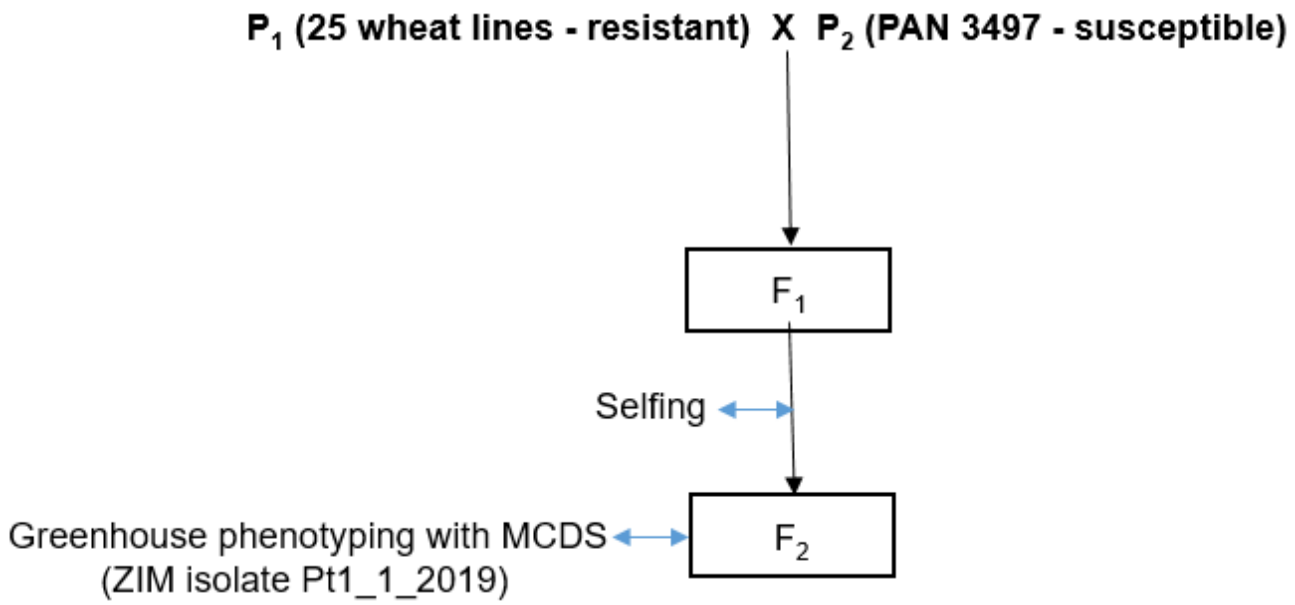

Figure 3.1: Inheritance study flowchart for developing F_1 seeds and F_2 progenies from crosses between 25 SeedCo wheat lines and PAN 3497 followed by greenhouse seedling phenotyping with *Puccinia triticina* (*Pt*) race MCDS (ZIM isolate Pt1_1_2019).

3.2.6 Molecular marker evaluation of known leaf rust resistance genes

3.2.6.1 Sample collection and DNA extraction

Scissors and tweezers were sterilized with 70% (v/v) ethanol to collect leaf material from four-week-old seedlings of the 72 Zimbabwean wheat varieties. Leaf samples were collected in 1.5 ml Eppendorf tubes and stored on ice during sample collection. Leaf material was freeze-dried using the Alpha 1-2 LDplus-Martin Christ (Harz, Germany) for three days and stored at -20°C before DNA isolation. Five 1-2 cm pieces of freeze-dried leaf material and two 5 mm stainless steel balls were placed into a 2 ml Eppendorf tube and ground to a fine powder at 30 r/s for 1 min using a Qiagen's TissueLyser (Haan, Germany). DNA was extracted using a modified CTAB isolation protocol (Saghai-Maroof *et al*., 1984). For each sample, 750 µl CTAB buffer [100 mM Tris-HCl pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl; 2% (w/v) CTAB; 0.2% (v/v) β-mercaptoethanol] was added and mixed until all the fine leaf powder was completely suspended. Samples were incubated for 1 h at 65°C with regular light shaking. A volume of 500 µl $ChCl_3$/IAA (24:1 v/v) was added to each sample and mixed, followed by centrifugation at 12 000 *g* for 5 min at 4°C. The supernatant was transferred to a new labelled 1.5 ml Eppendorf tube and DNA was precipitated with 500 µl (0.66 volumes) 2-isopropanol at room temperature (22°C) for 20 min and centrifuged at 12 000 *g* for 5 min at 4°C. DNA pellets were air-dried for 1 h at room temperature after discarding the supernatant and resuspended in 200 µl 1x TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) buffer.Thereafter, 200 µl/ml RNase was added to each sample and incubated for 2 h in a water bath at 37°C to digest the RNA. Quality of genomic DNA (gDNA) was determined on a 0.8% (w/v) agarose gel that was run at 100 V for 20 min in 1x UNTAN (40 mM Tris-HCl; 2 mM EDTA; pH adjusted to 7.4 with acetic acid) buffer. Ethidium bromide was used to visualise the gDNA under ultraviolet (UV) light (Bio-Rad Gel Doc^{Tm} EZ Imager 110, Bio-Rad, CA, USA). Using a Jenway 7315 spectrophotometer (Jenway, Staffordshire, UK), the absorbance was measured at A_{260}, A_{280}, and the $A_{260:280}$ ratios to calculate the DNA concentration for each sample. Using 1x TE buffer, pH 8.0, all gDNA samples were diluted to a working concentration of 20 ng/l.

3.2.6.2 Polymerase chain reaction genotyping

Twenty-five molecular markers linked to 17 *Lr* genes were evaluated. Marker names, primer sequences, annealing temperatures, expected product sizes and original references for the evaluated markers are summarised in Table 3.3 and 3.4. Polymerase chain reactions were performed in Bio-Rad T100™ thermal cyclers (Bio-Rad, CA, USA). Most PCR were set up to a final volume of 10 µl and contained 80 ng gDNA, 1 µM of each primer set and 1x KapaTaq Ready-mix (Sigma-Aldrich, USA). Marker *csSr2* was set to a final volume of 20 µl. The $MgCl_2$ concentration for marker *Lr25F20/Lr25R19* was increased to 2 mM to enhance enzymatic activity of DNA polymerase. Primers were sourced from Integrated DNA Technologies (Coralville, IA, USA) and PCR programmes consisted of a 5 min at 95°C denaturation step followed by 35-42 cycles of 30 s at 95°C, 30-60 s at a specific annealing temperature (Table 3.3) and 30-60 s at 72°C. The PCR programmes were concluded with a final elongation step of 5-10 min at 72°C and incubated at 15°C.

3.2.6.3 Visualisation of polymerase chain reactions

3.2.6.3.1 Agarose gel electrophoresis

The PCR products of markers *Lr25F20/Lr25R19, lag95, Lr29F24/Lr29R24, cssfr5* and *Aga7-759R* were visualised on a 1.5% (w/v) agarose gel, run at 100 V for 30 min in 1x UNTAN buffer. The PCR samples were mixed with Ficoll loading dye (15% (w/v) Ficoll, 0.24% (w/v) bromophenol blue) and a 1 kb DNA ladder (Promega, Madison, WI, USA) was loaded to every gel to determine the sizes of the amplified fragments. Ethidium bromide was used to visualise the gels using UV light (Bio-Rad Gel Doc™ EZ Imager 110, Bio-Rad, CA, USA).

3.2.6.3.2 Polyacrylamide gel electrophoresis

The remaining 20 markers were resolved using the Gel-Scan 3000 Real-Time DNA Fragment Analysis System (Corbett Research, Sydney, Australia). A 5% (w/v) non-denaturing polyacrylamide gel electrophoresis (PAGE) with a final volume of 25 ml consisting of 1x TBE buffer (2 mM EDTA; 89 mM Tris-HCl; 89 mM Boric acid), 5% (w/v) acrylamide: bis-acrylamide (19:1), 0.12% (v/v) N,N,N’,N’;-tetramethylethylenediamine (TEMED) and 0.08% (v/v) ammonium persulfate was prepared and polymerised for a minimum of 3 h.

Table 3.3: Molecular markers linked to targeted leaf rust (*Lr*) resistance genes/QTL used in this study.

Marker name	*Lr* gene	Positive control	Ta[1] (°C)	Marker type[2]	Estimated base pairs	Reference
J13-1/J13-2	*Lr9*	RL6010	55	STS	1100	Chełkowski *et al*. (2003)
Wmc24	*Lr11*	RL6053	53	SSR	152	Darino *et al*. (2015)
Wmc261	*Lr11*	RL6053	54	SSR	110	Darino *et al*. (2015)
STSLr19$_{130}$	*Lr19/Sr25*	Agatha	60	STS	130	Prins *et al*. (2001)
Gb	*Lr19/Sr25*	Agatha	50	STS	150	Prins *et al*. (2001)
PSY	*Lr19/Sr25*	Agatha	58	STS	200	Zhang and Dubcovsky (2008)
STSLr24	*Lr24*	Agent/Arkan	57	STS	700	Schachermayr *et al*. (1995)
Lr25F20/Lr25R19	*Lr25*	Transec	58	SCAR	750	Procunier *et al*. (1995)
lag95	*Lr26*	Fed*4/Kav	55	SCAR	210	Mago *et al*. (2002)
csSr2	*Lr27/Yr30/Sr2*	Kingbird	55	dCAPS	172	Mago *et al*. (2011)
Lr29F24/Lr29R24	*Lr29*	RL6080	58	SCAR	900	Procunier *et al*. (1995)
Barc135	*Lr32*	RL6086	51	SSR	239	Thomas *et al*. (2010)
cssfr5	*Lr34/Yr18/Sr57*	Kariega	55	SSR	751	Lagudah *et al*. (2009)
URIC/LN2	*Lr37/Yr17/Sr38*	Stylet	64	STS	285	Helguera *et al*. (2003)
Wmc44	*Lr46/Yr29*	Pavon	61	SSR	242	Martínez *et al*. (2001)
Gwm259	*Lr46/Yr29*	Pavon	55	SSR	105	MASWheat
Barc80	*Lr46/Yr29*	Pavon	52	SSR	115	MASWheat
PS10-L/-R	*Lr47*	KS90H450	55	dCAPS	450	Chełkowski *et al*. (2003)
Gwm382	*Lr50*	KS96WGRC36	60	SSR	139	Brown-Guedira *et al*. (2003)
Gdm87	*Lr50*	KS96WGRC36	60	SSR	110	Brown-Guedira *et al*. (2003)
Aga7-759R	*Lr51*	R 05	52	CAPS	422+397	Helguera *et al*. (2005)
TM4dcaps	*Lr67/Yr46/Sr55*	RL6077	54	dCAPS	329	Gao *et al*. (2021)
TM10dcaps	*Lr67/Yr46/Sr55*	RL6077	60	dCAPS	299	Gao *et al*. (2021)
Cs7	*Lr68*	Parula	60	dCAPS	738	Herrera-Foessel *et al*. (2012)
csGS	*Lr68*	Parula	60	dCAPS	385	Herrera-Foessel *et al*. (2012)

[1] Annealing temperature; [2] STS = Sequence-Tagged Sites; SSR = Simple Sequence Repeat; SCAR = Sequence Characterised Amplified Region; CAPS = Derived Cleaved Amplified Polymorphic Sequences; dCAPS = Derived Cleaved Amplified Polymorphic Sequences.

Table 3.4: Primer sequences of molecular markers linked to targeted leaf rust (*Lr*) resistance genes/QTL used in this study.

Marker name	*Lr* gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Reference
J13-1/J13-2	*Lr9*[1]	TCCTTTTATTCCGCACGCCGG	CCACACTACCCCAAAGAGACG	Chełkowski *et al*. (2003)
Wmc24	*Lr11*[1]	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	Darino *et al*. (2015)
Wmc261	*Lr11*[1]	GATGTGCATGTGAATCTCAAAAGTA	AAAGAGGGTCACAGAATAACCTAAA	Darino *et al*. (2015)
STSLr19$_{130}$	*Lr19/Sr25*	CATCCTTGGGGACCTC	CCAGCTCGCATACATCCA	Prins *et al*. (2001)
Gb	*Lr19/Sr25*[1]	CATCCTTGGGGACCT	CCAGCTCGCATACATCCA	Prins *et al*. (2001)
PSY	*Lr19/Sr25*[1]	CATCCTTGGGGACCTC	CCAGTCGCATACATCCA	Zhang and Dubcovsky (2008)
STSLr24	*Lr24*[1]	TCTAGTCTGTACATGGGGGC	TGGCACATGAACTCCATACG	Schachermayr *et al*. (1995)
Lr25F20/Lr25r19	*Lr25*[1]	CCACCCAGAGTATACCAGAG	CCACCCAGAGCTCATAGAA	Procunier *et al*. (1995)
lag95	*Lr26*[1]	GGTACCAACAACAACAACCC	GTTGCTGCTGAGGTTGGTTC	Mago *et al*. (2002)
csSr2	*Lr27/Yr30/Sr2*[2]	CAAGGTTGCTAGGATTGGAAAAC	AGATAACTCTTATGATCTTACATTTTTCTG	Mago *et al*. (2011)
Lr29F24/Lr29R24	*Lr29*[1]	GTGACCTCAGGCAATGCACACAGT	GTGACCTCAGAACCGATGTCCATC	Procunier *et al*. (1995)
Barc135	*Lr32*[1]	TAGCTCAACCACCACCCTACTG	ACTTCAACATCCAAACTGACCG	Thomas *et al*. (2010)
cssfr5	*Lr34/Yr18/Sr57*[2]	GTTGGTTAAGACTGGTGATGG	TGCTTGCTATTGCTGAATAGT	Lagudah *et al*. (2009)
URIC/LN2	*Lr37/Yr17/Sr38*[2]	GGTCGCCCTGGCTTGCACCT	TGCAGCTACAGCAGTATGTACACAAAA	Helguera *et al*. (2003)
Wmc44	*Lr46/Yr29/Sr58*[2]	GGTCTTCTGGGCTTTGATCCTG	GTTGCTAGGGACCCGTAGTGG	Martínez *et al*. (2001)
Gwm259	*Lr46/Yr29/Sr58*[2]	AGGGAAAAGACATCTTTTTTTC	CGACCGACTTCGGGTTC	MASWheat
Barc80	*Lr46/Yr29/Sr58*[2]	GCGAATTAGCATCTGCATCTGTTTGAG	CGGCAACCAACTACTGCACA C	MASWheat
PS10-L/-R	*Lr47*[1]	GCTGATGACCCTGACCGGT	GGGCAGGCGTTTATTCCAG	Chełkowski *et al*. (2003)
Gwm382	*Lr50*[1]	GTCAGATAACGCCGTCCAAT	CTACGTGCACCACCATTTTG	Brown-Guedira *et al*. (2003)
Gdm87	*Lr50*[1]	AATAATGTGGCAGACAGTCTTGG	CCAAGCCCCAATCTCTCTCT	Brown-Guedira *et al*. (2003)
Aga7-759R	*Lr51*[1]	GCATCAACAAGATATTCGTTATGACC	TGGCTGCTCAGAAAACTGGACC	Helguera *et al*. (2005)
TM4dcaps	*Lr67/Yr46/Sr55*[2]	ATCATCGGCAGGATCCTGATT	CTTTGATACTACCGTGGG	Gao *et al*. (2021)
TM10dcaps	*Lr67/Yr46/Sr55*[2]	GCCACGGGTGGGCCATACTG	ACCTTGTCGGTCATCTCCTC	Gao *et al*. (2021)
Cs7	*Lr68*[2]	AAGATTGTTCACAGATCCATGTCA	GAGTATTCCGGCTCAAAAAGG	Herrera-Foessel *et al*. (2012)
csGS	*Lr68*[2]	GAAGGAGTGCTTCCTCCACTG	CTTGGTTCTCCTGTTCTTCCC	Herrera-Foessel *et al*. (2012)

[1] All-stage resistance; [2] Adult plant resistance.

The system's upper buffer chamber (negative electrode) of the system contained 0.5x TBE buffer while the bottom buffer chamber (positive electrode) contained 0.5x TBE buffer and 1.5% (v/v) EtBr. PCR products were mixed with deionised formamide loading dye. Prior to sample loading a pre-run at 800 V for 45 min at 37°C was carried out. One µl of the PCR samples were loaded and run at 1 200 V for 45 min at 37°C. A 25 bp DNA ladder (HyperLadder V from Bioline, Taunton, MA, USA) was loaded for each gel and used to determine relative sizes of amplified fragments.

3.2.7 Data analysis

3.2.7.1 Inheritance studies

The Chi-square goodness of fit test was used to determine if the observed F_2 progenies segregation deviated significantly from the expected 3:1 ratio. The formula below was used to calculate the Chi-square statistic (χ^2) value with Microsoft Excel 2016. The *P* value was calculated from the Chi-square value using the online calculator "Quickcalcs" (GraphPad Software, Inc., San Diego, California, USA).

$$\chi^2_{(r-1)\,(c-1)} = \sum (O - E)^2/E$$

Where χ^2: Chi-square goodness of fit test; (r-1) (c-1): Chi-square goodness of fit test degrees of freedom (df) where r is the number of rows and c is the number of columns; O: Observed values and E: Expected values.

Hypothesis: **H_0**: Leaf rust resistance in Zimbabwean wheat lines is determined by a single dominant gene with a F_2 segregation ratio of 3:1; **H_1**: Leaf rust resistance in Zimbabwean wheat lines is determined by more than one single dominant gene.

Decision: Accept **H_0** if $\chi^2 < \chi^2_c$ critical value; Accept the **H_0** if *P* value > 0.05. Reject the **H_0** if $\chi^2 > \chi^2_c$ critical value; Reject the **H_0** if *P* value < 0.05.

3.2.7.2 Evaluation of molecular marker data

The data was tabulated in Microsoft Excel 2016. Samples were scored based on the presence (1) of the correct amplicon size or the absence (in case of a dominant marker) or incorrect amplicon size (0) compared to the positive control line that is known to carry the *Lr* gene/QTL.

3.3 Results

3.3.1 Race confirmation of *Puccinia triticina* isolates

The seedling ITs for the 27 standard differentials and 12 additional lines using nine *Pt* race isolates with different virulence profiles are shown in Table 3.5. Eight of the nine *Pt* race isolates typed according to their NA race codes as described in Table 2.3. The isolate of race SFDS had a low IT of ;1 on differential line (RL6004) with *Lr10* which differed from the expected high (IT = 3) reported by Visser *et al*. (2011) and thus typed as race SFDN. The isolate of *Pt* race CCPS typed low (IT = ;1C) on RL6047 (*Lr2c*) which deviated from the high IT (3+) reported by Visser *et al*. (2011) for isolates of this race. Seventeen differential lines with *Lr9*, *Lr11*, *Lr16*, *Lr19*, *Lr21*, *Lr25*, *Lr29*, *Lr33*, *Lr36*, *Lr37*+, *Lr38*, *Lr44*, *Lr45*, *Lr47*, *Lr50*, *Lr51* and *Lr52* had low ITs (≤3) to all tested *Pt* races. Differential lines with *Lr19* and *Lr47* had ITs ranging from 0; to fleck, while *Lr9*, *Lr21*, *Lr25*, *Lr29*, *Lr38*, *Lr44*, *Lr45*, *Lr50*, *Lr51* and *Lr52* had ITs ranging from fleck to ;1+X. Infection types ranging from ;1 to 2+ were observed on differential lines with *Lr11*, *Lr16*, *Lr33*, *Lr36* and differential line Trident with *Lr37*+. Three differential lines with *LrB*, *Lr14a* and *Lr17a*, were considered susceptible to all tested *Pt* races with ITs ranging from 3X to 4. The remaining 19 differential lines with *Lr* genes *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr15*, *Lr17b*, *Lr18*, *Lr20*, *Lr23*, *Lr24*, *Lr26*, *Lr28, Lr30*, *Lr32* and *Lr27+31* varied in their low and high ITs.

Table 3.5: Infection types (ITs) of 27 standard differentials and 12 additional lines inoculated with eight South African (3SA) isolates and one Zimbabwean (ZIM) representative isolate of *Puccinia triticina* (*Pt*) races.

Entry[2]	*Lr* gene	ITs to *Pt* races[1]								
		3SA140[3] **UVPt13**[4] **SFDS**[5]	**3SA144 UVPt19 SDDN**	**3SA145 UVPt20 CCPS**	**3SA146 UVPt21 MCDS**	**3SA147 UVPt22 FBPT**	**3SA115 UVPt25 CBPS**	**3SA248 UVPt26 CFPS+*Lr20***	**3SA100 UVPt30 MFPS**	**ZIM Pt1_1_2019 MCDS**
RL6003	*1*	4	3+	;	3+	;	;	;	3+	3+
RL6016	*2a*	4	3+	0;	;	;1=	;	;	;	;
RL6019	*2b*	4	3+	;	;	;1	;1-	0;	;	;
RL6047	*2c*	4	3	;1C	;	3+X	;1-	;	;	;
RL6002	*3a*	0;	;	3++	3+	4	3+	4	3+	3+
RL6042	*3bg*	0;	;	4	3+	4	3+	3+	3+	3+
RL6007	*3ka*	;	;	3++	1+	4	3+X	4	3	;1
RL6051	*B*	3+	3+	3+	3+	3X	3+	4	3+	3+
RL6010	*9*	;1-	;1	;	;	;	;	;	;	;
RL6004	*10*	;1	;1-	3++	3+	4	3+	3+	3+	3+
RL6053	*11*	2+	12C	;1	;1+	;1	;1	;1	;1	;1+
RL6013	*14a*	4	3X	4	3+	4	3+	4	3	3+
RL6052	*15*	4	3+	4	3+	;	;	3+	3+	3+
RL6005	*16*	1CN	;1CN	2C	2CN	12CN	2CN	2CN	12CN	2CN
RL6008	*17a*	4	3+	4	4	3X	3+X	3+	4	3+
Harrier	*17b*	3+	3+	4	4	12+X	;12+X	3+	3+X	3X
RL6009	*18*	1	;1-	;1+	;1	3X	;1	;1	;1+	;1-
Agatha	*19*	;	0;	;	;	;	;	;	;	;
Thew	*20*	3+	3+	;N	3+	;	;	4	4	3+
RL6043	*21*	;1	;1C	;1	;1+	;1	;1	;1	;1+	;1+
Gaza	*23*	3X	12C	;1CN	12C	;1-	;1=	;1CN	;1	12C
Agent	*24*	4	3+	;	;	;	;	3++	3+	;
RL6084	*25*	;1	;N	;	;1N	;1	;	;	;	;
RL6078	*26*	3++	;	33+X	3+	;	;	3+	3X+	3+
CS_2A/2M	*28*	3X	3X	;	;	;	;	;	;	;

Table 3.5 (cont.): Infection types (ITs) of 27 standard differential- and 12 additional lines inoculated with eight South African (3SA) isolates and one Zimbabwean (ZIM) representative isolate of *Puccinia triticina* (*Pt*) races.

Entry[2]	***Lr* gene**	**ITs to *Pt* races**[1]								
		3SA140[3] **UVPt13**[4] **SFDS**[5]	**3SA144 UVPt19 SDDN**	**3SA145 UVPt20 CCPS**	**3SA146 UVPt21 MCDS**	**3SA147 UVPt22 FBPT**	**3SA115 UVPt25 CBPS**	**3SA248 UVPt26 CFPS+*Lr20***	**3SA100 UVPt30 MFPS**	**ZIM Pt1_1_2019 MCDS**
RL6080	*29*	;1	;	;	;	;1-	;	;	;	;
RL6049	*30*	2	;	4	;1+	3+	3+	4	3+	;1
Gatcher	*27+31*	;1	;	12	4	;1N	;1N	3+	4	4
RL6086	*32*	;1-	3+	;1	;1+	;1	;1	;1	;1	;1+
RL6057	*33*	2+	2+C	2+C	2+C	2+C	2+C	2+C	2+C	2+C
E84081	*36*	;1-	;N	12X	;1N	;1-	1CN	12X	12X	;1=
Trident	*37+*	;1+	;1CN	12+X	;1+X	;1	;1	12+	12+	;1+X
RL6097	*38*	;1	;	;	;	;	;	;	;	;
RL6147	*44*	;1	;1=	;1CN	;	;1CN	1CN	;1=	;1	;
RL6144	*45*	;1	;	;1-	;1=	;1=	;	;	;	;1=
KS90H450	*47*	;	;	;	;	;	;	;	;	;
KS96WGRC36	*50*	;1	;1+X	;1+	;1+X	;1+X	;1+X	;1+X	;1+X	;1+X
R05	*51*	;1	;1C	;1-	;1N	;1-	;1-	;N	;1-	;1=
RL6017	*52*	;1	;	;	;	;1-	;1-	;1=	;1-	;

[1] According to the 0 to 4 by McIntosh *et al.* (1995); [2] Seed source as indicated in Chapter 2 in Table 2.2; [3] Agricultural Research Council-Small Grain race notation; [4] University of the Free State race notation; [5] North American race notations.

3.3.2 Seedling infection types recorded for 72 Zimbabwean wheat varieties

The ITs of the 72 Zimbabwean wheat varieties assessed using nine *Pt* race isolates are presented in Table 3.6. Fifty-eight tested wheat varieties showed low ITs (≤2+) ranging from fleck to 12+X over all *Pt* race isolates. From these 49 produced only flecks. The remaining nine varieties; SC003, SC014, SC019, SC033, SC038, SC039, SC047, SC050 and SC056 produced ITs ranging from fleck to 12+X to the different *Pt* race isolates.

The seedling data for the commercial varieties Runde, SST 875, PAN 3402 and MRI 834 revealed high ITs, varying from 3X to 3+, to at least five *Pt* race isolates. Runde showed susceptibility to six race isolates, including segregation to race SFDS, and intermediate ITs (;12X) to isolates of races FBPT and CBPS and lastly produced a fleck to race CFPS+*Lr20*. Similarly, SST 875 produced high ITs for six race isolates and low to intermediate ITs towards races SDDN (;1-), FBPT (;1N) and CBPS (;12+X).

The variety PAN 3402 showed susceptibility to seven race isolates, including segregation to race SDDN, and low to intermediate ITs towards isolates of races FBPT (;1N) and CBPS (;12X). The variety MRI 834 produced a low seedling IT to only race SDDN (;1-) and consistently produced moderately susceptible ITs (3X) to the remaining eight race isolates. This was followed by varieties PAN 3494 and K215W119 (high ITs ≥3X to four races); SC015 and Peregrine (ITs ≥3X to three races); SC051, SC052 and SC Shungu (ITs ≥3X to two races); SC048 and SC063 (ITs ≥3X to one race) and lastly SC Nduna that segregated (ITs 12X to 3+) to the two isolates of race MCDS.

Fifty-four varieties showed low ITs of fleck to ;N when evaluated with Zimbabwean representative isolate (Pt1_1_2019) of race MCDS. Furthermore, the varieties SC003, SC019, SC033, SC038, SC039, SC048, SC052 and SC056 showed low to intermediate IT responses varying from ;1- to 12+X. The varieties SC Nduna (segregating), K215W119, Runde, SC051, SST 875, PAN 3494, Peregrine, PAN 3402, SC Shungu and MRI 834, all commercially available in Zimbabwe except for SC051 and K215W119, produced high ITs to the Zimbabwean isolate of race MCDS. Except for minor deviations in seedling ITs recorded for SC014 (; 1N), SC015 and SC033 (; to ;1-N), and SC038 (;1-N to ;1N) the MCDS isolates from SA (UVPt21) and Zimbabwe (Pt1_1_2019) produced largely identical seedling responses. Figure 3.2 shows the IT responses of the 13 Zimbabwean commercial varieties to isolate Pt1_1_2019 of race MCDS ranging from fleck to 3+.

Table 3.6: Seedling infection types (ITs) and adult plant field responses recorded at Napier (2020 and 2021) and Greytown (2021) for 72 Zimbabwean wheat accessions to nine *Puccinia triticina* (*Pt*) races.

Entry	ITs to *Pt* races[1]									Field response[2]		
	3SA140 UVPt13 SFDS	**3SA144 UVPt19 SDDN**	**3SA145 UVPt20 CCPS**	**3SA146 UVPt21 MCDS**	**3SA147 UVPt22 FBPT**	**3SA115 UVPt25 CBPS**	**3SA248 UVPt26 CFPS+*Lr20***	**3SA100 UVPt30 MFPS**	**ZIM Pt1_1_2019 MCDS**	**Napier 2020 CFPS[3]**	**Napier 2021 CFPS[3]**	**Greytown 2021 CFPS[3] & MFPS**
SC001	;	;	;	;	;	;	;	;	;	0	0	0
SC002[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC003[4]	;1-N	;1+N	;	;1+	;	;	;	;1N	;1+	0	0	10MR
SC004	;	;	;	;	;	;	;	;	;	0	0	0
SC005	;	;	;	;	;	;	;	;	;	0	0	0
SC006	;	;	;	;	;	;	;	;	;	0	0	0
SC W9101[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC008[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC009[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC010[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC011	;	;	;	;	;	;	;	;	;	0	0	0
SC012	;	;	;	;	;	;	;	;	;	0	0	0
SC013[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC014	;1N	;	;;N	;, 1N	;	;1-	12+X	;1N	;	0	0	0
SC015	3+	;1-	;	;N	;	;1-	33+	3	;N	0	0	20MR
SC016	;	;	;	;	;	;	;	;	;	0	0	TMR
SC017	;	;	;	;	;	;	;	;	;	0	0	0
SC018	;	;	;	;	;	;	;	;	;	0	0	0
SC019	;12X	;	;	;	;	;	;1CN	;1CN	;1-N	0	0	0
SC020	;	;	;	;	;	;	;	;	;	0	0	0
SC Select[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC Nduna	;1CN	;	2C	3+, 12X	;1+	;	22+C	;1+	3+, 12+X	0	0	40MRMS
SC Serena	;	;	;	;	;	;	;	;	;	0	0	0
K215W119	;12+X	;N	;12X	3+	;1+	3	;12X	3+	3+	0	0	20MRMS
Runde	;, 3	3X	3+	3+	;12X	12X	;	3+	3+	0	0	0, 30MRMS

Table 3.6 (cont.): Seedling infection types (ITs) and adult plant field responses recorded at Napier (2020 and 2021) and Greytown (2021) for 72 Zimbabwean wheat accessions to nine *Puccinia triticina* (*Pt*) races.

Entry	ITs to *Pt* races[1]									Field response[2]		
	3SA140 UVPt13 SFDS	**3SA144 UVPt19 SDDN**	**3SA145 UVPt20 CCPS**	**3SA146 UVPt21 MCDS**	**3SA147 UVPt22 FBPT**	**3SA115 UVPt25 CBPS**	**3SA248 UVPt26 CFPS+*Lr20***	**3SA100 UVPt30 MFPS**	**ZIM Pt1_1_2019 MCDS**	**Napier 2020 CFPS[3]**	**Napier 2021 CFPS[3]**	**Greytown 2021 CFPS[3] & MFPS**
SC026	;	;	;	;	;	;	;	;	;	0	0	0
SC027[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC028[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC029[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC030	;	;	;	;	;	;	;	;	;	0	0	0
SC031	;	;	;	;	;	;	;	;	;	0	0	0
SC032	;	;	;	;	;	;	;	;	;	0	0	0
SC033	;1+	;	;	;	;	;	;	;1+	;1-	0	0	30MRZ
SC034	;	;	;	;	;	;	;	;	;	0	0	0
SC035[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC036	;	;	;	;	;	;	;	;	;	0	0	0
SC037	;	;	;	;	;	;	;	;	;	0	0	0
SC038[4]	;1=N	;1N	;1-N	;1-N	;1=N	;1=N	;N	;1-N	;1N	0	0	5MR
SC039	;1-N	;1N	;N	;1N	;N	;1-N	;1-N	;1-N	;1N	0	0	0
SC040[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC041	;	;	;	;	;	;	;	;	;	0	0	0
SC042	;	;	;	;	;	;	;	;	;	0	0	0
SC043	;	;	;	;	;	;	;	;	;	0	0	0
SC044	;	;	;	;	;	;	;	0;	;	0	0	0
SC045	;	;	;	;	;	;	;	;	;	0	0	0
SC046[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC047[4]	;, 1N	;	;	;	;	;, ;1+	;	;	;	0	0	0
SC048	;1CN	;1	;	;1	;1N	;1-N	3X	12X	;1	0	0	20MRMS
SC049[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC050[4]	;	;	;	;	;	;	;	;12X	;	0	0	0

Table 3.6 (cont.): Seedling infection types (ITs) and adult plant field responses recorded at Napier (2020 and 2021) and Greytown (2021) for 72 Zimbabwean wheat accessions to nine *Puccinia triticina* (*Pt*) races.

Entry	ITs to *Pt* races[1]									Field response[2]		
	3SA140 UVPt13 SFDS	**3SA144 UVPt19 SDDN**	**3SA145 UVPt20 CCPS**	**3SA146 UVPt21 MCDS**	**3SA147 UVPt22 FBPT**	**3SA115 UVPt25 CBPS**	**3SA248 UVPt26 CFPS+*Lr20***	**3SA100 UVPt30 MFPS**	**ZIM Pt1_1_2019 MCDS**	**Napier 2020** CFPS +*Lr20*[3]	**Napier 2021** CFPS+ *Lr20*[3]	**Greytown 2021** CFPS+*Lr20*[3] & MFPS
SC051	;1+N	;12X	;12X	3+	;1N	;12X	;12X	;12X	3+	0	0	10MRR
SC052	33+	;	;N	;	;	;	3	;	;1-	40S	0	20MSZ
SC053	;	;	;	;	;	;	;	;	;	0	0	0
SC054[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC055[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC056	;1N	;	;1CN	;12+X	;1CN	12+X	12+X	;12X	12+X	0	0	5MRR
SC057	;	;	;	;	;	;	;	;	;	0	0	0
SC058[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC059	;	;	;	;	;	;	;	;	;	0	0	0
SC060	;	;	;	;	;	;	;	;	;	0	0	0
SC061[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC062[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC063	;12X	;	;	;	;	;	3X	;12X	;	0	10MR	30MRMS
SC064[4]	;	;	;	;	;	;	;	;	;	0	0	0
SC065[4]	;	;	;	;	;	;	;	;	;	0	0	0
SST 875	33+	;1-	3+	3X	;1N	;12+X	3X	3X	3X	0, 15S	0, 20S	20MRMS
PAN 3494	3X	;12+X	;1+	3X	;1CN	;12X	3X	;1+	3X	0	0	10MR
Peregrine	;12X	;	;1N	3X	;	;	3X	;1CN	3X	0	0	40MRMS
PAN 3402	3X	;1CN, 3X	3X	3X	;1N	;12X	3X	3X	3X	0	5MS	30MRMS
SC Sicho	;	;	;	;	;	;	;	;	;	0	0	0
SC Shungu	;12X	;1-N	;12X	3X	;1CN	;1CN	;12X	;12X	3X	0	0	20MR
MRI 834	3X	;1-	3X	3X	3X	3X	3X	3X	3X	20S	0	40MRMS

[1] According to McIntosh *et al.* (1995); [2] Field responses include percentage flag leaf severity followed by reaction type R = Resistant, MR = Moderately resistant, MRR = Moderately resistant to resistant, MRMS = Moderately resistant to moderately susceptible, MS = Moderately susceptible, S = Susceptible, T = trace, Z = infection (pustules) concentrated at the flag leaf base; [3] Representing *Puccinia triticina* race CFPS+*Lr20*; [4] Varieties that was selected for crossing with PAN 3497 for inheritance studies in section 3.2.5.

Figure 3.2: Seedling infection types (ITs) for 13 Zimbabwean wheat varieties to *Puccinia triticina* (*Pt*) race MCDS, isolate Pt1_1_2019, (from left to right) SC W9101, SC Select, SC Nduna, SC Serena, K215W119, Runde, SST 875, PAN 3494, Peregrine, PAN 3402, SC Sicho, SC Shungu and MRI 834.

Comparative seedling ITs recorded for the experimental varieties SC002, SC038 and SC052, selected based on their different pedigree groups (Appendix 3.1), revealed flecks only for SC002 from group 3 as depicted in Figure 3.3 for six of the *Pt* races. Similarly, SC038 from group 4 produced low ITs with necrosis and rare minute pustules to some of the six race isolates. Variety SC052 from pedigree group 2 produced high seedling ITs to isolates of races SFDS and CFPS+*Lr20* with low ITs to isolates CCPS, MCDS, FBPT and CBPS (Figure 3.3).

Figure 3.4 summarises the low and high IT responses of the 72 Zimbabwean wheat varieties tested with the nine *Pt* race isolates. Race isolate MCDS and the Zimbabwean representative isolate of MCDS were the most virulent with 10 varieties showing susceptibility, followed by race CFPS+*Lr20* (eight varieties), and SFDS and MFPS (six varieties each). Races CCPS, CBPS, SDDN, and FBPT were the least virulent with four, two, one and one varieties showing susceptibility, respectively.

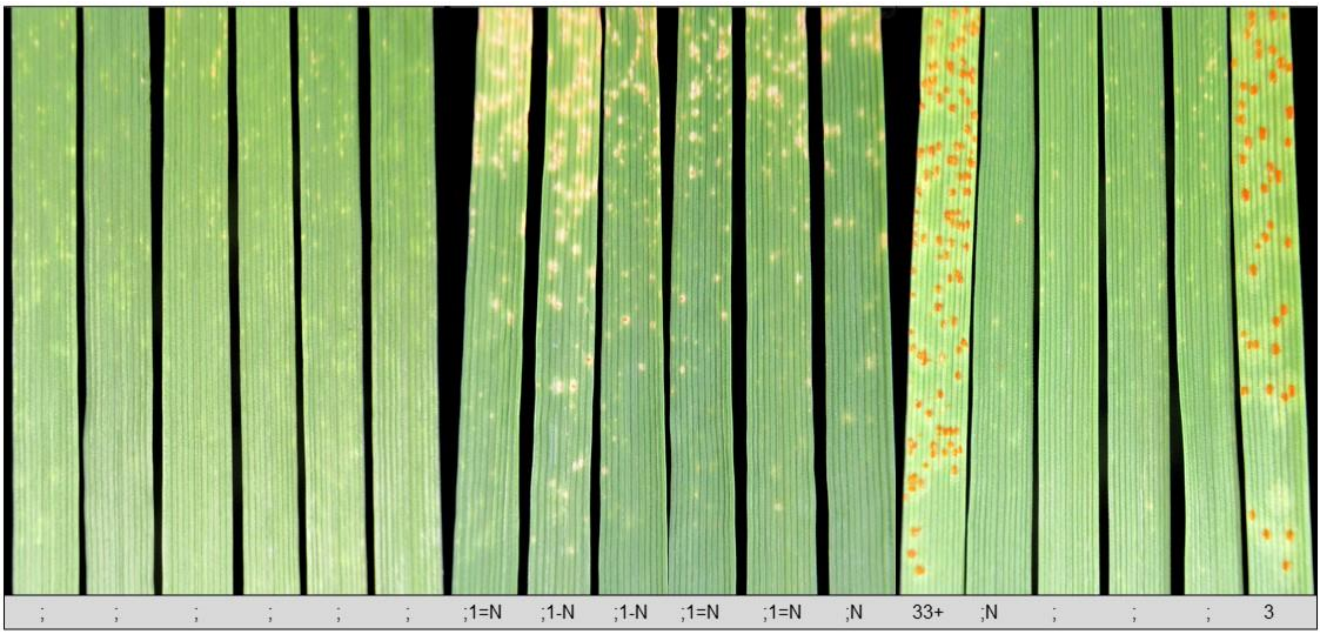

Figure 3.3: Seedling infection types (ITs) for three SeedCo experimental lines (from left to right) SC002 (group 3), SC038 (group 4) and SC052 (group 2) to six *Puccinia triticina* (*Pt*) race isolates (from left to right) SFDS, CCPS, MCDS, FBPT, CBPS and CFPS+*Lr20*.

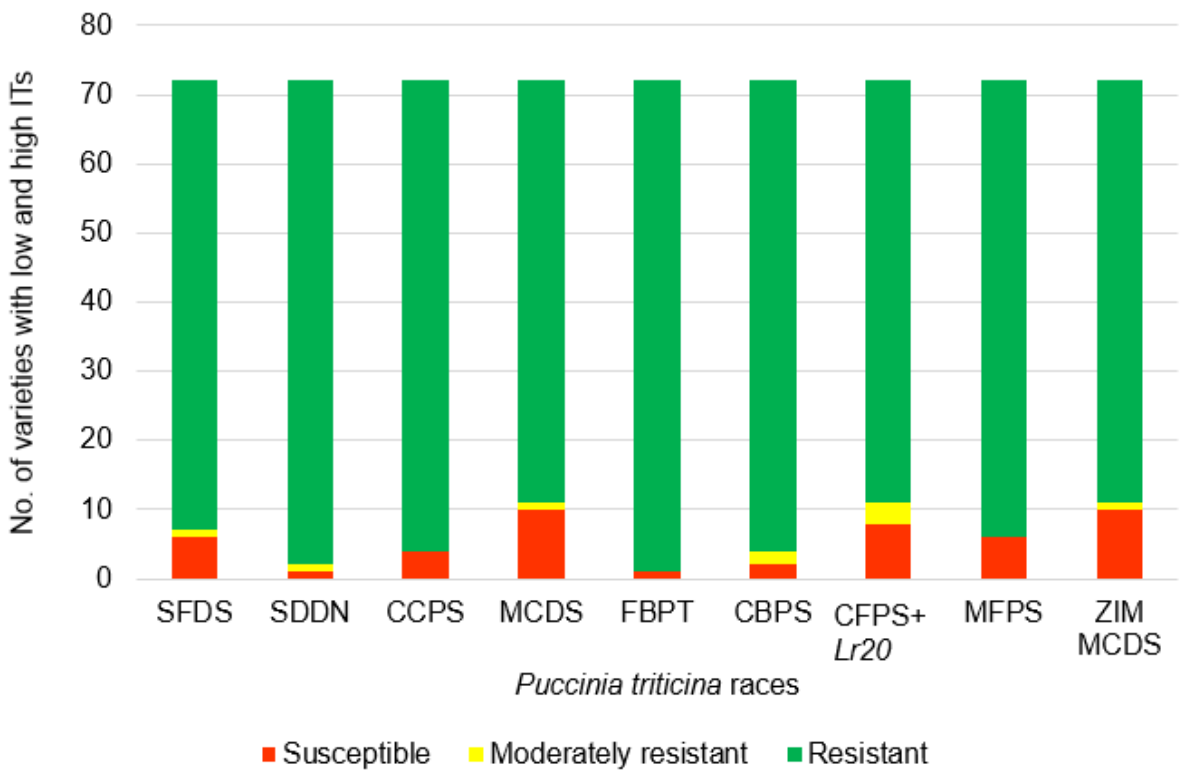

Figure 3.4: Summary of seedling infection type (IT) responses for 72 Zimbabwean wheat varieties tested to nine *Puccinia triticina* race isolates. ITs ≥ 3 = Susceptible; ≥ 2 = Moderately resistant; < 2 = Resistant.

3.3.3 Field response recorded for 72 Zimbabwean wheat varieties

Leaf samples infected with leaf rust, were collected from the Napier trial entries during the 2020 and 2021 seasons and race typing was performed as described in Chapter 2. *Puccinia triticina* race CFPS+*Lr20* was confirmed as the dominant race that occurred through natural infection in both seasons. Similarly, the occurrence of *Pt* races CFPS+*Lr20* and MFPS was confirmed in the Greytown trial following artificial inoculation during the 2021 season. The highest flag leaf rating recorded for each of the 72 Zimbabwean varieties at Napier (2020 and 2021) and Greytown (2021) are listed in Table 3.6. At the time of rating the susceptible control PAN 3497 rated 80S (2020) and 30S (2021) at Napier, respectively. In both 2020 and 2021 growing seasons at Napier, 67 Zimbabwean wheat varieties showed no leaf rust infection. Susceptible reaction types were produced by SC052 (40S, 2020); SST 875 (0 to 15S 2021; 0 to 20S, 2021) and MRI 834 (20S, 2020). Two varieties SC063 (10MR, 2021) and PAN 3402 (5MS, 2021), produced intermediate reaction types. Overall, the severities recorded at Napier were low with the highest 40% flag leaf area affected recorded for SC052. Except for varieties SC052 (2020), SST 875 (segregation 2020 and 2021) and MRI 834 (2020) all entries with intermediate to high seedling ITs produced low (mostly nil R) field responses at Napier indicating the presence of APR.

The highest flag leaf rating for the susceptible check, PAN 3497, reached 100% at Greytown (Figure 3.5) at time of the first rating indicating an adequate disease pressure during 2021 at this locality. Despite this, 54 of the Zimbabwean wheat varieties produced low field responses of 0 to trace to isolates of *Pt* races MFPS and CFPS+*Lr20*. Varieties SC003, SC033, SC038, SC051, SC056 and SC Shungu (Figure 3.6) showed resistant field responses in accordance with their intermediate to low seedling ITs produced to isolates of races MFPS and CFPS+*Lr20*. Variety SC014 produced a nil field response and PAN 3494 (10MR) showed higher levels of field resistance considering their intermediate to high seedling responses to *Pt* race CFPS+*Lr20*. The variety SC052 produced a 20MSZ field response often associated with APR sources of leaf rust resistance. The remaining varieties produced MRMS field reaction types indicating the presence of APR compared to the S reaction type of the susceptible control PAN 3497.

Figure 3.5: Flag leaf infection responses observed at an early rating time (from left to right) for SC Select (0), SC Nduna (10MRMS), SC Serena (0, showing infection from *Puccinia striiformis* f. sp. *tritici* race 6E22A+), Runde (10MRMS) and PAN 3497 (100S) to *Puccinia triticina* (*Pt*) races CFPS+*Lr20* and MFPS in a field trial outside Greytown during the 2021 season.

Figure 3.6: Flag leaf infection responses observed at an early rating time (from left to right) for SC Sicho (0), Peregrine (10MRMS), MRI 834 (20MRMS), and SC Shungu (10MR, showing infection from *Puccinia striiformis* f. sp. *tritici* race 6E22A+) to *Puccinia triticina* (*Pt*) races CFPS+*Lr20* and MFPS in a field trial outside Greytown during the 2021 season.

3.3.4 Postulation of leaf rust resistance genes

Variations of ITs on the 27 standard differentials and 12 additional lines with known *Lr* genes inoculated with nine *Pt* race isolates (Table 3.4) indicated the possibility of identifying 19 *Lr* genes (*Lr1*, *Lr*2a, *Lr2b*, *Lr2c*, *Lr*3a, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr15*, *Lr17b*, *Lr18*, *Lr20*, *Lr23*, *Lr24*, *Lr26*, *Lr27+31*, *Lr28*, *Lr30* and *Lr32*). From these, 12 differentials with *Lr1*, *Lr2c*, *3ka*, *Lr18*, *Lr20*, *Lr23*, *Lr24*, *Lr26*, *Lr28, Lr30*, *Lr32* and *Lr27+31* produced unique low and high patterns useful for gene postulation. From the remaining seven differentials, similar low and high IT patterns were produced by lines with *Lr2a* and *Lr2b*; *Lr3a*, *Lr3bg* and *Lr10* as well as *Lr15* and *Lr17b* and are therefore considered less useful in gene postulation. Postulation of *LrB*, *Lr14a* and *Lr17a* was impossible because only high ITs (3 to 4) were recorded for all nine race isolates. Forty-nine varieties produced only flecks like Agatha (*Lr19*) and KS90H450 (*Lr47*) which did not allow any gene postulation (Table 2.4). Similarly, varieties SC003, SC014, SC019, SC033, SC038, SC039, SC047, SC050 and SC056 produced ITs ranging from fleck to 12+X to the different *Pt* race isolates excluding the possibility of any gene postulation. The remaining fourteen varieties, SC015, SC Nduna, K215W119, Runde, SC048, SC051, SC052, SC063, SST 875, PAN 3494, Peregrine, PAN 3402, SC Shungu and MRI 834 were seedling susceptible (IT ≥3) to at least one *Pt* race isolate, which potentially allow for *Lr* gene postulation. However, from these only two varieties SST 875 and PAN 3402 produced a similar pattern to RL6078 (*Lr26*), ignoring the segregation in IT responses observed for PAN 3402 to *Pt* race SDDN. The remaining 12 varieties produced unique low and high profiles to those observed in Table 3.4 for the differential lines containing single *Lr* genes. Furthermore, the varieties SC Nduna (to the two MCDS race isolates) and Runde (SFDS) showed segregation in their seedling ITs. The varieties SC048 and SC063 produced an identical low and high profile with similar ITs indicating that they may carry the same unknown *Lr* gene(s).

3.3.5 Determining the segregation ratios for 25 F_2 populations

The 25 F_2 populations, including the selected SC-parents and PAN 3497, were successfully assessed for their seedling ITs to *Pt* race MCDS (ZIM isolate Pt1_1_2019) using the McIntosh *et al.* (1995) rating scale. Seedling ITs including combinations of 0 to 2 were considered resistant (low ITs) and 3 to 4 or combinations thereof as susceptible (high ITs). The 25 Zimbabwean parental lines were confirmed resistant to the *Pt* race MCDS expressing similar low ITs to that reported in Table 3.5 with the susceptible parent PAN 3497 consistently producing high ITs of 3+. Segregation for low and high ITs in the individual F_2 populations was in accordance with the seedling responses of the resistant (Table 3.5) and susceptible (PAN 3497, 3+) parents. This is illustrated in Figure 3.7 for the crosses PAN3497/SC003,

PAN3497/SC009, PAN3497/SC010 and PAN3497/SC038. The calculated critical value for Chi-square (X^2_c) for each cross at one degree of freedom (df) and 0.05 significance level is 3.841. The H_0 that resistance in Zimbabwean varieties is determined by dominant single genes can be accepted for all segregating populations indicating a ratio of 3:1 with X^2_c value less than 3.841 and *P* value greater than 0.05.

Based on these calculations, 23 F_2 populations including crosses PAN3497/SC009 ($X_{3:1}$ = 0.131, *P* = 0.7174), PAN3497/SC010 ($X_{3:1}$ = 0.021, *P* = 0.8848) and PAN3497/SC038 ($X_{3:1}$ = 0.167, *P* = 0.6828) (Figure 3.7, Table 3.6) indicated the presence of a single dominant *Lr* gene. For the remaining two populations of SC003 and SC065 the H_0 hypothesis for a 3:1 segregation ratio was rejected. The F_2 seedlings from cross PAN3497/SC003 segregated in a three high (IT = 3+) one low (IT = ;1+), 1:3 ratio ($X_{1:3}$ = 378.08, *P* = <0.0001, Figure 3.7, Table 3.7). The progenies from the cross PAN3497/SC065 indicated the presence of two dominant *Lr* genes for leaf rust resistance, 15:1 ratio ($X_{15:1}$ = 30.37, *P* = <0.0001, Table 3.6).

Figure 3.7: Images for seedling infection types (ITs) to *Puccinia triticina* (*Pt*) race MCDS (isolate Pt1_1_2019**)** for F_2 progenies from crosses (left to right) PAN3497/SC003 (3+ to ;1+), PAN3497/SC009 (fleck to 3+), PAN3497/SC010 (fleck to 3+) and PAN3497/SC038 (;1+ to 3+).

Table 3.7: Segregation ratios of F_2 populations determined in the greenhouse on seedlings inoculated with the Zimbabwean isolate Pt1_1_2019 of *Puccinia triticina* (*Pt*) race MCDS.

F_2 cross	Resistant	Susceptible	Expected R:S ratio	χ^{21}	*P* value
PAN3497/SC002	204	81	3:1	1.799	0.1798
PAN3497/SC003[2]	51	192	3:1	378.08	<0.0001
PAN3497/SCW9101	206	56	3:1	1.837	0.1753
PAN3497/SC008	218	65	3:1	0.623	0.4299
PAN3497/SC009	188	66	3:1	0.131	0.7174
PAN3497/SC010	188	64	3:1	0.021	0.8848
PAN3497/SC013	223	70	3:1	0.192	0.6613
PAN3497/SCSelect	200	58	3:1	0.873	0.3501
PAN3497/SC027	217	65	3:1	0.572	0.4495
PAN3497/SC028	212	60	3:1	1.255	0.2626
PAN3497/SC029	229	84	3:1	0.563	0.4531
PAN3497/SC035	189	71	3:1	0.738	0.3903
PAN3497/SC038	213	75	3:1	0.167	0.6828
PAN3497/SC040	195	62	3:1	0.105	0.7459
PAN3497/SC046	188	54	3:1	0.931	0.3346
PAN3497/SC047	297	79	3:1	3.192	0.0740
PAN3497/SC049	254	69	3:1	2.28	0.1311
PAN3497/SC050	231	67	3:1	1.007	0.3156
PAN3497/SC054	228	70	3:1	0.362	0.5474
PAN3497/SC055	231	76	3:1	0.001	0.9748
PAN3497/SC058	196	56	3:1	1.037	0.3085
PAN3497/SC061	217	65	3:1	0.572	0.4495
PAN3497/SC062	212	60	3:1	1.255	0.2626
PAN3497/SC064	180	59	3:1	0.013	0.9092
PAN3497/SC065[3]	212	22	3:1	30.37	<0.0001

[1] Significant limit of χ^2 (P = 0.05, df = 1); [2] Cross indicating segregation ratio 1:3 and did not conform to the predicted 3:1 ratio; [3] Cross indicating segregation ratio 15:1 and did not conform to the predicted 3:1 ratio.

3.3.6 Molecular marker analysis linked to leaf rust resistance genes

An important application of molecular markers in wheat disease resistance breeding is genotypic profiling of specific markers linked to resistance genes. This application was successfully applied to the set of 72 Zimbabwean adapted varieties to evaluate the genetic profiles for 17 *Lr* genes. Molecular marker data indicated that 12 *Lr* genes were detected in different varieties: seven ASR genes (*Lr9, Lr11, Lr19, Lr26, Lr29, Lr50* and *Lr51*) and five APR genes (*Lr27, Lr34, Lr37, Lr46* and *Lr68*). Four ASR genes (*Lr24, Lr25, Lr32*, *Lr47*) and a single APR gene *(Lr67*) were not detected in the set of 72 Zimbabwean varieties (Figure 3.8, Table 3.8).

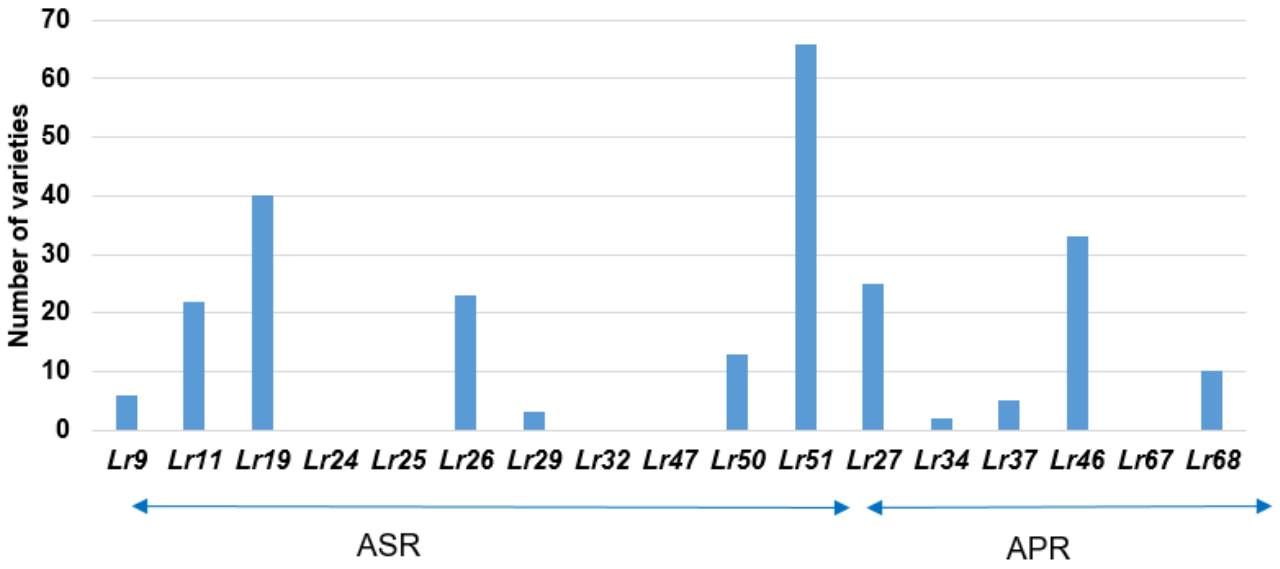

Figure 3.8: Distribution of molecular markers linked to leaf rust (*Lr*) resistance genes among the 72 Zimbabwean wheat varieties as genotyped by molecular markers and grouped into all-stage resistance (ASR) and adult plant resistance genes (APR).

Table 3.8: Presence (1) or absence (0) of leaf rust resistance (*Lr*) genes in 72 Zimbabwean wheat accessions based on molecular marker(s) assessment.

Entry	*Lr* genes																
	***Lr9*[1]**	***Lr11*[1]**	***Lr19*[1]**	***Lr24*[1]**	***Lr25*[1]**	***Lr26*[1]**	***Lr27*[2]**	***Lr29*[1]**	***Lr32*[1]**	***Lr34*[2]**	***Lr37*[2]**	***Lr46*[2]**	***Lr47*[1]**	***Lr50*[1]**	***Lr51*[1]**	***Lr67*[2]**	***Lr68*[2]**
SC001	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
SC002	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC003	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
SC004	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0
SC005	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0
SC006	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SC W9101	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0
SC008	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
SC009	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0
SC010	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC011	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC012	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0
SC013	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0
SC014	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
SC015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC016	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
SC017	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1
SC018	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1
SC019	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0
SC020	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1
SC Select	1	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1
SC Nduna	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
SC Serena	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
K215W119	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1
Runde	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Table 3.8 (cont.): Presence (1) or absence (0) of leaf rust resistance (*Lr*) genes in 72 Zimbabwean wheat accessions based on molecular marker(s) assessment.

Entry	*Lr* genes																
	***Lr9*[1]**	***Lr11*[1]**	***Lr19*[1]**	***Lr24*[1]**	***Lr25*[1]**	***Lr26*[1]**	***Lr27*[2]**	***Lr29*[1]**	***Lr32*[1]**	***Lr34*[2]**	***Lr37*[2]**	***Lr46*[2]**	***Lr47*[1]**	***Lr50*[1]**	***Lr51*[1]**	***Lr67*[2]**	***Lr68*[2]**
SC026	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC027	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0
SC028	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0
SC029	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0
SC030	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
SC031	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0
SC032	0	0	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0
SC033	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
SC034	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC035	0	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0
SC036	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC037	0	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0
SC038	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
SC039	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC040	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC041	0	1	1	0	0	1	1	0	0	0	0	1	0	1	0	0	0
SC042	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC043	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0
SC044	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	0	0
SC045	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0
SC046	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC047	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
SC048	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
SC049	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0
SC050	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0

Table 3.8 (cont.): Presence (1) or absence (0) of leaf rust resistance (*Lr*) genes in 72 Zimbabwean wheat accessions based on molecular marker(s) assessment.

Entry	*Lr* genes																
	***Lr9*[1]**	***Lr11*[1]**	***Lr19*[1]**	***Lr24*[1]**	***Lr25*[1]**	***Lr26*[1]**	***Lr27*[2]**	***Lr29*[1]**	***Lr32*[1]**	***Lr34*[2]**	***Lr37*[2]**	***Lr46*[2]**	***Lr47*[1]**	***Lr50*[1]**	***Lr51*[1]**	***Lr67*[2]**	***Lr68*[2]**
SC051	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
SC052	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
SC053	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC054	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC055	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0
SC056	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC057	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1
SC058	0	1	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0
SC059	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC060	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
SC061	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC062	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SC063	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
SC064	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0
SC065	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0
SST 875	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
PAN 3494	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Peregrine	0	0	0	0	0	1	1	0	0	1	1	1	0	0	1	0	0
PAN 3402	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SC Sicho	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1
SC Shungu	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1
MRI 834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

[1] All-stage resistance gene; [2] Adult plant resistance gene.

3.3.6.1 Markers linked to all-stage resistance genes

Each marker was evaluated against a positive control carrying the *Lr* gene and amplicon size based on literature (Table 3.3) to score for the presence or absence. Marker *J13-1/J13-2* was used to evaluate the presence of *Lr9*, with six varieties containing the correct fragment size compared to the positive RL6010 control line (Table 3.7). Two markers, *Wmc24* and *Wmc261* were used to detect the *Lr11* gene. Marker *Wmc261* was not informative for the varieties tested in this study due to non-separation of amplified fragments to distinguish between the positive control and susceptible varieties thus polymorphism between the resistant and susceptible allele was not obtained (maswheat.ucdavis.edu). Marker *Wmc24* was informative, and 22 varieties produced the same fragments as the control line used for *Lr11*. Markers *PSY* and *Gb* linked to gene *Lr19/Sr25* detected the gene in 40 of the 72 varieties. The STS marker, *STSLr24*, linked to *Lr24* did not detect the presence of *Lr24* in any of the varieties. The SCAR marker *Lr25F20/Lr25R19,* linked to *Lr25* also did not detect the presence of *Lr25* in the Zimbabwean varieties. The SCAR marker, *lag95,* detected the presence of *Lr26* in 23 varieties. Molecular marker *Lr29F24/Lr29R24* linked to *Lr29* was detected in three varieties: SC035, SC044 and SC045. The SSR marker *Barc135* did not detect the presence of *Lr32* in any of the tested varieties. Marker analysis also confirmed the absence of *Lr47* in all the varieties genotyped with marker *PS10-L/-R.* Flanking markers *Gwm382* and *Gdm87*, were used to detect the presence of *Lr50* in Zimbabwean varieties*.* Marker *Gdm87* was not informative due to monomorphic fragment amplification for both the susceptible and resistant control varieties. The informative marker, *Gwm382,* detected the presence of *Lr50* in 13 varieties. The marker *Aga7-759R* linked to *Lr51* was amplified in 66 varieties, therefore the most commonly detected *Lr* gene in the selected Zimbabwean wheat varieties (Figure 3.9).

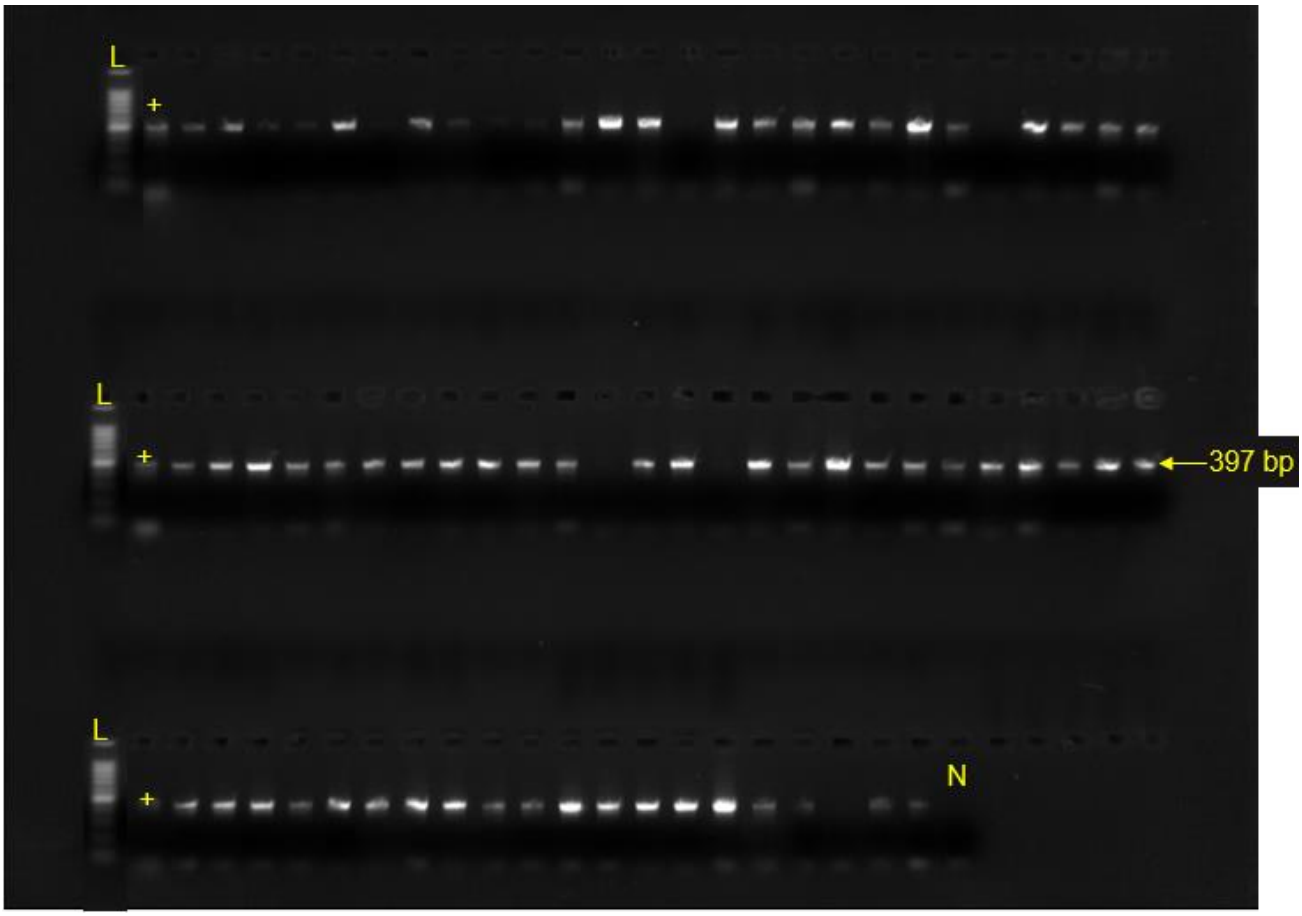

Figure 3.9: Polymerase chain reaction amplification of marker *Aga7-759R* linked to *Lr51* at 397 bp for 72 Zimbabwean wheat varieties. L = 1 kb DNA ladder; + = Positive control R05; N = Non template control.

3.3.6.2 Markers linked to adult plant resistance gene

Figure 3.8 summarises the distribution of the *Lr* genes within the Zimbabwean wheat varieties according to molecular marker genotyping, while Table 3.7 shows the genotyping results for the different varieties denoted in binary notation: 0 for the absence of the linked *Lr* gene and 1 for the presence of the linked *Lr* gene. The CAPS marker *csSr2* was used to detect the pleiotropic gene *Lr27/Yr30/Sr2* within the Zimbabwean wheat varieties and Kingbird was used as the positive control. Twenty-five varieties were identified to carry the APR gene *Lr27/Yr30/Sr2*. The co-dominant SSR marker, *cssfr5*, was used to detect *Lr34*/*Yr18*/*Sr57* which was identified in only two varieties namely SC033 and Peregrine (Figure 3.10). Marker URIC/LN2, linked to *Lr37*, was detected in five varieties namely K215W119, SC045, SC047, SC065 and Peregrine. Three markers *Wmc44*, *Gwm259* and *Barc80* were used to detect *Lr46*/*Yr29* and line Pavon was added as the positive control. Markers *Wmc44* and *Gwm259* were uninformative for the varieties used in this study due to similar mobility of the amplicons. Marker *Barc80* was informative and the *Lr46*/*Yr29* gene was detected in 33 varieties, making it the most commonly detected APR gene in the selected Zimbabwean wheat varieties. The

APR gene *Lr67* was not detected in any of the 72 varieties using the two derived CAPS (dCAPS) markers *TM4dcaps* and *TM10dcaps* linked to the gene. The APR gene *Lr68* was detected in 10 varieties namely SC017, SC018, SC020, SC Select, SC Nduna, K215W119, SC057, SC Sicho, SC Shungu and MRI 834 using two linked markers *Cs7* and *csGS*.

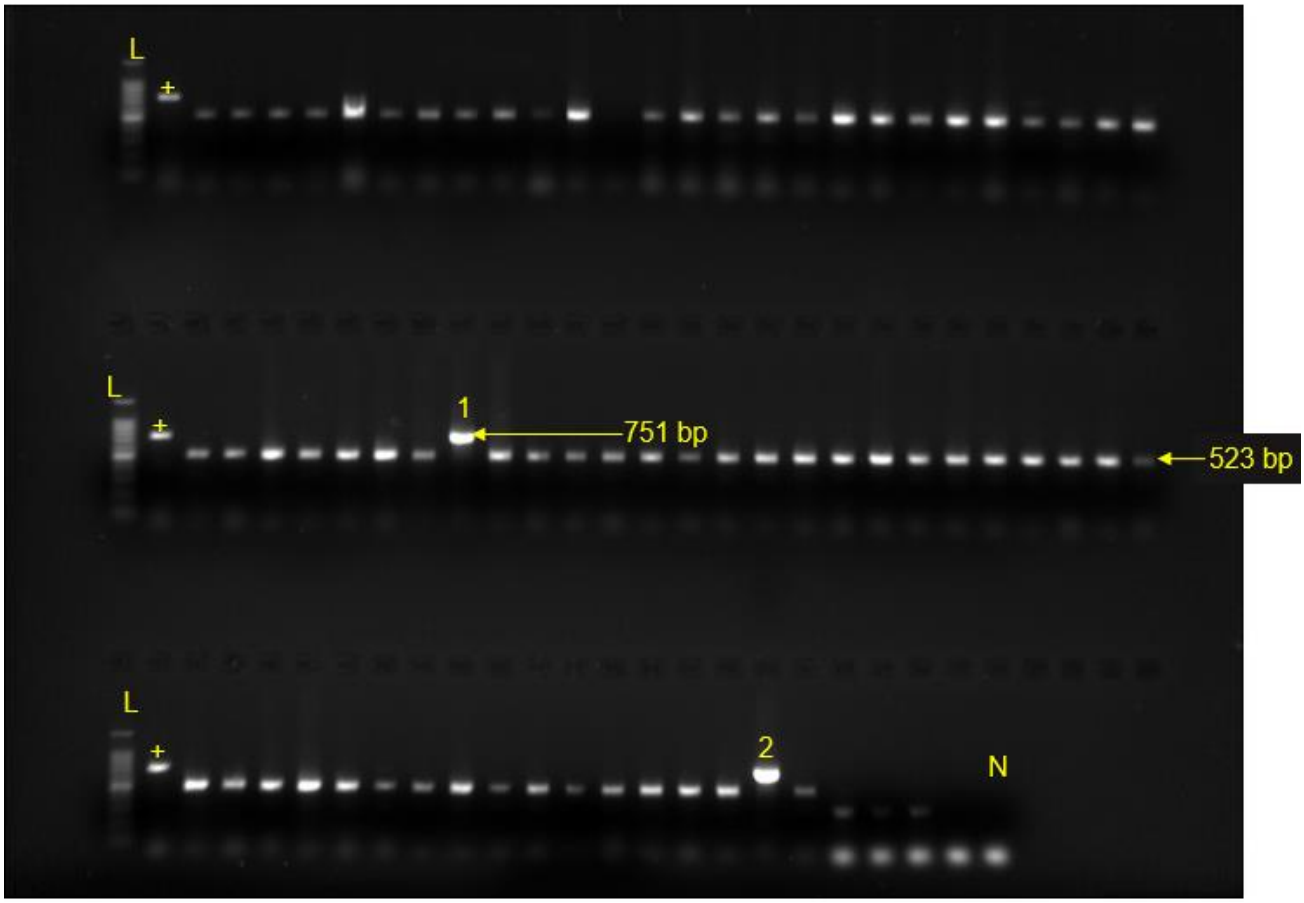

Figure 3.10: Polymerase chain reaction amplification of marker *cssfr5* linked to *Lr34*/*Yr18*/*Sr57* for 72 Zimbabwean wheat varieties. L = 1 kb ladder marker; + = positive control Kariega; N = non template control; 1 = SC033; 2 = Peregrine.

3.3.6.3 Comparison of all-stage resistance gene postulation, molecular marker evaluation and results from outcrosses

The varieties SST 875 and PAN 3402 produced the same seedling IT patterns over isolates representative of the nine *Pt* races to that of RL6078 carrying *Lr26*. However, considering their low ITs to *Pt* races SDDN, FBPT and CPBS, it varied from ;1 to ;12+X compared to the flecks produced by isolates of these races on RL6078 (Table 3.6). This discrepancy question whether *Lr26* is indeed present, but its absence was confirmed by the SCAR marker, *Iag95,* in both varieties (Table 3.8). However, the marker did detect the presence of *Lr26* in 23 varieties. The *Lr26* gene is ineffective against both South African and Zimbabwean isolates of *Pt* race MCDS showing high ITs of 3+. Two varieties, SC051 and Peregrine were susceptible to both MCDS isolates while the molecular markers confirmed that both varieties carried the *Lr26* gene.

For the remaining 21 varieties, nine (SC005, SC009, SC013, SC020, SC Select, SC032, SC041, SC055 and SC Sicho) with fleck IT responses to both MCDS isolates were genotyped to carry *Lr19* which is effective against *Pt* race MCDS. The other 12 varieties also showed fleck IT responses to MCDS isolates but the effective *Lr19* gene was not detected in these varieties indicating the presence of another effective, but uncharacterised, ASR gene(s).

Twenty-one of the 25 varieties selected for outcrosses showed fleck IT responses to all nine *Pt* pathotypes, except for SC003, SC038, SC047 and SC050 (Table 3.6). The F_2 offspring of 20 crosses showed a segregation ratio of 3:1 indicating monogenic resistance, while SC003/PAN3497 showed a segregation ratio of 1:3 indicating the presence of a monogenic recessively inherited resistance gene and SC0065/PAN3497 showed a ratio of 15:1 indicating at least two dominant genes. The fleck response to all nine *Pt* pathotypes was consistent with the presence of *Lr19* and *Lr47* in the differential lines. However, in the 72 Zimbabwean wheat entries molecular markers confirmed the presence of only *Lr19* with no amplification for *Lr47*. The *Lr9* gene showed fleck responses to seven pathotypes and an IT response of ;1- to the SFDS isolate and ;1 to SDDN. According to molecular marker data, three outcrosses carried *Lr9* and 20 carried *Lr19*. Genes *Lr11*, *Lr50* and *Lr51* were also detected by molecular markers in ten, six and twenty-four of the outcrosses respectively (Table 3.9). However, the IT responses of these genes were low to intermediate to all nine races: *Lr11* (;1 to 2+), *Lr50* (;1 to ;1+X) and *Lr51* (;1 to ;1N) which was not consistent with the fleck responses of the Zimbabwean varieties in which these genes were detected by molecular markers (Table 3.5, Table 3.9). The presence of *Lr9*, *Lr11*, *Lr50* and *Lr51* in the tested varieties was therefore questionable based on the observed discrepancies.

Table 3.9: Summary of seedling infection types (ITs) and field response (from Table 3.5) as well as leaf rust (*Lr*) genes detected with molecular markers for 25 Zimbabwean wheat varieties.

Entry	Group[1]	ITs	Field response		Molecular marker data
		Pt1_1_2019	Napier[2]	Greytown[3]	
		MCDS	CFPS+*Lr20*	CFPS+*Lr20* and MFPS	*Lr* genes detected
SC002	3	;	0	0	*11*, *19*, *46*, *51*
SC003	2	;1+	0	10MR	*27*,*51*
SC W9101	4	;	0	0	*9*, *19*, *27*, *50*, *51*
SC008	1	;	0	0	*19*, *27*, *51*
SC009	1	;	0	0	*19, 26*, *27*, *51*
SC010	3	;	0	0	*9*, *11*, *19*, *51*
SC013	1	;	0	0	*19*, *26*, *27*, *51*
SC Select	3	;	0	0	*9*, *11*, *19*, *26*, *46*, *51*, *68*
SC027	1	;	0	0	*26*, *27*, *46*, *51*
SC028	4	;	0	0	*19*, *27*, *46*, *51*
SC029	4	;	0	0	*19,27*, *50*, *51*
SC035	4	;	0	0	*11*, *19*, *29*, *50*, *51*
SC038	4	;1N	0	5MR	*11*, *46*
SC040	3	;	0	0	*19*, *51*
SC046	4	;	0	0	*19*, *51*
SC047	4	;	0	0	*37*, *51*
SC049	3	;	0	0	*11*, *19*, *46*, *50*, *51*
SC050	1	;	0	0	*46*, *51*
SC054	3	;	0	0	*11*, *19*, *46*, *51*
SC055	4	;	0	0	*19*, *26*, *50*, *51*
SC058	1	;	0	0	*11*, *19*, *27*, *46*, *51*
SC061	3	;	0	0	*19*, *46*, *51*
SC062	1	;	0	0	*19*, *46*, *51*
SC064	4	;	0	0	*11*, *19*, *46*, *50*, *51*
SC065	4	;	0	0	*11*, *19*, *37*, *46*, *51*

[1] Group allocated based on proprietary pedigree info; [2] Highest field response scores recorded at Napier during the 2020 and 2021 growing seasons; [3] Field response scores at Greytown during the 2021 growing season.

Five (SC008, SC009, SC013, SC058 and SC062) of the seven varieties used in outcrosses from pedigree group 1 containing both *Lr19/Sr25* and *Lr27/Yr30/Sr2* backgrounds, were confirmed to carry *Lr19*. *Lr27* was detected in four of them, SC008, SC009, SC027 and SC058. As expected *Lr27* was detected in SC003 from group 2 with *Lr27/Yr30/Sr2* pedigree background. All seven outcrosses from group 3 from the *Lr19/Sr25* background were confirmed to carry *Lr19* as expected. Ten outcrosses in group 4, except for SC038 and SC047, were confirmed to carry *Lr19* while only three outcrosses from the same group (SC W9101, SC028 and SC029) carried *Lr27/Yr30/Sr2*. Based on the results, the pedigree data was predictive. Thus, in summary, except for SC003 (*Lr51* detected), SC027 (unknown *Lr* gene as the low IT of fleck is inconsistent with the detected *Lr51*), SC038 (*Lr11*), SC047 and SC050 (unknown *Lr* gene(s) as the low ITs of fleck is inconsistent with the detected *Lr51*), phenotypic and molecular data is in support of the presence of *Lr19* in 20 of the 25 varieties

used in outcrosses. The variety SC065 can possibly carry either *Lr11* or *Lr51* in addition to *Lr19*.

The 25 varieties used in outcrosses to PAN 3497 were immune (0) in the field, except for SC003 (10MR) and SC038 (5MR). The presence of APR genes was masked by ASR genes present in each of these entries under field conditions. Applying molecular markers, the APR genes *Lr27* and *Lr46* were predicted to occur in nine and 13 of the 25 varieties, respectively. The marker data for varieties SC027, SC028 and SC058 used in outcrosses indicated the presence of both *Lr27* and *Lr46*. In addition, these two genes were also detected in SC004, SC017, SC041, SC043, SC044, Peregrine and SC Sicho. The *Lr34*/*Yr18*/*Sr57* gene was not present in any of the varieties used in outcrosses but was present in two varieties namely SC033 and Peregrine. Only one variety, SC065 used in outcrosses, carried *Lr37*, while four additional varieties SC045, SC047, K215W119 and Peregrine, carried the *Lr37* gene. Only SC Select used in outcrosses carried *Lr68*. In addition, the *Lr68* gene was detected in nine more varieties, namely SC017, SC018, SC020, SC057, SC Nduna, SC Sicho, SC Shungu, K215W119 and MRI 834. In 20 of the 72 varieties, molecular markers failed to detect any APR genes. From these 20 varieties, SC015, SC048, Runde, SST 875 and PAN 3402 had high seedling ITs with MRMS field responses, indicating the presence of unidentified APR sources against the leaf rust races used.

3.4 Discussion

In this study, 72 Zimbabwean wheat varieties were tested to identify their *Lr* gene status using a combination of pedigree grouping, gene postulation, F_2 segregation ratios and molecular marker genotyping. Applying closely linked molecular markers, a total of 12 *Lr* genes namely *Lr9*, *Lr11*, *Lr19*, *Lr24*, *Lr26*, *Lr27*, *Lr34*, *Lr37*, *Lr46*, *Lr50*, *Lr51* and *Lr68* were identified either singly or in combination in the 72 Zimbabwean wheat varieties. The results from the F_2 segregation ratios in combination with the molecular data however revealed several varieties with false positives for *Lr9*, *Lr11*, *Lr50* and *Lr51*. This questions the presence of these genes in the rest of the varieties based on molecular marker data. Forty-nine varieties showed resistant R seedling ITs to all nine *Pt* races in the greenhouse and at least 53 varieties were strongly resistant with immune responses to races CFPS+*Lr20* and MFPS in the field.

Most of the resistant varieties were breeding lines from the SeedCo breeding programme belonging to the pedigree groups 1-4 (Appendix 3.1) that were selected under naturally occurring leaf rust infections over several planting seasons at each filial generation. The used SeedCo breeding lines were selected from an advanced F_7 nursery of a previous breeding project that focused on the introgression of ASR gene *Sr25/Lr19* and APR gene *Lr46* with

donors from CIMMYT in México (Soko, 2018) where they showed moderate to high levels of field resistance. However, MAS was not applied to confirm if the successful introgression of these genes and selection were based on field responses following natural infection from uncharacterised *Pt* isolates. Pedigree group 5, which contributed most varieties with high seedling ITs to at least some *Pt* race isolates in the greenhouse and which produced mostly MRMS reaction types in the field, were not developed by SeedCo and were selected based on their presence in the Zimbabwean commercial market, hence no known breeding history.

The nine *Pt* races and 46 differential lines did not permit gene postulation for any ASR genes. The presence of the *Lr26* gene in the two varieties SST 875 and PAN 3402 which showed the same high and low IT patterns than RL6078 (*Lr26*), could not be confirmed with the SCAR marker *lag95*. Thus, the gene postulation method failed to determine the *Lr* gene profiles of the selected varieties used in this study, which can be ascribed to the lack of *Pt* isolates with virulence to the ASR gene(s) present in most varieties. The remaining varieties where low and high seedling IT patterns were recorded either carried unique genes not present, or gene combinations not accounted for, in the 46 differential lines. This limitation of gene postulation was also highlighted in studies done by Mebrate *et al*. (2008) and Dakouri *et al*. (2013).

Determination of the number of ASR genes using F_2 segregation ratios revealed that 23 of the 25 selected F_2 crosses showed a segregation ratio of 3:1, indicating inheritance of a single dominant *Lr* gene. Differential line Agatha had fleck IT responses to all nine races tested. Molecular markers detected *Lr19* in 20 varieties used in outcrosses which also had fleck responses to all the nine races and immune responses in the field. Variety SC Select used in the outcrosses and SC Serena were previously genotyped to carry *Lr19* and immune responses in the field by Pretorius *et al*., 2015. These results therefore indicated to *Lr19* as the single inherited dominant gene. Furthermore, *Lr19* was previously not detected in SC Nduna and SC Shungu. Results from this study confirms these earlier findings. This was also supported by the pedigree grouping based on the previous genotyping results for the *Sr25* stem rust resistance gene with a tight linkage to *Lr19*. Groups 1 and 3 that had *Sr25* parentage background contributed 12 outcrosses carrying *Lr19*, while group 4 had eight outcrosses with *Lr19* as expected from their known pedigrees.

Other ASR genes detected by molecular markers (*Lr9*, *Lr11*, *Lr26*, *Lr29*, *Lr50* and *Lr51*) had low to intermediate (; to 2+) to high (*Lr26*) IT responses. The *Lr26* gene was not effective against the confirmed dominant *Pt* race MCDS. The remaining ASR genes that occurred with a higher frequency and effective against MCDS (*Lr9*, *Lr11*, *Lr50* and *Lr51*) did not show fleck responses to all nine race isolates as observed for *Lr19*. Two varieties used in outcrosses,

SC003 and SC038, deviated from the fleck IT response and showed MR field responses at Greytown.

Variety SC003 from group 2 carrying *Lr27/Yr30/Sr2* and SC038 from group 4 carrying the *Lr46* introgression both lacked *Lr19* and are predicted to carry *Lr51* and *Lr11*, respectively. Based on the false positives observed for *Lr11* and *Lr51*, it is also possible that they carry unique ASR genes. A high number of group 4 entries carried *Lr19* as they were selected from an F_7 nursery from a previous breeding project that focused on introgression of *Sr25/Lr19*, and their pedigree info has parentage that was previously genotyped to carry *Sr25*. Molecular markers confirmed the presence of APR gene *Lr27* in SC003 which is supported by the pedigree info. An MR response in the field was therefore expected due to their none-immune responses at seedling stage.

Since some of the markers used in this study were flanking markers (Vida *et al*., 2009) and not tightly linked (<1 centimorgan) to the genes of interest (maswheat.ucdavis.edu), the detection of *Lr9*, *Lr11*, *Lr50* and *Lr51* could be false positives. The presence of *Lr19* as a single dominant gene is supported based on the pedigree grouping of the *Lr19*/Sr25 gene, which was actively introgressed into the selected population from known stem rust resistance donors. Therefore, the F_2 outcrosses showing segregation ratios of 3:1, together with the confirmation by molecular marker genotyping, revealed that *Lr19* could be relied upon as the single dominant ASR gene in question.

Due to their poor diagnostic power and high rate of false positives, single assays of molecular marker are not always suitable, according to Maccaferri *et al*. (2022). Since assayed markers still recombine with the target gene, this disparity is proportional to their distance from the target locus. To limit the influence of recombination, it is consequently required to rely on at least a few markers flanking the target locus/QTL. In this instance, it is possible to forecast the frequency of false positives using the product of the distances between the two markers flanking the targeted locus.

According to Terracciano *et al*. (2013), the more tightly associated the markers are to the gene of interest, the more they are considered as ‘diagnostic’ or ‘predictive’ in various genetic backgrounds or crosses. To achieve this, the gene of interest is cloned either by positional cloning or functional examination of candidate genes in the locus region and the desired polymorphisms are identified. Thus, it is possible to design the so-called ‘perfect marker’ that is not subjected to recombination. The number of cloned wheat disease resistance loci/QTLs to develop highly diagnostic ‘perfect markers’ which are not subjected to recombination has grown steadily in the last five years (Thudi *et al*., 2021). Notable examples include *Fhb1*,

Cdu1, *Fhb7*, *MIWE18*, *Lr14*, *Pm4*, *SSt1* and *Yr15*, as well as several other rust genes such as *Lr9* (Wang *et al*., 2022), *Lr34* (Krattinger *et al*., 2009), *Sr61* (Zhang *et al*., 2021) and *Yr27* (Athiyannan *et al*., 2022).

The *Lr9* gene was detected in six varieties of which three has been confirmed as likely false positives through outcrosses. The effectiveness of this gene against all the tested race isolates confirms *Lr9* as a potential source of resistance when deployed in combination with complementary ASR genes such as *Lr11, Lr19* and *Lr51*. Recent studies by Wang *et al*. (2022) revealed the cloning of *Lr9* gene from the wild grass species *Ae. umbellulata*. The gene encodes an unusual tandem kinase fusion protein, a trait important in disease resistance. The *Lr9* gene imparted effective resistance to 24 *Pt* races in China (Li *et al*., 2010). However, using this gene in commercial cultivars has been limited due to potential negative effects on yield (Gultyaeva *et al*., 2021). Similarly, *Lr9* was also detected in low frequency in some European countries and the USA (Urbanovich *et al*., 2006; Kolmer *et al*., 2007).

The *Lr11* gene was detected in 22 varieties, which showed several responses. They displayed either uncharacteristic low (fleck) and high (≥3) ITs in the greenhouse and R and MR responses in field trials. Previous studies on Zimbabwean germplasm did not report the presence of *Lr11*. Darino *et al*. (2015) reported that *Lr11* has seldom been detected in worldwide studies aimed at genotyping germplasm collections. Huerta-Espino (1992) reported high frequencies of virulence to *Lr11* among *Pt* isolates from Nepal, Romania, Bulgaria and South America, and moderate frequencies of virulence in several other countries. However, there is no evidence to support that this was a consequence of the use of *Lr11* in local varieties in these countries.

The *Lr19* gene (derived from *T. ponticum* and transferred to the long arm of wheat chromosome 7D) is closely linked to *Sr25* which confers resistance to the African *Pgt* race Ug99 has been commonly used in Zimbabwean wheat breeding programmes. Similarly, *Lr19/Sr25* has been used in Australia, Egypt, Pakistan, Afghanistan and CIMMYT breeding programmes. Soko (2018) reported the presence of *Lr19* in 12 varieties in a panel of 45 Zimbabwean varieties that were genotyped. Pretorius *et al*. (2015) also confirmed the presence of *Lr19* in 18 of 49 Zimbabwean varieties tested. The dominant STS marker, $STSLr19_{130}$, linked to *Lr19* did not show any amplicons for the set of 72 varieties and to confirm the data, two additional markers, *PSY* and *Gb*, linked to the pleiotropic gene *Lr19*/*Sr25* were evaluated. Forty varieties were confirmed to carry *Lr19* using markers *Gb* and *PSY* closely linked to *Lr19*/*Sr25*. Despite the undesirable yellow pigment trait in bread wheat associated with the gene, *Lr19* is still widely deployed in new varieties due to its association

with higher yield. Singh *et al*. (2006) reported a yield increase of 10-15% in CIMMYT germplasm carrying *Lr19*.

The *Lr24* ASR gene derived from *Th. ponticum* (syn. *Agropyron elongatum*), is closely linked with *Sr24* on the 3DL/3Ag translocation (McIntosh *et al*., 1977) and was previously detected in three Zimbabwean varieties (Soko, 2018). In our study, the *STSLr24* marker did not detect the presence of *Lr24* in any of the Zimbabwean wheat varieties. Similarly, Mutari *et al*. (2018) reported the absence of *Sr24*/*Lr24* among a set of Zimbabwean genotypes using molecular markers *J09/1J09/2* and *Sr24#/12*. Mago *et al*. (2005) reported that the *Sr24/Lr24* gene is extensively deployed in Australian wheat varieties. In the Zimbabwean context, *Lr24* can still be deployed since it is effective against the currently dominant *Pt* race MCDS. However, it might be risky in the long-term, since South African races SDDN, SFDS, MFPS and CFPS+*Lr20* are virulent to *Lr24*.

The *Lr26* gene was derived from rye and exists in bread wheat as the 1BL/1RS translocation. This translocation also carries *Sr31*, *Yr9* and *Pm8* (Rajaram *et al*., 1983) and has been used as a source of disease resistance in wheat breeding programmes at CIMMYT where most of the Zimbabwean wheat germplasm was sourced in the 1980s and 1990s for trait introgression. In the current study, *Lr26* was detected in 23 varieties using molecular markers. The 1BL/1RS translocation is no longer effective to a wide range of *Pt* races. In this study, isolates of *Pt* races SFDS, CCPS, CCPS+*Lr20*, MFPS and both Zimbabwean and SA MCDS isolates were virulent to *Lr26*. Gultyaeva *et al*. (2021) also reported on the loss of effectiveness of *Lr26* when deployed individually in varieties but in combination, the gene can broaden the level of field resistance to avirulent races. Wheat varieties carrying the *Pm8* powdery mildew gene have been widely planted around the world, but *Pm8* has subsequently lost resistance to newly emerged races of the wheat powdery mildew biotrophic fungus *B. graminis* f. sp. *tritici* (Cheng *et al*., 2022) and is therefore not recommended to be deployed singly.

Varieties SC035, SC044 and SC045 were detected to carry *Lr29,* but a false positive is predicted in SC035 considering the F_2 segregation results from a cross to PAN 3497. Varieties SC044 and SC045 had low ITs (mostly flecks) similar to RL6080 (*Lr29*) for all the tested *Pt* races and showed resistance in all field trials. This gene is a potential resistance source to new *Pt* races that might occur in Zimbabwe and neighbouring countries. Huerta-Espino (1992) reported that only two isolates from Turkey and one from Pakistan were virulent on seedlings that contain *Lr29* in a global survey. The *Lr29* gene was transferred from *Th. ponticum* to chromosome 7DS of common wheat (Procunier *et al*., 1995).

The *Lr50* ASR gene, transferred to line KS96WGRC36 from TA 870 of *T. timopheevii* subsp. *armeniacum* (Brown-Guedira *et al*., 2003), showed low seedling ITs to all the *Pt* race isolates in the greenhouse. The gene has not been previously reported in the Zimbabwean wheat varieties. The gene was predicted to occur in 13 varieties using flanking markers *Gwm382* and *Gdm87*. However, these markers produced false positives in varieties used in outcrosses namely SC W9101, SC029, SC035, SC049, SC055 and SC064. Varieties SC004, SC032, SC037, SC041 and SC060 produced low ITs (fleck) inconsistent with *Lr50*. The variety K215W119 was clearly susceptible to some *Pt* race isolates and although SC048 produced low ITs similar to *Lr50*, it was also predicted to carry *Lr51*. The presence of *Lr50* in the current set of germplasm could not be confirmed.

The highest frequency of detected ASR genes by molecular markers, was *Lr51*, which was predicted to occur in 66 of the Zimbabwean varieties. Unfortunately, these did include 21 likely false positives as predicted from the F_2 segregation ratios. Based on low ITs, *Lr51* may be present as predicted with marker *Aga7-759R* in SC003. However, its predicted presence in SC047 and SC050 needs confirmation as this is contradicted by their fleck low ITs. This gene, formerly known as *LrF7*, was transferred from *T. speltoides* to common wheat and is located on the long arm of chromosome 1B (Helguera *et al*., 2005). In tests for resistance to *Pt* race 5, plants homozygous for *Lr51* were highly resistant with hypersensitive response (Helguera *et al*., 2005). The *Lr51* gene was previously not commonly deployed in breeding programmes, despite the good levels of resistance it conferred, but can be useful in the SeedCo breeding programme.

The segregation results for the F_2 populations revealed the presence of single dominant ASR genes in the majority of the populations fitting the theoretically expected ratios of 3:1. However the presence of a single recessive gene was also revealed in a population from cross PAN3497/SC003 with a segregation ratio of 1:3. Pretorius *et al*. (1988) reported similar results indicating that the APR gene *Lr22a* was inherited as a partially recessive single gene (ratio 1:3) in an F_2 population from crosses between line RL6044 (*Lr22a*) and spring wheat varieties Zaragoza and SST 33. In the current study, molecular markers detected the presence of *Lr51*, however there is no previous evidence to suggest that *Lr51* can be inherited in a recessive manner, thus an unknown gene could be present. Recently, El-Orabey *et al*. (2020) also reported the presence of two independent recessive genes (ratio 1:15) in some Egyptian varieties.

The population from cross PAN3497/SC065 revealed the presence of two independent resistance genes. The combination of several effective resistance genes into a single wheat

variety can extend the period of resistance against the development of new virulent races. Molecular markers detected the presence of the ASR genes *Lr11*, *Lr19* and *Lr51.* Based on the ITs and pedigree background, *Lr19* is highly likely to be one of the dominant genes, with either *Lr11*, *Lr51* or an unknown gene, being the other.

Two (SC033 and Peregrine) out of the 72 Zimbabwean varieties that contained *Lr34*, concurred with findings by Pretorius *et al*. (2015) that identified four out of 50 varieties from Zimbabwe that tested positive for *Lr34*. Studies by Soko (2018) also reported six varieties out of a panel of 45 to contain *Lr34*. The *Lr34* gene is located on the short arm of chromosome 7D and has been cloned (Krattinger *et al*., 2009) and provides race non-specific APR to wheat stripe rust (*Yr18*), stem rust (*Sr57*), powdery mildew (*Pm38*) as well as leaf tip necrosis (*Ltn1*). The resistance phenotype displayed by this gene includes a longer latent period, fewer and smaller uredinia. The *Lr34* gene is widely deployed in many wheat cultivars around the world (Rinaldo *et al*., 2017). Variety SC033 showed a typical *Lr34* expression of MRZ (MR reaction type with pustules concentrated at the leaf base) in the field at Greytown in 2021 to races CFPS+*Lr20* and MFPS. Results from this study shows that *Lr34* is not fully utilized in the Zimbabwean wheat breeding programmes and increased deployment of *Lr34* in combination with ASR gene *Lr19* can be valuable to improve durable leaf rust resistance in Zimbabwe.

The *Lr68* APR gene was detected in ten Zimbabwean wheat varieties which showed varying responses during greenhouse and field tests*. The Lr68 gene* was first described in CIMMYT's spring bread wheat Parula in 1981 that also contains *Lr34* and *Lr46* (William *et al*., 2007). Herrera-Foessel *et al*. (2012) showed in field tests in México that the combined effect of *Lr34*, *Lr46* and *Lr68* in Parula resulted in near immunity. Similar, to these findings, *Lr68* was detected in nine Zimbabwean varieties SC017, SC018, SC020, SC Select, SC Nduna, K215W119, SC057, SC Sicho and SC Shungu in combination with at least one more APR gene. The combination of *Lr68*, *Lr46* and the ASR gene *Lr19* in varieties SC018, SC020, SC Select, SC057 and SC Sicho had low seedling ITs to all nine *Pt* races and R field reaction types. The varieties SC Nduna and SC Shungu that lacked *Lr19* showed high seedling ITs to race MCDS and MRMS and MR field reaction types to races CFPS+*Lr20* and MFPS respectively, despite carrying *Lr68* in combination with *Lr46*. K215W119 with *Lr68* in combination with *Lr37* showed high ITs to isolates of *Pt* races MCDS, CBPS and MFPS in the greenhouse and a MRMS reaction type in the field. Variety MRI 834 with only *Lr68* occurring singly was susceptible to race CFPS+*Lr20* in the field at Napier and showed high seedling ITs to eight of the nine *Pt* race isolates. These examples emphasized the importance of combining resistance genes.

For this study, *Lr27* had the second highest frequency of APR genes in the 72 Zimbabwean varieties and was detected in 25 varieties. Soko (2018) identified 20 varieties carrying *Sr2*/*Yr30*/*Lr27* in a panel of 45 Zimbabwean wheat varieties. Some lines from that study were used as parental lines to develop entries in group 2. The stem rust resistance gene *Sr2* is tightly linked to *Lr27,* yellow rust resistance (*Yr*) gene *Yr30* and pseudo-black chaff (*Pbc1*) (Spielmeyer *et al*., 2003). The varieties PAN 3494 and Peregrine carrying *Lr27* showed MR and MRMS field reaction types respectively, further stressing the need for combining *Lr27* with other APR genes to improve leaf rust resistance at adult plant stage.

The *Lr46* gene, a slow rusting gene, was originally reported in Pavon 76 on chromosome 1BL (Singh *et al*., 1998). William *et al*. (2003) reported that *Lr46* is widely deployed in CIMMYT wheat varieties. At present, *Lr46* still maintains good resistance that makes it an ideal candidate to combine with other resistance genes to achieve durable leaf rust resistance. The *Lr46* gene was the most common APR source in the Zimbabwean wheat varieties and was detected in 33 varieties. Varieties that combined ASR gene *Lr19* and *Lr46* showed strong resistance in the field to *Pt* races CFPS+*Lr20* and MFPS, whereas varieties such as Peregrine and SC Shungu where *Lr46* occurred individually and not masked by *Lr19*, were recorded as 40MRMS and 20MR, respectively, at Greytown during 2021. Four commercial varieties that did not carry both *Lr19* and *Lr46* recorded varying responses with SST 875, PAN 3402 and MR1834 scoring MRMS and PAN 3494 scoring an MR reaction type. The *Lr46* gene is the most utilised APR gene in the Zimbabwean breeding programmes, but its frequency can still be improved. A low frequency of 16 varieties carrying *Lr46* has been previously reported in a panel of 75 Zimbabwean varieties (Mutari *et al*., 2018).

Only five varieties carried *Lr37* in the tested panel of Zimbabwean varieties. A similarly low number of varieties carrying *Lr37* was reported by Mutari *et al*. (2018). The *Lr37* gene is closely linked to *Yr17* and *Sr38* and was translocated from the short arm of *T. ventricosum* 2NS to the 2AS chromosome of bread wheat (Bariana and McIntosh, 1993). Rust races virulent to the *Lr37* and *Yr17* resistance genes have been reported (Robert *et al*., 1999). However, resistance donors known to carry *Lr37* have not been extensively used in the Zimbabwean wheat breeding programme. Low ITs of ;1+X were recorded for the differential line Trident carrying *Lr37* when inoculated with both the Zimbabwean and South African isolates of race MCDS which indicates the presence of additional *Lr* gene(s) in this variety. The stem rust variants of Ug99, namely TTKSF, TTKSF+ and PTKST previously reported in Zimbabwe, are virulent to *Sr38* (Terefe, 2021). In a paper by Cruz *et al*. (2016) it was reported that the 2NS translocation is also associated with a significant reduction in wheat blast caused by the fungus *Magnaporthe oryzae* in both spring and winter wheat indicating that there is

value in utilising the 2NS translocation carrying *Lr37/Yr17/Sr38* in combination with other resistance genes.

A thorough analysis of the prevalence and distribution of both ASR and APR genes is necessary for the effective utilisation of genetic resistance to leaf rust. Using F_2 segregation ratios and molecular markers, *Lr19* and five APR genes were detected in a collection of Zimbabwean wheat germplasm. Additional sources of ASR may be present but could not be postulated, as most varieties carried ASR sources effective to all the *Pt* races. Secondly the low and high IT patterns for most varieties did not match that of the single gene differential lines. This indicated, although defeated in some, the presence of more complex sources of resistance in the Zimbabwean wheat collection. The identified resistant varieties are a potential source of both ASR and APR *Lr* genes in Zimbabwe. Pedigree information of the varieties tested in this study was useful in support of *Lr19* as the dominant ASR gene present in the Zimbabwean panel, as well as the APR genes *Lr27* linked to stem rust genes *Sr2* and *Lr46.*

Results from this study confirmed that there is an overreliance on the *Lr19* ASR and *Lr46* and *Lr27* APR genes. Therefore, an urgent need to combine more APR genes and increase the frequency of *Lr34* and *Lr68,* exists to extend the duration of leaf rust resistance. The slow rusting *Lr67* APR gene which was not detected in the current set needs to be incorporated in the Zimbabwean wheat breeding programmes to develop durable resistance. The findings of this study emphasised the significance of APR genes in providing durable leaf rust resistance in wheat, as well as the necessity of gene pyramiding.

3.5 Chapter 3 references

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3.5.1 Website references

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Chapter 4: Field evaluation of 72 Zimbabwean bread wheat varieties for yield stability, leaf rust response and quality traits

4.1 Introduction

Wheat (*T. aestivum* L.) is a staple food for 35% of the world's population providing more than 21% of the required calories and 20% of protein (FAO, 2020). It ranks as the second most strategic food crop in Zimbabwe after maize with its main products being wheat flour and bran. Wheat flour is the main ingredient for making bread and confectionaries, consumed by over eight million households daily (Zimstat, 2021). Wheat bran is used for making stock feeds in the manufacturing sector. Thus, the wheat industry in Zimbabwe contributes substantially to food security and employment. Despite these socio-economic advantages, wheat production is still low due to unstable yields with the occurrence of diseases being one of the constraints. An average annual production of approximately 200 000 tonnes from a cultivated area of 66 000 ha is insufficient to provide in the local demand. Zimbabwe imports wheat to decrease the deficit of the required 450 000 tonnes per annum (Zimstat, 2021).

Among the biotic constraints of wheat production in Zimbabwe is the annual presence of one of the most commonly occurring fungal pathogens of wheat, *Pt* that causes leaf rust. Most farmers rely on fungicides to protect susceptible varieties against the disease, thereby increasing the production cost. The underlying importance of resistance to wheat leaf rust in commercial farming is continuously highlighted by the widespread use of fungicides. However, planting resistant varieties can be equally efficient and more environmentally friendly to minimise losses. This provides an opportunity to develop widely adapted high yielding and leaf rust resistant wheat varieties. For example, the CIMMYT variety "Attila" with a unique combination of genes for increased yield (*QYld.dms-2D.2*), test weight density (*QTwt.dms-5A* and *QTwt.dms-5B*), kernel weight (*QTkw.dms-4A*, *QTkw.dms-6A.1*, *QTkw.dms-6D.2* and *QTkw.dms-7B.1*) and with a wide adaptation to low yield potential environments and with rust resistance, became a popular choice in South Asia, Middle East, North Africa and East Africa (Zou *et al*., 2017). With the discovery of *Pgt* r*ace* Ug99 in East Africa (Pretorius *et al*., 2000), "Attila" and its derivative varieties were shown to lack durable resistance when they succumbed to the new race. Furthermore, "Atilla" also became susceptible to *Pst* with the emergence of *Yr27*-virulent races in South Asia (Solh *et al*., 2012). This demonstrated that achieving durable resistance combined with high yield relies on continuous and consistent efforts to combine genes for these traits in new wheat varieties (Singh *et al*., 2016). For instance, Martin *et al*., (2003) described that leaf rust resistant lines

with *Lr41* and *Lr42* increased grain yield by 63% and 26%, test weight by 5% and 3%, and kernel weight by 14% and 9%, respectively under high disease pressure.

In addition to high grain yield, disease resistance and wide adaptability, an ideal wheat variety needs to retain high end-use quality attributes required by millers, bakers and consumers (Guzman *et al*., 2016; Sandhu *et al*., 2021). In Zimbabwe, farmers are interested in wheat varieties with stable high yield and good agronomic traits including early maturity, lodging tolerance and semi-dwarf types considering plant height. The millers expect varieties with good milling qualities such as high flour yield, while the bakeries require varieties with good baking qualities such as strong flours with high gluten content. Consumers prefer a good quality loaf consisting of a crispy crust, not too dense with a glossy interior and of good flavour. To identify varieties that can meet these criteria, there is a need for METs across the main representative wheat growing regions of Zimbabwe. These trials allow for the prediction of variety yield performance based on experimental data, identification of stable varieties that maintain their superior rankings across environments and aid in decision making on variety deployment for current and future markets (Crossa, 1990). After field evaluations the wheat quality assessments based on the minimum threshold set by the milling and baking industry are critical to ensure that only varieties that combine high yield, with adequate levels of disease resistance, and desirable agronomic and quality traits are commercialised.

Crop scientists focus on breeding, testing and selecting genotypes that show superior performance in different environments. This objective is often complicated by GxE. Hongyu *et al*. (2014) referred to GxE as changes in genotype rankings across environments with cross-over interactions being the extreme form. Cross-over interaction is whereby a genotype is superior in one environment but a poor performing genotype in another environment. Inefficiency in GxE analysis can present problems to breeders and plant pathologists leading to wrong varietal recommendations. This can result in low variety adoption by farmers following the release of such a variety, decreased income and potential loss in market share and trust in the brand of the breeding company. Poor performing or unstable varieties can contribute to decreased food security in the long run. Several statistical methods are available that can be used to understand GxE effects. However, two multivariate analyses such as AMMI and GGE analysis allow for analysis of mega-environments (MEs), evaluation of environments and, evaluation of genotypes (Yan *et al*., 2007). The AMMI model is very efficient, accurate, and cost-effective in analysing GxE resulting in better variety recommendations, enhanced genetic gains by breeding programmes and is superior for agricultural reasons because it analyses all three sources of variation: genotype (G), environment main effects (E) and GxE effects (Gauch, 2006). The AMMI analysis can also

be used to determine the stability of genotypes across locations using the PCA (principal component axis) scores and AMMI CSI values. Moreover, the GGE analysis is an effective method which is based on the PCA to fully explore MET.

The aim of this chapter was to analyse multi-location and season trial data for 72 Zimbabwean wheat varieties to draw varietal recommendations for commercialisation. The specific objectives were 1) to evaluate 72 Zimbabwean wheat varieties for leaf rust response, quality traits and stability over MET for two seasons; 2) to evaluate GxE effects on the performance of 72 Zimbabwean wheat varieties using AMMI analysis and GGE-biplots.

4.2 Materials and methods

4.2.1 Plant materials

The set of 72 Zimbabwean wheat varieties (Table 4.1) used in this study consisted of 13 commercial varieties (six from SeedCo and seven from competitor companies) and 59 SeedCo experimental breeding lines as described in section 3.2.1.

4.2.2 Trial site information

Four sites in Zimbabwe were used for MET in 2020 and 2021 (Table 4.2). These were the Rattray Arnold Research Station (RARS) in Mashonaland province, Agricultural Research Trust Farm (ART Farm) in Harare metropolitan province, Sisal Farm in Manicaland province and Panmure in Mashonaland central province (Figure 4.1). These sites were selected based on the main commercial wheat production areas in Zimbabwe and are geographically located on the highveld (>1200 masl), and middleveld (800 to 1200 masl).

Table 4.1: List of 72 Zimbabwean wheat varieties planted in multi-environment trials (METs) in Zimbabwe during 2020 and 2021.

Genotype	Name	Status	Source
G1	SC001	Breeding line	SeedCo
G2	SC002	Breeding line	SeedCo
G3	SC003	Breeding line	SeedCo
G4	SC004	Breeding line	SeedCo
G5	SC005	Breeding line	SeedCo
G6	SC006	Breeding line	SeedCo
G7	SC W9101	Commercial cultivar	SeedCo
G8	SC008	Breeding line	SeedCo
G9	SC009	Breeding line	SeedCo
G10	SC010	Breeding line	SeedCo
G11	SC011	Breeding line	SeedCo
G12	SC012	Breeding line	SeedCo
G13	SC013	Breeding line	SeedCo
G14	SC014	Breeding line	SeedCo
G15	SC015	Breeding line	SeedCo
G16	SC016	Breeding line	SeedCo
G17	SC017	Breeding line	SeedCo
G18	SC018	Breeding line	SeedCo
G19	SC019	Breeding line	SeedCo
G20	SC020	Breeding line	SeedCo
G21	SC Select	Commercial cultivar	SeedCo
G22	SC Nduna	Commercial cultivar	SeedCo
G23	SC Serena	Commercial cultivar	SeedCo
G24	K215W119	Commercial cultivar	Klein Karoo
G25	Runde	Commercial cultivar	Crop Breeding Institute
G26	SC026	Breeding line	SeedCo
G27	SC027	Breeding line	SeedCo
G28	SC028	Breeding line	SeedCo
G29	SC029	Breeding line	SeedCo
G30	SC030	Breeding line	SeedCo
G31	SC031	Breeding line	SeedCo
G32	SC032	Breeding line	SeedCo
G33	SC033	Breeding line	SeedCo
G34	SC034	Breeding line	SeedCo
G35	SC035	Breeding line	SeedCo
G36	SC036	Breeding line	SeedCo
G37	SC037	Breeding line	SeedCo
G38	SC038	Breeding line	SeedCo
G39	SC039	Breeding line	SeedCo
G40	SC040	Breeding line	SeedCo
G41	SC041	Breeding line	SeedCo

Table 4.1 (cont.): List of 72 Zimbabwean wheat varieties planted in multi-environment trials (METs) in Zimbabwe during 2020 and 2021.

Genotype	Name	Status	Source
G42	SC042	Breeding line	SeedCo
G43	SC043	Breeding line	SeedCo
G44	SC044	Breeding line	SeedCo
G45	SC045	Breeding line	SeedCo
G46	SC046	Breeding line	SeedCo
G47	SC047	Breeding line	SeedCo
G48	SC048	Breeding line	SeedCo
G49	SC049	Breeding line	SeedCo
G50	SC050	Breeding line	SeedCo
G51	SC051	Breeding line	SeedCo
G52	SC052	Breeding line	SeedCo
G53	SC053	Breeding line	SeedCo
G54	SC054	Breeding line	SeedCo
G55	SC055	Breeding line	SeedCo
G56	SC056	Breeding line	SeedCo
G57	SC057	Breeding line	SeedCo
G58	SC058	Breeding line	SeedCo
G59	SC059	Breeding line	SeedCo
G60	SC060	Breeding line	SeedCo
G61	SC061	Breeding line	SeedCo
G62	SC062	Breeding line	SeedCo
G63	SC063	Breeding line	SeedCo
G64	SC064	Breeding line	SeedCo
G65	SC065	Breeding line	SeedCo
G66	SST 875	Commercial cultivar	Sensako
G67	PAN 3494	Commercial cultivar	Corteva
G68	Peregrine	Commercial cultivar	Klein Karoo
G69	PAN 3402	Commercial cultivar	Corteva
G70	SC Sicho	Commercial cultivar	SeedCo
G71	SC Shungu	Commercial cultivar	SeedCo
G72	MRI 834	Commercial cultivar	Syngenta

Table 4.2: Information on sites used during multi-environment trials (METs) in 2020 and 2021.

Site	Province	Altitude[1]	Latitude (S)[2]	Longitude (E)[2]
RARS[3]	Mashonaland East	1351	17°67'35"	31°21'24"
ART Farm[4]	Harare Metro	1521	17°70'88"	31°06'15"
SISAL Farm	Manicaland	1070	18°54'95"	32°34'67"
Panmure	Mashonaland Central	871	17°29'97"	31°61'98"

[1] Altitude: height above sea level in metres (masl) including two high yield potential (≥1200 masl) and two medium yield potential (800 to 1200 masl) localities; [2] Latitude in degrees (°), minutes (') and seconds (") south (S) and Longitude to the east (E); [3] Rattray Arnold Research Station; [4] Agriculture Research Trust Farm.

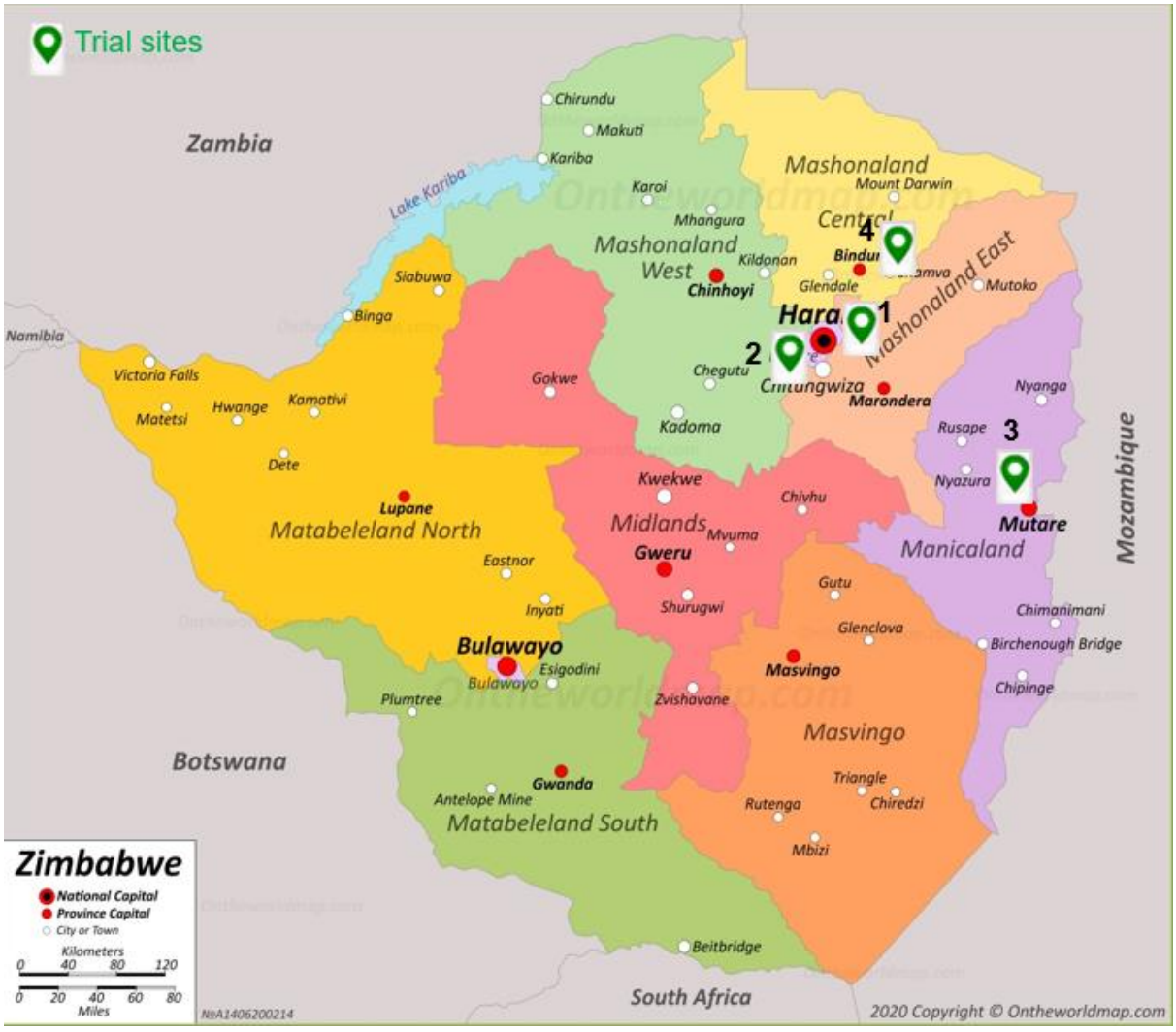


Figure 4.1: Multi-environment trial sites (MET) in Zimbabwe during the 2020 and 2021 winter seasons. 1 = RARS, 2 = ART Farm, 3 = Sisal Farm and 4 = Panmure.

4.2.3 Trial management

Trial sites underwent ploughing, discing and rolling to make a perfect seedbed. Since plots were machine planted, the fine seedbed was required to provide an equal sowing depth and ease of movement of the machinery during planting. Prior to discing, base fertiliser was dispersed using a Vicon spreader. Additionally, the discing helped the soil absorb basal fertiliser. The seeding rate was 120 kg/ha for all plots over two seasons. The objective was to achieve a plant stand of 250 to 300 plants per m^2.

In two split applications, ammonium nitrate (NH4NO3, 34.5% N) was utilised as a top-dressing fertiliser. The first spray was made four to five weeks following crop emergence, and the second application was made six to eight weeks later. Each plot received a hand-applied top dressing of fertiliser, which was immediately followed by a light (20 mm) irrigation. Weed management was done by a combination of herbicides and hand weeding where necessary. Aphids were controlled using Dimethoate where necessary, as recommended on the label, and bird scarers were engaged at all sites from grain filling up to harvesting. Overhead sprinkler irrigation was used at all sites. Table 4.3 summarises the agronomic management at all the sites.

Genotypes were planted in 10 rows 6 m in length with an inter-row spacing of 20 cm using a modified small plot tractor drawn seed drill. The initial plot sizes were 12 m^2 and were reduced four weeks after emergence to 11 m^2 (10 rows by 5.5 m by 20 cm) by removing 25 cm on either end of each plot to avoid overlaps and create 0.5 m pathways between plots. At harvesting a nett plot of 6.6 m^2 (six middle-rows by 5.5 m x 0.2 m) was harvested for each experimental unit at all sites using a Hege 125C small plot combine (Hege Company, Waldenburg, Germany). Harvested grain was collected into 5 kg harvesting bags labelled with the plot numbers and trial site name.

Table 4.3: Summary of management practices at trial sites used in Zimbabwe during the 2020 and 2021 winter seasons.

Year	Activity	RARS	ART	SISAL	Panmure
2020	Previous crop	Soybean	Soybean	Soybean	Maize
	Date of planting	15/5	4/5	6/5	8/5
	Date of harvest	8/10	19/10	14/10	6/10
	NPK (13:26:23) kg/ha	250	250	250	250
	AN (34.5:0:0) kg/ha	460	496	580	540
	Irrigation (mm)	340	396	660	428
	Pesticides	Dimethoate	Dimethoate	Dimethoate	Dimethoate
	Herbicides	MCPA+Banvel	MCPA+Banvel	MCPA+Banvel	MCPA+Banvel
2021	Previous crop	Soybean	Soybean	Soybean	Sorghum
	Date of planting	20/5	5/5	6/5	10/5
	Date of harvest	2/10	7/10	5/10	10/10
	NPK (13:26:23) kg/ha	42	36	28	21
	AN[1] (34.5:0:0) kg/ha	180	191	166	159
	Irrigation (mm)	330	396	507	549
	Pesticides	Dimethoate	Dimethoate	Dimethoate	Dimethoate
	Herbicides	MCPA+Banvel	MCPA+Banvel	MCPA+Banvel	MCPA+Banvel

[1] AN = Ammonium nitrate.

4.2.4 Experimental design

A 9x8 alpha-lattice randomised design with two replications was used in 2020 and 2021 seasons at all testing sites.

4.2.5 Data collection

Agronomic, leaf rust response, yield and quality data were collected as follows:

Days to anthesis: Number of days from planting until the net plot plants had 50% of their anthers shedding pollen.

Leaf rust scores: Disease severity (%) using the modified Cobb scale (Peterson *et al*., 1948) representing the percentage area of leaf tissue affected by leaf rust and host reaction types (Roelfs *et al*., 1992) where immune = 0, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible (Table 3.6) were recorded for each entry per plot over two replicates in all four environments.

Net plot yield: Harvested grain from each net plot was weighed using an electronic scale (DPYH Platform, Nicolas Scale, Harare, Zimbabwe) to determine the grain in kilograms.

Grain moisture (%), test weight density (kg/hl), starch content (%), wet gluten (%) and grain protein content (%, at 12.5% moisture basis): A near-infrared analysing machine NIR9500 (Labotec Pvt (Ltd), Pretoria, RSA) was used to record these variables on all samples from each plot in two replicates at the four trial sites.

The following important factors required by Zimbabwean millers and bakers were considered when interpreting the data from recording these variables. Variety SC Nduna used as the biological standard for parameters required by millers and bakers in Zimbabwe:

Grain protein content (%) at 12.5% moisture basis: minimum accepted for premium wheat is ≥11%.

Starch content (%): A trait that is normally not a primary quality trait in Zimbabwe during wheat trade, although ≥60% is considered desirable.

Wet gluten content (%): A wet gluten value of ≥25% is desirable.

Test weight density (kg/hl): The minimum acceptable test weight density is 75 kg/hl.

4.2.5.1 Derived data

The net plot yield (kg) was adjusted to yield at 12.5% moisture basis (mb, %) in tonnes per ha using the formula:

$$\text{Yield (t/ha) at 12.5\% mb} = \frac{(10*(\text{Net yield (kg)}*\%\text{ moisture @ harvesting})}{(\text{net plot } (m^2))*(100 - 12.5\%)}$$

Leaf rust coefficient of infection (LRCI) as a percentage: Consisting of the field disease severity score (%) and a constant for field host reaction where immune = 0.0, resistant = 0.2, moderately resistant = 0.4, moderately susceptible = 0.8 and susceptible = 1.

4.2.6 Data analysis

A combined MET analysis using the AMMI model in GenStat 18th edition (VSN International, Hemel Hempstead, UK) was performed on the days to anthesis (flowering), yield (kg/ha), LRCI, test weight density (kg/hl), starch content (%), wet gluten (%), and grain protein content at 12.5% moisture basis (%). Data from all four environments collected over two seasons were used. The collected and derived data (yield and LRCI) was subjected to analysis of variance (ANOVA) to determine differences between means. The AMMI analysis of variance summarises most of the magnitude of GxE into one or few interaction principal component axes (IPCA) (Zobel *et al*., 1988, Crossa, 1990). Fisher's least significant difference (LSD) at 1% and 5% probability levels were used to separate the variable means of G, E and GxE. The broad sense heritability (H^2), defined as the proportion of phenotypic variance that is attributable to an overall variance for the genotype (Schmidt *et al*., 2019), for each trait across environments was calculated from the expression:

$$H^2 = \sigma_G^2 / \left(\sigma_G^2 + \left(\frac{\sigma_{G\times E}^2}{ny} \right) + \left(\frac{\sigma_e^2}{ny \times nr} \right) \right)$$

where σ^2_G is the genotypic variance, σ^2_{GXE} is the variance for GxE, *ny* is the number of years and *nr* number of replications.

To determine the general performance of an experimental genotype in relation to the optimum response averaged over all locations, the variety superiority measure (P_i) was calculated. The variety CSI is the sum of the squares of the difference between the genotypic mean in each environment and the mean of the best genotype divided by twice the number of environments. GenStat stability coefficient macro was used to calculate the variety CSI using the formula by Lin and Binns (1988):

$$P_i = \sum^{n}_{j=1}(X_{ij} - M_j)^2/(2n)$$

Where: P_i = variety superiority measure index

n = number of environments (n = 1 …….4)

X_{ij} = yield of i^{th} genotype at environment j (i = 1….72)

M_j = maximum response among all genotypes in the j^{th} location

Genotypes with the smallest values of the superiority measure tends to be more stable, and closer to the best genotype in each environment. In addition, genotypes were ranked according to the mean performance at all the tested environments for each trait. Pearson's *r* correlation coefficient was used to test the relationship between variables.

4.3 Results

4.3.1 AMMI analysis of variance results for agronomic, quality traits and leaf rust response for 72 wheat varieties over four sites in Zimbabwe during the 2020 and 2021 growing seasons

The general management of the trials during the 2020 and 2021 growing seasons was satisfactory with weeds, insects and bird damage all under control. No problems were experienced with germination and seedling establishment and the plant stand was maintained optimally throughout the two growing seasons. No fungicides were sprayed to control fungal diseases to allow for natural occurrence and spread of *Pt* in the trials. Natural appearance of leaf rust was observed annually in trials at the onset of flowering. Figure 4.2 below shows images of the field trials.

Mean values of the tested genotypes and the range of corresponding traits across the four tested environments are presented in Table 4.3. The presence of the GxE was indicated by changes in mean rankings of genotypes for different traits over trial sites. Significant differences between genotypes were observed for all the tested traits based on the LSD values at 0.05 significance level. The varieties SC Shungu, MRI 834 and SC047 flowered in less than 85 days and varieties SC048, SC013 and SC030 flowered later than 93 days after planting. Varieties MRI 834, SC030 and SC052 had high protein content of more than 12% whereas varieties SC057, SC050 and SC054 fell short of the minimum bakers' and millers' industry requirement of 11%.

Figure 4.2: Field layout of plots at RARS (left) and Panmure (right) during the 2021 winter season.

All the varieties met the minimum industry requirements for wet gluten but varieties SC002, SC030 and MRI 834 ranked top with wet gluten content of more than 30% whereas SC027, SC054 and SC029 were below 27%. Similarly, all varieties met the minimum industry requirements for starch and test weight densities of 60% and 75 kg/hl respectively (Table 4.4). Varieties, SC026, SC027and SC059 were exceptional with more the 70% starch content and SC051, MRI 834 and SC020 were below the mean average of 68.9%. The varieties SC030, SC51 and SC063 had high test densities above 80 kg/hl whereas SC014, SC042 and SC048 were below the average of 78.96 kg/hl but above the minimum requirement of 75 kg/hl.

Genotype, E and GxE were estimated by the additive main effect and AMMI model (Table 4.4). Variance analysis of the AMMI model detected significant effects ($P \leq 0.05$) for G, E and GxE for all traits: grain protein content, starch, test weight densities, LRCI, flowering (days to anthesis) and wet gluten content. The GxE effects contributed highly to the variance observed for LRCI and wet gluten. The environment had greater effect on the variance in days to anthesis, grain protein content, starch and test weight density. Relatively higher heritability was estimated for grain protein content (0.82), starch (0.87) wet gluten (0.7) and LRCI (0.9), whereas moderate heritability was estimated for anthesis (0.61) and test weight densities (0.59).

Table 4.4: Means and ranks of agronomic and quality traits, and leaf rust response (LRCI) for 72 wheat varieties tested at four sites in Zimbabwe during the 2020 and 2021 growing seasons.

Genotype	Name	Anthesis (days)	Rank[1]	Protein %	Rank	Starch %	Rank	Test weight (kg/hl)	Rank	Wet gluten %	Rank	LRCI	Rank
G1	SC001	89.67	44	11.65	19	69.83	5	78.10	57	27.42	62	**0.06**	**3**
G2	SC002	90.61	55	12.30	4	69.83	6	77.39	68	**33.11**	**2**	1.54	39
G3	SC003	86.12	13	10.87	60	68.97	35	79.94	17	27.98	55	11.57	61
G4	SC004	90.55	54	11.56	26	68.63	53	78.73	45	29.67	19	1.34	32
G5	SC005	91.90	62	10.56	68	68.94	37	77.80	63	27.10	66	0.56	17
G6	SC006	88.29	26	10.66	67	69.34	12	79.55	21	27.32	65	2.91	44
G7	SC W9101	89.80	46	11.18	43	69.51	11	79.10	30	28.71	41	3.44	45
G8	SC008	89.58	43	11.64	22	69.76	8	79.38	25	29.66	21	0.53	16
G9	SC009	90.14	52	10.74	65	68.76	43	77.36	69	27.49	60	1.47	35
G10	SC010	90.83	56	11.04	50	69.18	21	79.41	23	28.44	47	1.49	37
G11	SC011	87.55	23	10.75	64	69.19	18	77.71	67	27.66	58	3.82	49
G12	SC012	87.03	20	11.58	25	68.71	45	79.00	33	29.56	24	1.53	38
G13	SC013	94.40	71	11.06	49	69.25	16	78.98	34	27.80	57	0.88	25
G14	SC014	91.79	61	10.89	57	69.12	24	76.47	71	28.65	44	1.20	31
G15	SC015	93.10	69	10.84	62	69.27	15	78.98	35	27.37	64	0.24	7
G16	SC016	85.93	10	11.34	35	68.97	34	79.15	29	28.89	38	0.31	12
G17	SC017	92.80	66	10.88	58	68.45	60	78.18	53	28.23	48	0.35	13
G18	SC018	89.09	36	11.32	37	68.53	56	78.09	58	29.18	34	0.24	8
G19	SC019	88.74	33	11.52	28	68.43	61	78.63	47	29.54	26	**0.00**	**1**
G20	SC020	91.41	58	11.68	17	68.00	70	78.94	37	29.79	18	1.19	30
G21	SC Select	89.56	42	11.82	13	68.34	64	77.78	65	29.96	13	3.55	46
G22	SC Nduna	90.06	51	11.56	27	68.71	46	80.08	11	29.58	23	51.67	67
G23	SC Serena	85.12	8	10.98	53	68.99	33	78.55	50	28.03	52	1.47	36
G24	K215W119	86.92	19	11.24	40	69.04	31	78.73	46	28.87	39	44.43	65
G25	Runde	88.85	35	10.92	56	69.18	19	78.11	56	27.95	56	69.97	72
G26	SC026	91.44	59	11.51	29	**71.46**	**1**	80.61	5	29.14	36	7.93	58
G27	SC027	92.12	63	11.33	36	**70.03**	**3**	78.38	51	26.60	70	0.27	10

Table 4.4 (cont.): Means and ranks of agronomic and quality traits, and leaf rust response (LRCI) for 72 wheat varieties tested at four sites in Zimbabwe during the 2020 and 2021 growing seasons.

Genotype	Name	Anthesis (days)	Rank[1]	Protein %	Rank	Starch %	Rank	Test weight (kg/hl)	Rank	Wet gluten %	Rank	LRCI	Rank
G28	SC028	89.30	39	11.30	39	69.10	29	79.60	20	28.70	43	0.59	18
G29	SC029	87.30	22	11.10	46	69.80	4	78.90	40	26.10	72	0.27	11
G30	SC030	93.50	70	**12.60**	**2**	68.30	65	**81.30**	**1**	**31.90**	**3**	6.50	54
G31	SC031	89.90	50	11.10	47	68.70	48	78.60	48	28.50	46	0.11	5
G32	SC032	92.70	65	11.80	12	68.30	66	79.00	32	29.20	35	0.64	20
G33	SC033	91.50	60	11.00	51	69.20	22	80.40	8	28.10	50	0.26	9
G34	SC034	86.00	12	11.90	10	68.50	58	77.90	61	30.00	14	1.01	29
G35	SC035	89.40	40	11.50	32	68.80	39	79.30	26	29.30	31	0.08	4
G36	SC036	87.90	24	11.90	9	68.60	54	78.30	52	30.10	9	1.42	34
G37	SC037	88.40	28	11.10	48	68.80	42	78.60	49	29.60	22	0.38	14
G38	SC038	92.40	64	11.60	20	68.40	62	78.20	54	29.80	16	6.83	56
G39	SC039	91.00	57	11.50	30	68.30	68	78.90	38	29.30	33	7.84	57
G40	SC040	89.10	37	12.10	5	69.00	36	80.10	9	31.10	5	0.71	22
G41	SC041	88.60	32	10.90	55	69.10	27	78.90	41	28.10	51	0.83	24
G42	SC042	85.10	7	11.00	52	69.30	14	77.10	70	28.20	49	2.66	43
G43	SC043	88.40	29	10.90	61	69.20	17	79.50	22	28.00	53	0.91	27
G44	SC044	89.80	49	10.70	66	68.80	40	79.10	31	27.50	61	1.70	41
G45	SC045	92.90	68	12.00	6	68.40	63	77.80	62	29.30	30	0.64	21
G46	SC046	88.40	27	11.80	14	69.00	30	77.70	66	30.00	11	3.78	47
G47	SC047	**84.10**	**3**	11.60	23	69.00	32	80.50	7	29.50	27	11.23	59
G48	SC048	97.50	72	11.50	31	69.20	23	75.50	72	27.40	63	6.80	55
G49	SC049	89.80	48	11.20	42	69.80	7	78.00	60	28.70	42	4.78	52
G50	SC050	86.40	15	10.50	70	68.70	50	78.10	55	26.80	68	3.80	48
G51	SC051	90.40	53	11.80	11	67.70	71	**80.90**	**2**	30.20	8	13.17	62
G52	SC052	89.50	41	**12.40**	**3**	68.70	44	80.10	10	31.70	4	1.78	42
G53	SC053	84.90	6	11.20	41	69.60	10	79.40	24	30.40	6	5.03	53
G54	SC054	86.70	17	10.30	72	69.70	9	77.80	64	26.60	71	0.23	6

Table 4.4 (cont.): Means and ranks of agronomic and quality traits, and leaf rust response (LRCI) for 72 wheat varieties tested at four sites in Zimbabwe during the 2020 and 2021 growing seasons.

Genotype	Name	Anthesis (days)	Rank[1]	Protein %	Rank	Starch %	Rank	Test weight (kg/hl)	Rank	Wet gluten %	Rank	LRCI	Rank
G55	SC055	89.80	47	10.50	69	68.70	49	79.30	27	26.90	67	0.62	19
G56	SC056	85.90	9	11.70	18	69.10	26	79.60	19	29.80	17	11.23	60
G57	SC057	86.00	11	10.40	71	68.50	59	78.70	44	26.70	69	**0.01**	**2**
G58	SC058	92.90	67	11.70	16	69.10	28	80.00	15	29.90	15	0.40	15
G59	SC059	86.50	16	11.30	38	**70.00**	**2**	78.80	43	28.90	37	4.14	50
G60	SC060	87.20	21	10.80	63	69.10	25	79.80	18	27.50	59	1.61	40
G61	SC061	88.00	25	10.90	59	68.90	38	79.20	28	28.00	54	0.79	23
G62	SC062	86.20	14	11.50	33	68.30	67	80.10	12	29.40	29	0.89	26
G63	SC063	88.50	30	11.60	24	68.60	55	**80.80**	**3**	29.50	25	0.94	28
G64	SC064	86.90	18	11.90	8	68.70	51	80.00	14	30.00	12	4.57	51
G65	SC065	89.80	45	11.60	21	69.30	13	78.80	42	29.70	20	1.40	33
G66	SST 875	84.20	4	11.20	45	68.80	41	80.70	4	28.60	45	62.52	69
G67	PAN 3494	89.30	38	11.50	34	68.70	47	78.90	39	29.30	32	44.16	64
G68	Peregrine	84.50	5	11.20	44	68.50	57	78.00	59	28.80	40	69.97	71
G69	PAN 3402	88.80	34	11.80	15	68.60	52	80.60	6	30.10	10	47.71	66
G70	SC Sicho	88.50	31	11.90	7	68.20	69	80.00	13	30.20	7	13.87	63
G71	SC Shungu	**81.10**	**1**	11.00	54	69.20	20	79.90	16	29.50	28	57.80	68
G72	MRI 834	**81.70**	**2**	**13.70**	**1**	66.90	72	79.00	36	**35.10**	**1**	67.46	70
Minimum		81.70		10.30		66.90		75.47		26.12		0.00	
Maximum		97.50		13.70		71.50		81.33		35.10		69.97	
Mean		88.89		11.29		68.94		78.96		29.94		9.15	
$LSD_{0.05}$		2.73		0.88		1.09		1.20		3.13		8.39	

[1]Mean performance and ranking of a genotype across environments.

Table 4.5: AMMI analysis of variance for agronomic and quality traits, and leaf rust response (LRCI) for 72 wheat varieties tested at four sites in Zimbabwe during 2020 and 2021 growing seasons.

Trait	Mean square			Variance %				
	G	E	GxE	G	E	GxE	CV%	H^2
LRCI	1272.2***	273.0***	38.20***	33.33	7.15	46.68	81.72	0.90
Anthesis	34.0***	1033.6***	3.90***	8.61	261.78	11.46	4.19	0.61
Protein	1.42***	16.95***	0.41***	3.45	41.18	7.19	5.46	0.82
Starch	1.52***	4.16***	0.62***	2.46	6.71	0.85	0.91	0.87
Test density	4.73***	77.57***	0.77***	6.16	101.04	1.76	1.96	0.59
Wet Gluten	292.9***	241.1***	103.8***	2.82	2.32	18.96	36.61	0.70

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; LRCI = Leaf rust coefficient index; G = Genotype; E = Environment; GxE = Genotype-by-environment; CV% = coefficient of variation; H^2 = Broad sense heritability.

4.3.2 AMMI analysis of variance for grain yield performance of 72 wheat varieties tested at four sites in Zimbabwe during 2020 and 2021

Variance analysis of the AMMI model for grain yield detected significant G, E and GxE interaction effects. The E had a significant effect ($P < 0.001$) accounting for 65.91%, followed by variability among G with 27.52% and GxE interaction with 6.57% (Table 4.6). Results from the AMMI analysis of variance of the 72 Zimbabwean wheat varieties revealed that the mean sum of square (ss) of the first IPCA 1 axis was highly significant ($P < 0.001$) and accounted for 55.44% of the total interaction effects in the model. Following, the IPCA 2 explained 32.08% of the interaction effects. The first two IPCA axes best explained the interaction sum of squares (Zobel *et al*., 1988), while the residual of 12.48% is considered as noise.

The variety superiority measure was computed to determine stable and superior varieties (Table 4.7). With the CSI method, a genotype with the lowest CSI indicates the most stable and superior variety. Accordingly, the top five stable varieties across the four tested environments were SC001, SC027, SC002, SC004 and SC W9101. Varieties PAN 3402, PAN 3494, SC Shungu, Runde and MRI 834 were the most unstable. Variety SC001 was the highest yielding variety and MRI 834 was ranked lowest in grain yield.

Table 4.6: ANOVA table for AMMI model of grain yield at four sites over two seasons.

Source	Df	Ss	MS	Explained %
Genotypes	71	99.93	1.41***	27.52
Environments	7	239.35	79.78***	65.91
Interactions (GxE)	213	23.88	0.11***	6.57
IPCA 1	73	13.24	0.18***	55.44
IPCA 2	71	7.66	0.11***	32.08
Residuals	69	2.98	0.04ns	12.48

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns = not significant; df = degrees of freedom; SS = sum of square; ms = mean square.

Table 4.7: Means of grain yield and variety superiority index (CSI) value for 72 wheat varieties tested at four sites in Zimbabwe during the 2020 and 2021 growing seasons.

Genotype	Name	Grain yield (ton/ha)	Rank[1]	CSI
G1	SC001	**9.15**	**1**	0.06
G27	SC027	**9.11**	**2**	0.03
G2	SC002	**9.00**	**3**	0.10
G4	SC004	**8.99**	**4**	0.11
G7	SC W9101	**8.98**	**5**	0.11
G43	SC043	**8.90**	**6**	0.13
G49	SC049	**8.87**	**7**	0.13
G23	SC Serena	**8.87**	**8**	0.13
G16	SC016	**8.83**	**9**	0.16
G10	SC010	**8.78**	**10**	0.27
G13	SC013	**8.76**	**11**	0.19
G41	SC041	**8.73**	**12**	0.24
G21	SC Select	**8.73**	**13**	0.22
G35	SC035	**8.72**	**14**	0.20
G9	SC009	8.69	15	0.21
G3	SC003	8.69	16	0.31
G65	SC065	8.68	17	0.24
G44	SC044	8.64	18	0.27
G29	SC029	8.60	19	0.41
G60	SC060	8.55	20	0.48
G12	SC012	8.54	21	0.32
G17	SC017	8.51	22	0.38
G47	SC047	8.50	23	0.43
G57	SC057	8.48	24	0.43
G64	SC064	8.47	25	0.54
G20	SC020	8.45	26	0.40
G54	SC054	8.41	27	0.47
G14	SC014	8.39	28	0.51
G50	SC050	8.38	29	0.50
G61	SC061	8.38	30	0.49
G55	SC055	8.35	31	0.50
G31	SC031	8.35	32	0.55
G59	SC059	8.33	33	0.54
G46	SC046	8.32	34	0.55
G15	SC015	8.30	35	0.57
G26	SC026	8.29	36	0.56
G19	SC019	8.28	37	0.60
G5	SC005	8.28	38	0.57
G18	SC018	8.27	39	0.64
G38	SC038	8.27	40	0.59
G32	SC032	8.26	41	0.59
G45	SC045	8.23	42	0.68
G58	SC058	8.23	43	0.65

Table 4.7 (cont.): Means of grain yield and variety superiority index (CSI) value for 72 wheat varieties tested at four sites in Zimbabwe during the 2020 and 2021 growing seasons.

Genotype	Name	Grain yield (ton/ha)	Rank[1]	CSI
G62	SC062	8.22	44	0.65
G37	SC037	8.22	45	0.68
G28	SC028	8.18	46	0.68
G8	SC008	8.15	47	0.74
G33	SC033	8.13	48	0.76
G36	SC036	8.09	49	0.81
G39	SC039	8.06	50	0.84
G22	SC Nduna	8.01	51	0.92
G63	SC063	8.00	52	1.05
G30	SC030	7.95	53	0.97
G11	SC011	7.93	54	1.00
G34	SC034	7.91	55	1.06
G42	SC042	7.87	56	1.09
G53	SC053	7.81	57	1.21
G24	K215W119	7.81	58	1.24
G6	SC006	7.74	59	1.32
G40	SC040	7.74	60	1.28
G51	SC051	7.62	61	1.49
G68	Peregrine	7.56	62	1.57
G70	SC Sicho	7.51	63	1.70
G48	SC048	7.35	64	1.98
G56	SC056	7.31	65	2.11
G66	SST 875	7.16	66	2.41
G52	SC052	7.10	67	2.52
G69	PAN 3402	7.06	68	2.76
G67	PAN 3494	7.00	69	2.75
G71	SC Shungu	6.95	70	2.92
G25	Runde	6.91	71	3.04
G72	MRI 834	6.20	72	4.96
Minimum		6.20		
Maximum		9.15		
Mean		8.20		
LSD		0.44		
Heritability		0.75		
CV%		8.02		
P Value		0.00***		

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns = not significant; [1] Mean performance ranking of a genotype across environments.

4.3.3 GGE-biplot analysis for grain yield

The GGE-biplots were used to analyse ME among the test environments, to evaluate both the test environments and genotypes. The GGE-biplot analysis for four test environments (RARS, ART Farm, Sisal Farm and Panmure) is shown in Figure 4.3. The biplot explained 93.73% of the total variation observed, of which 83.43% was explained by the first principal component (PC1), while the second principal component explained 10.31%.

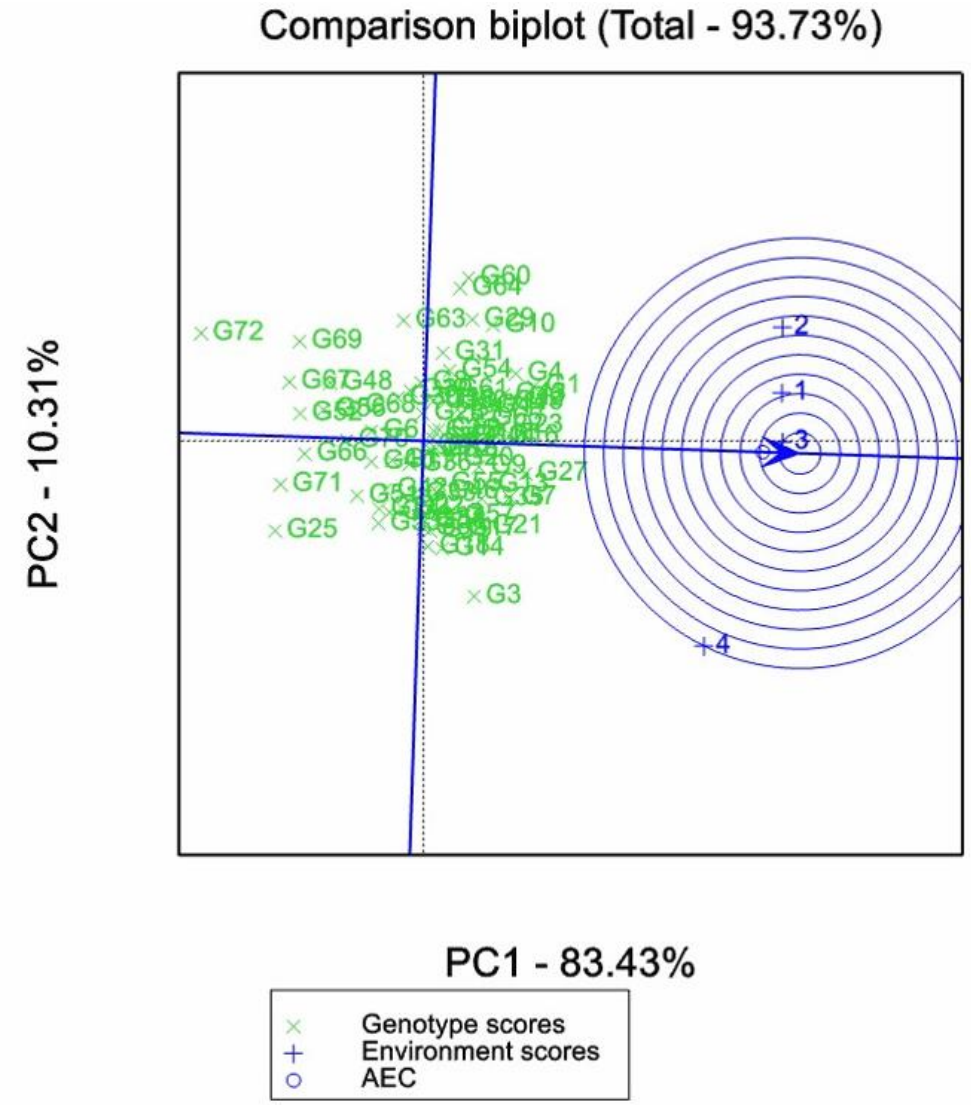


Figure 4.3: The genotype-by-environment interaction (GGE) genotype comparison biplot showing the mean *versus* stability performance of 72 Zimbabwean wheat varieties tested at four sites.

4.3.4 Mega-environment analysis (Which-won-where model)

To prominently display the yield performance of varieties across different environments, the 'which-won-where' model uses polygon view to highlight the interactions. The scatter plot produced six sectors whereas all four test environments were distributed into two sectors in two ME (Figure 4.3). The polygon view showed that the panel of 72 test varieties were distributed across all the sectors. This GxE indicates that the test environments could be delineated into distinct ME (Yan *et al*., 2007). The GGE-biplots demarcated environments into two major ME namely, ME1 and ME2. ME1 consisted of three environments with similar characteristics: environment 1 (RARS), environment 2 (ART Farm) and environment 3 (Sisal Farm) covering sector 2 and sector 3 while ME2 consisted of environment 4 (Panmure) covering only sector 1. Panmure was shown to be a unique environment further away from the other three. According to Figure 4.3, genotypes G1, G4 and G27 were the highest yielding genotypes in ME1. In ME2 genotypes G3 and G21 were the winning genotypes. Genotypes G72, G69, G67, G71, G25 and G66 fell in sectors with no defined environmental conditions, indicating poor adaptation to the ME.

4.3.5 Comparison of genotypes to the "ideal genotype"

The average environment coordination (AEC) is the average of the first and second PC values test conditions. It is represented by the line with a single arrowhead passing through the origin in Figure 4.4 and the perpendicular to AEC is considered as ordinate. The yield performance of genotypes is determined by the length of the abscissa where above average is on the right and below average on the left. Greater absolute length of the ordinate of a genotype indicates lower stability with more variability and shorter length indicates higher stability and less variability. Figure 4.4 shows that genotypes G1, G2, G4, G27, G7, G21 and G23 were the most stable with above average yield, whereas G3, G60 and G64 were above average yielders but with lower stability. Genotypes; G72, G69, G67, G66, G71, and G25 were both below average yield and less stable.

4.3.6 Comparison of genotypes to the "ideal environment"

The comparison indicated that environment 2 (ART Farm) was the closest test environment to the ideal followed by environment 1 (RARS) and environment 3 (Sisal Farm) as indicated by the AEC view that shows the ideal environment. Environment 4 (Panmure) was the furthest from the ideal environment among the four tested environments (Figure 4.5).

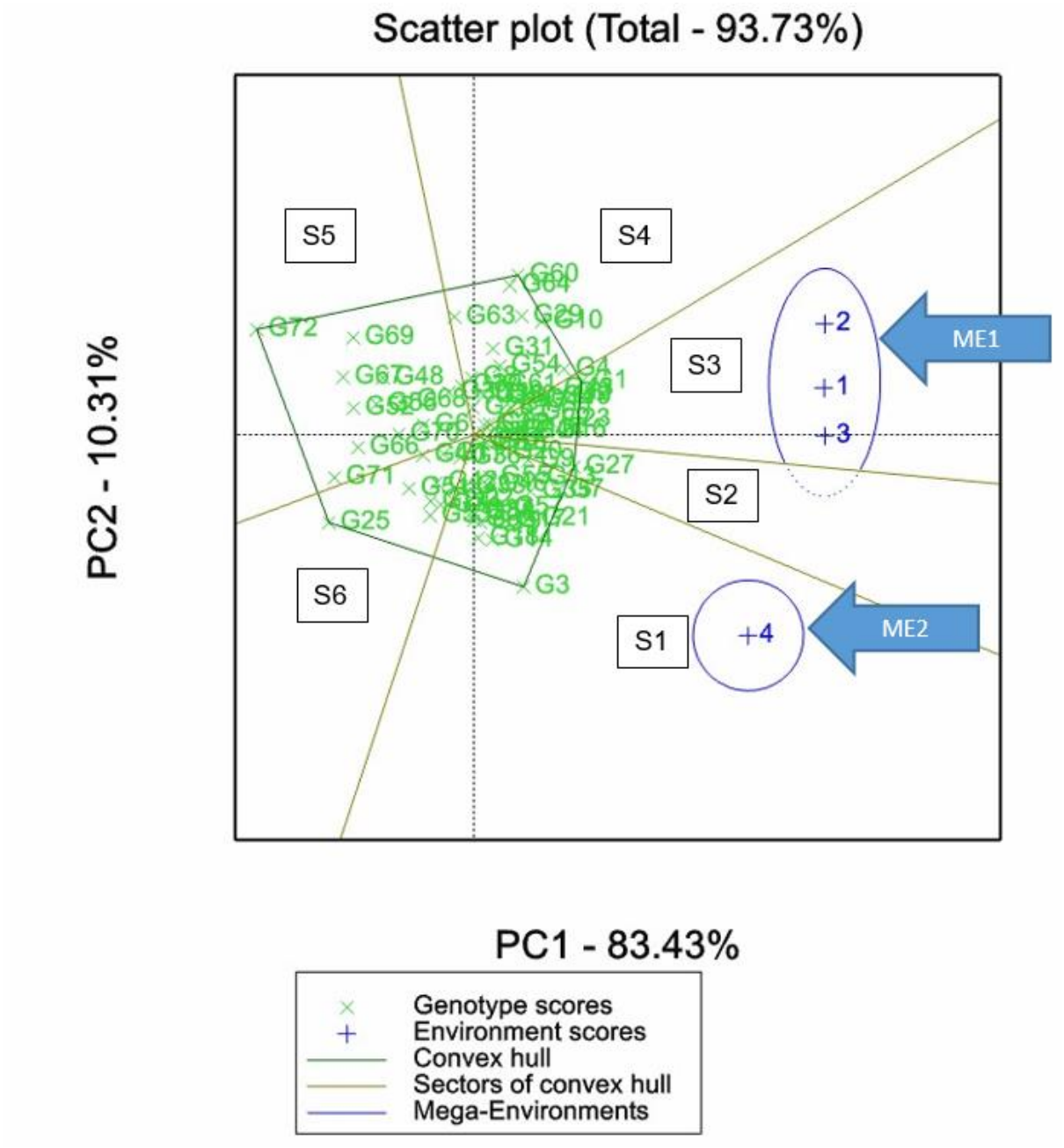


Figure 4.4: Which-won-where model of genotypic effect and genotype-by-environment interaction (GxE) biplot based on grain yield of 72 Zimbabwean wheat varieties tested at four environments in Zimbabwe: RARS, ART Farm, Sisal Farm and Panmure over two seasons.

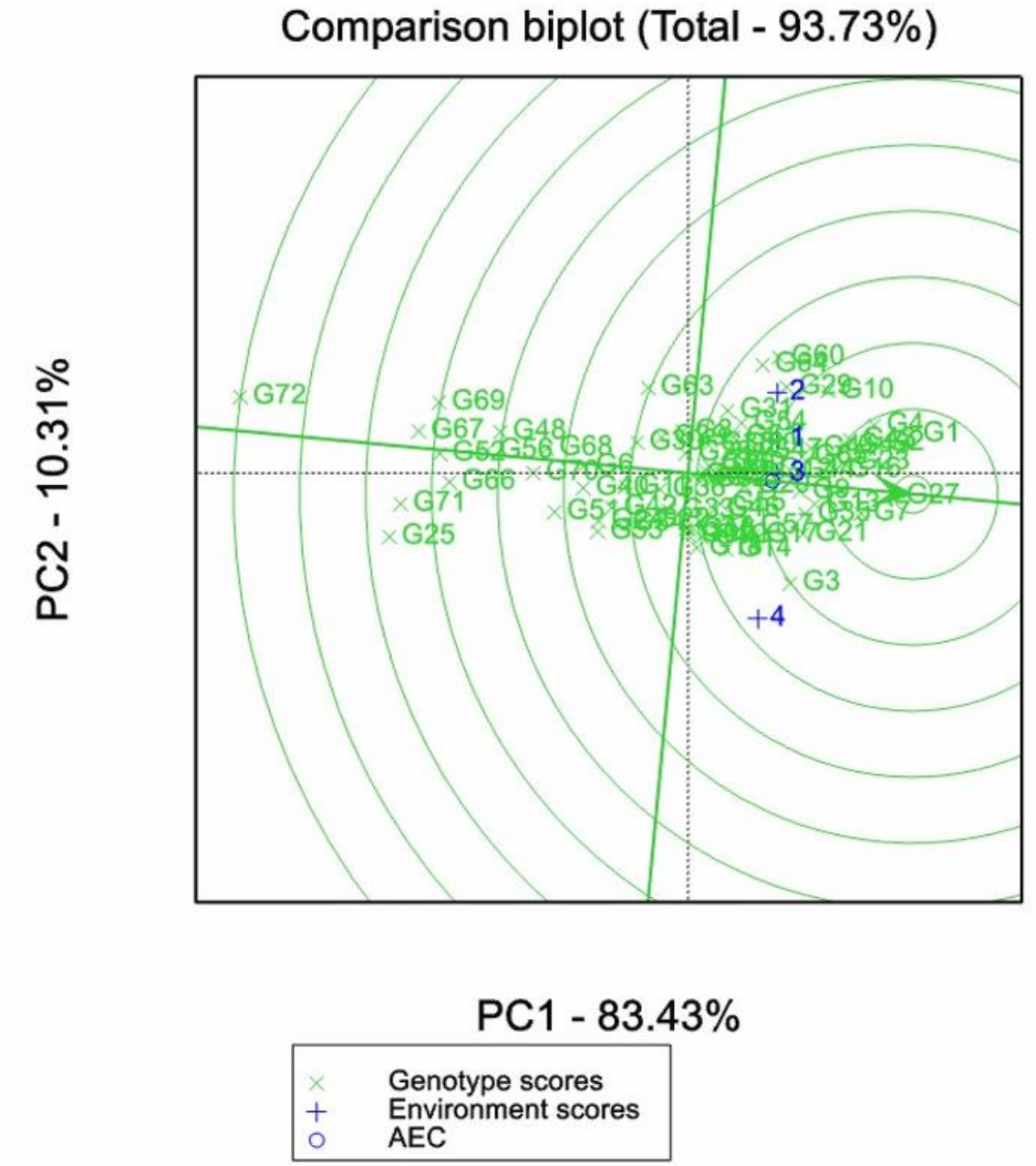
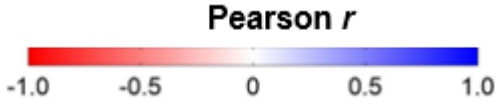


Figure 4.5: The genotype and genotype-by-environment interaction (GGE) biplot for 72 Zimbabwean wheat varieties tested at four trial sites.

4.3.7 Pearson's *r* correlation coefficients among tested agronomic traits

Pearson's *r* correlation coefficients among agronomic- and quality traits were studied. Results revealed that protein content and wet gluten showed a strong positive (0.86) and significant ($P \leq 0.001$) correlation (Figure 4.6). Furthermore, small but significant positive correlations were observed between protein content and days to anthesis (0.22; $P \leq 0.001$); grain yield and starch (0.15; $P \leq 0.05$); grain yield and days to anthesis (0.22; $P \leq 0.001$) and wet gluten and leaf rust coefficient index (0.13; $P \leq 0.05$). Strong negative correlations ($P \leq 0.001$), were revealed between protein content and starch (-0.85) and between wet gluten and starch (-0.82). Analysis further revealed moderate but significantly negative correlations ($P \leq 0.001$), between leaf rust response and grain yield (-0.43) as well as days to anthesis (-0.34). Small negative correlations were detected between yield and test weight density (-0.16; $P \leq 0.01$) and grain yield and wet gluten (-0.18; $P \leq 0.01$). Protein content was negatively correlated to yield (-0.10) although not significant ($P > 0.05$). Starch content showed a small but significant positive correlation with grain yield (0.15; $P \leq 0.05$). However, there was no significant effect of the variety responses between leaf rust response and protein content (0.05) or starch content (-0.06) and test weight densities (0.09). The small but significant positive correlation revealed between leaf rust response and wet gluten, indicate that varieties with higher LRCI values had higher gluten content.

Protein %	1	-						
Starch %	2	-0.85***	-					
Grain yield	3	-0.10ns	0.15**	-				
Days to anthesis	4	0.22***	-0.13*	0.22***	-			
Leaf rust response (LRCI)	5	0.05ns	-0.06ns	-0.43***	-0.34***	-		
Test weight density	6	0.04ns	0.00ns	-0.16**	0.00ns	-0.09ns	-	
Wet gluten content	7	0.86***	-0.82***	-0.18**	0.01ns	0.13**	0.09ns	-
		1	2	3	4	5	6	7



*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns = not significant.

Figure 4.6: Pearson's *r* correlation coefficients among different agronomic and quality traits of 72 Zimbabwean wheat varieties across four environments.

4.4 Discussion

Genotype, E and GxE significantly affected all the traits analysed. In this study these effects were demonstrated by a change in genotype ranking across the trial sites for all seven traits. Grain yield, days to anthesis, protein content, test weight densities and starch were affected more intensely by E than G and GxE. LRCI and wet gluten content depended more on genotypic variation. From the analysis the GxE effects, although remaining significant, were lower than the effects of G and E, for all the traits. This is supported by similar findings from a study by Mladenov *et al*. (2001). Heritability for the traits analysed varied from moderate to high, ranging from 0.59 to 0.9. This implies that more than half of the differences observed for LRCI, days to anthesis, protein and starch content, test weight density and wet gluten content are from genetic factors.

Heritability data assists plant breeders to predict the behaviour of the succeeding generation and increase the chance of making desirable selections. The higher the heritability for a trait, the more straightforward the selection process and the greater the response to selection. Heritability estimates presented in this study will enable SeedCo breeders to make sound predictions about the possible progress that can be achieved from future crosses as the moderate to high heritability for all the analysed traits suggests that effective and proper selection for these traits is possible. These results agree with the findings reported by Chowdhry *et al*. (1997) and Khan *et al*. (2003).

In Zimbabwe, an ideal bread wheat variety (typical ideotype that breeders strive to develop) is defined as one with high grain yield, disease resistance, wide adaptation across environments while maintaining high end-use quality attributes required by millers, bakers and consumers. Grain yield is a quantitative trait which significantly interacts with the environment. Hence, the selection of the best performing varieties with wider adaptability is a cumbersome task. GGE-biplot analysis assists in selecting the best and most stable performing genotypes in MET (Yan and Tinker, 2006). Previous studies also used GGE-biplots to characterise different water regimes treated as environments and identified most stable and high water use efficient wheat genotypes (Meena *et al*., 2019).

Based on the objectives of this study, three different biplot views of GGE analysis were used to assess the stability of 72 Zimbabwean wheat varieties for grain yield and select responsive varieties for leaf rust resistance while meeting the required end-use quality characteristics. The results showed that GGE analysis efficiently explained 93.73% of the total variation to plot the most responsive varieties for grain yield for each tested environment under natural disease development resulting from *Pt* infection. Yang *et al*. (2009) stated that more than 60%

total variance (PC1+PC2) is required for GGE analysis to be useful in assessing conditions. For effective interpretation of a GGE-biplot, the 'which-won-where' model also termed as polygon view, is quite handy as it depicts interaction patterns between environments and genotypes (Yan and Kang, 2003). The model explains the existence of cross-over and non-cross-over GxE interactions by graphically representing ME differentiation and specific genotype adaptation resulting in rank changes of genotypes (Rakshit *et al*., 2014; Oral *et al*., 2018).

This view, in combination with the mean genotype ranking and CSI, clearly demonstrated that the top five high yielding and stable varieties are SC001, SC002, SC004, SC0027 and SC W9101. In addition, these varieties also met the end-use attributes of grain protein of ≥11%, starch content of 60%, test weight density of ≥75 kg/hl, wet gluten content of ≥25%, medium to late in days to flowering and were resistant considering their leaf rust responses. Varieties SC041 and SC043 were also high yielding and stable across environments but with their grain protein content below the required 11%, cannot be selected as ideal varieties. MRI 834 was the top variety considering its excellent grain protein and wet gluten content but had the lowest grain yield and showed a high LRCI value to leaf rust and could therefore not be selected as an ideal variety for all stakeholders in the bread wheat value chain of Zimbabwe.

The GGE scatter plot grouped the four environments into two MEs where ART Farm (environment 2) had the highest mean grain yield and Panmure (environment 4) the lowest. This environment analysis presents an opportunity to reduce the number of testing environments when several environments produce the same information as shown by similar characteristics for ART Farm, RARS and Sisal Farm. In this case, ART Farm can represent ME 1 and Panmure can represent ME 2. Results from this study have shown that higher yields were recorded in environments at high altitudes (≥1200 masl); ART Farm and RARS and lower yields were recorded at Panmure lying at a lower altitude (871 masl). Similar findings were observed by Easterling *et al*. (2007). Panmure being a lower altitude site also has higher temperatures which shortens the wheat growth period by accelerating flowering development, resulting in reduced yield (You *et al*., 2005; Asseng *et al*., 2011). By plotting the multiple simulation studies from low-latitude areas, Easterling *et al*. (2007) reported a linear relationship between an increase in temperature and the reduction in wheat yield with a 7% decrease for a 2°C rise in temperature.

Susceptibility to diseases like leaf rust induces cell tissue death, early senescence, reduced water use efficiency and photosynthetic rate, thereby causing yield loss due to shrivelled seeds (Simón *et al*., 2021). The moderate but significant negative correlation between yield

and LRCI was therefore expected. However, this was accompanied by non-significant correlations between LRCI and protein and starch content, test weight density as well as wet gluten content. This can be explained by 51 of the 72 varieties with low LRCI values (≤5) indicating strong resistance responses. Only ten of the 72 varieties had LRCI values between 42 and 70 indicating moderate susceptibility to *Pt*. From these five varieties namely SC Shungu, MRI 834, SST 875, Peregrine and SC042 were also early flowering with low mean yield performances. A significant negative correlation was also observed between LRCI and days to anthesis. These results for both *Pt* and earliness contrast with findings by Msundi *et al*. (2021) who observed that earliness is desirable as an escape strategy to biotic and abiotic stresses. In support of the performance of longer growers in our study it has been reported that late maturity is associated with more accumulation of dry matter due to prolonged growth period, which translates into high grain yield (Asseng *et al*., 2011). High yielding varieties in this study were mostly medium to late flowering, which explains the small but significant positive correlation between anthesis and yield performance.

Previous studies have shown that moisture stress increases grain protein and wet gluten content, because less starch is formed (Schierenbeck *et al*., 2016). However, genotypes planted in this study had adequate moisture at all growth stages from irrigation meaning more starch was formed therefore leading to a small but significant positive correlation that was observed between starch and yield. It was therefore not surprising to observe a strong and significant negative correlation between starch with both protein and wet gluten content. Similarly, a small but significant negative correlation was observed between yield and wet gluten content although the similar small negative correlation between yield and protein content was not significant. Kaya and Akcura (2014) obtained similar results and noted that the negative relationship between grain yield and protein content is highly undesirable for the development of varieties eligible for marketing grades. Negative correlations between yield and test weight density are often influenced by stress factors occurring during the grain-filling period resulting in lower hectolitre mass. These stressors could include drought, excessive soil moisture, a shortage of nutrients, too little sunlight and too low or too high temperatures (Johansson, 2002).

The results showed a significant positive correlation between wet gluten and grain protein content. Similarly, Sourour *et al.* (2018) determined a positive correlation between the two traits and recommended that researchers consider these components when developing new varieties. This positive association was expected since the influence of protein content on wet gluten is well known. Wet gluten content is significantly influenced by protein content, and the magnitude of the effect varies according to the genotype (Cubadda *et al*., 2007). Flaete and

Uhlen (2003) also reported that the *Glu-B3* allele that plays a key role in gluten strength positively affects protein and wet gluten content. This indicates that MAS for *Glu-B3* can be useful in selecting genotypes with desirable protein and wet gluten content.

In this study we observed that the early flowering variety MRI 834 with moderate susceptibility to *Pt* in combination with a low yield had higher protein and wet gluten content. The small but significant positive correlation between LRCI with both gluten and protein content cannot be attributed to *Pt* infection only. Early flowering accompanied by low yield also appear to be contributing factors, especially considering that most genotypes in the current study were resistant to leaf rust. Leaf rust disease resulted in lower yield and test weight densities, as low-test weight densities result in lower endosperm to bran ratio (Dimmock and Gooding, 2002). Lower endosperm to bran ratio results in a drop in starch content hence higher concentration of protein in bran than endosperm (Schierenbeck *et al*., 2016).

Therefore, it is expected that a leaf rust susceptible variety would have higher protein concentration from bran but with low yield due to shrivelled grain affected by the disease. This could aid in explaining the high protein content observed in MRI 834 which is moderately susceptible, early flowering and low yielding. However, the same cannot be derived for some other varieties moderately susceptible to leaf rust such as SC Shungu, Peregrine, SC Nduna, PAN 3494, SST 875, PAN 3402, Runde and K215W119 expected to have lower test weight densities and higher protein content similar to MRI 834. The reasons might be that the disease came in or peaked late during grain filling with minimum effect on the test weight densities, protein and starch content. Furthermore, irrigation allowing for sufficient moisture also limited the stress caused by late disease infection.

A shortcoming of the current study is that a leaf rust check variety like PAN 3497 or SC Shine was not included in the trials. This would have highlighted that, although some varieties recorded higher LRCI values, they carry known or unknown slow rusting genes that delayed disease development reducing the negative impacts on quality traits.

Grain quality is important as it defines the end-use of wheat and contributes to maximise profit across the wheat value chain. It adds value to the rest of the breeding activities as it is a key set of characteristics for the commercialisation and trading of wheat. Grain quality should form an integral part of the breeding process and be considered within the variety development process. In breeding programmes, end-use quality phenotyping is laborious, expensive, time-consuming and can require a large amount of grain. Consequently, selection for end-use quality is often delayed until the later breeding stages (Kiszonas *et al*., 2013; Jernigan *et al*., 2018).

Genomic selection (GS) is a promising tool for the prediction of wheat quality (Battenfield *et al*., 2016). Using GS, wheat quality traits could be predicted with an accuracy ranging from ±60% for traits like gluten strength, to ±40% for traits highly influenced by the environment such as protein content and dough extensibility. In contrast to single-locus molecular markers, GS can capture the genetic complexity of the different quality traits at once, thus making the selection process more efficient and accurate. Another benefit of GS is it enables a pre-selection of high performing lines in a much broader population 2-3 years before conducting costly industrial tests, thereby promoting the selection of lines that combine desirable quality characteristics and grain yield (Sallam and Smith, 2016). It is thus a recommended method for adoption by Zimbabwean researchers in future.

In conclusion, this study showed that grain yield and quality traits were determined mainly by E effects followed by G influence. Genotype-by-environment interactions had less effect on the studied traits. The observed negative association between high yield and some quality traits should be an important target for future breeding efforts in Zimbabwe. From the trial results over two seasons, the identified ideotype varieties SC001, SC002, SC004, SC0027 and the recently commercialised variety SC W9101 should form the basis of the future wheat crop in Zimbabwe, including desirable traits for the whole value chain. However, the downside of these varieties is that only SC002, SC004 and SC027 are predicted to carry one effective APR gene, *Lr46*, against the Zimbabwean *Pt* race MCDS (Chapter 3). Furthermore, although showing high yield performance in field trials, variety SC001 is not a good choice for release as it carries no known APR genes. Therefore, this indicates that the Zimbabwean scenario can be considered vulnerable especially if the ASR genes, these top performing varieties carry, becomes ineffective to the current or new races of *Pt*. Therefore, there is a need to introgress a combination of effective ASR and APR genes into new wheat varieties.

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Chapter 5: Conclusions and Recommendations

Studies on the genetic diversity, distribution and relatedness of *Pt* race isolates present in Zimbabwe with SA races have not been carried out comprehensively before. Furthermore, there is a lack of adequate information on the identity and number of *Lr* genes present in the current commercial wheat varieties and advanced breeding lines of the SeedCo wheat breeding programme. The Zimbabwean wheat breeding programmes have relied mainly on conventional breeding. In this method, disease screening is based on phenotypic responses from natural infection, which is not always reliable, costly and time-consuming. The avirulence/virulence profiles of rust pathogens dominating in particular seasons have not been available to breeders. Furthermore, breeding methods like backcrossing and gene stacking are almost impossible without applying closely linked molecular markers to track the presence of the genes of interest. Above all, in addition to achieving good disease resistance in a particular variety, the variety needs to be widely adapted with stable yields across environments whilst maintaining high end-use quality attributes required by millers, bakers, retailers and consumers.

The purpose of this study was to address the following knowledge gaps:

- To characterise *Pt* isolates from Zimbabwe collected between 2019 and 2021 for their avirulence/virulence and genotypic profiles compared to selected race isolates identified in SA.
- To characterise selected Zimbabwean commercial wheat varieties and SeedCo advanced breeding lines for their seedling ITs and field response to the dominant *Pt* race(s) found in Zimbabwe and selected South African races.
- To determine the number of effective resistance genes based on the F_2 segregation ratios from crosses made between selected leaf rust resistant lines and a susceptible wheat variety.
- To detect ASR and APR *Lr* genes present in selected Zimbabwean wheat varieties using molecular markers.
- To identify promising experimental varieties that meet the ideotype variety criteria by all stakeholders in the wheat value chain in Zimbabwe when evaluated for *Pt* response, quality traits, yield and stability over MET for two seasons.

Both greenhouse phenotypic studies and genotypic analysis revealed that the Zimbabwean *Pt* population is currently dominated by isolates that typed to race MCDS. However, the genotypic profiling indicated a low frequency of distinct *Pt* isolates from Zimbabwe that typed to race MCDS with genetic similarity to South African races SDDN and SCDS.

The microsatellite analysis also revealed close genetic relationship between isolates of *Pt* race MCDS and isolates of the South African races MCDS, MCPS and MFPS. A recent *Pt* race survey carried out in SA between 2017 and 2020, revealed that race MCDS was the most common with an average incidence of 18% among isolates. Findings of this study demonstrated a close genetic similarity between *Pt* isolates from Zimbabwe and SA and supports previous studies suggesting the movement of urediniospores between wheat fields in the region. The detection of five genetically distinct *Pt* isolates among the Zimbabwean isolates (19_1_2019, 24_3_2019, 5_1_2020, 20_1_2020, 23_2_2020) indicates genetic variations that could have arisen due to either asexual recombination, or through new foreign introductions.

The wheat leaf rust population in Zimbabwe can therefore be described as clonal, stable and small considering that only four races have been described for the period 1986 to 2021. This can be attributed to a combination of factors that include regular use of fungicides, small hectarage planted, lack of the alternate host during the summer and the deployment of effective *Lr* genes. Furthermore, the wheat seed market in Zimbabwe is currently dominated by SeedCo varieties accounting for more than 80% of the total area planted. These have been developed from a relative narrow genetic background as the sharp decline in wheat hectarage planted over the last two decades did not allow for expansion in wheat research and variety development.

However, with the recent upward trend in the area planted with wheat from 2018 and the current adoption of growing summer wheat which requires different varieties, it is likely that more *Pt* races might emerge from Zimbabwe in the near future. Therefore, coordinated and regular rust surveys are recommended in Zimbabwe and its neighbouring countries (including SA, Zambia, Malawi and Mozambique) in order to be proactive to early identify any new, more virulent races posing a threat to wheat production in the SADC region. With improved molecular technologies becoming available, surveys should include genotyping of *Pt* isolates at a central laboratory in the region which would lower the risk of the spread of isolates through phenotyping. Selected isolates can be phenotyped in support of the genotypic data.

The 46 differential lines and nine *Pt* races used did not allow gene postulation for any ASR genes. The presence of *Lr26* in two varieties SST 875 and PAN 3402 that showed similar high and low IT patterns to the differential line RL6078 carrying *Lr26*, could not be confirmed with the SCAR marker, *lag95*. Thus, the gene postulation method was unsuccessful in determining the *Lr* gene profiles of the selected varieties used in this study. This drawback could be explained by the lack of *Pt* isolates with virulence to the ASR gene(s) present in most

Zimbabwean varieties. Twenty-three of the 25 varieties selected for outcrossing displayed an F_2 segregation ratio of 3:1, indicating the inheritance of a single dominant gene. Molecular markers detected *Lr19* in 20 varieties used in the outcrosses which also had fleck IT responses to *Pt* isolates representative of all nine races and immune responses to races CFPS+*Lr20* and MFPS in the field. These results point to the presence of *Lr19* as the singly inherited ASR gene dominant in SeedCo germplasm.

In support of the findings in the current study, in a previous study two current commercial varieties SC Select (used in the current outcrosses) and SC Serena were genotyped to carry *Lr19* at experimental line stage before commercialisation while similar to this study, SC Nduna and SC Shungu were confirmed not to carry *Lr19*. Furthermore, the pedigree information based on the previous genotypic results for *Sr25*, with a tight linkage to *Lr19*, was predictive of the findings. Groups 1 and 3 with *Sr25* parentage background contributed 12 outcrosses carrying *Lr19*, while group 4 had eight outcrosses with *Lr19* as expected from their known pedigrees.

Other ASR genes detected with molecular markers were *Lr9*, *Lr11*, *Lr26*, *Lr29*, *Lr50* and *Lr51*. Using differential lines for gene postulation, *Lr26* gene was not effective against the confirmed dominant *Pt* race MCDS. The other ASR genes detected in higher frequency and effective against *Pt* race MCDS (*Lr9*, *Lr11*, *Lr50* and *Lr51*) did not show fleck responses to all nine race isolates as observed for *Lr19*. Based on these discrepancies, the detection of *Lr9*, *Lr11*, *Lr26*, *Lr29*, *Lr50* and *Lr51* could include false positives and require further verification especially for SeedCo lines not used in test crosses to PAN 3497.

Previous studies have suggested that the linkage between flanking markers and the gene of interest is not complete compared to diagnostic markers developed from cloned genes. Hence the occurrence of false positives when using flanking markers is expected in different genetic backgrounds. Nevertheless, *Lr* gene flanking markers can be used effectively in a marker-assisted backcrossing programme in conjunction with regular phenotypic monitoring if satisfactory parental genotypes are to be selected. Findings from this study have shown that Zimbabwean and SeedCo breeding programmes rely heavily on the monogenic resistance of *Lr19.* The deployment of a combination of *Lr19* with APR genes in a single variety is therefore highly recommended. Five APR genes (*Lr27*, *Lr34*, *Lr37*, *Lr46* and *Lr68*) were detected in the Zimbabwean germplasm, with *Lr46* being the most common among them and *Lr34* the least common. From the results, *Lr37* was not effective to *Pt* race MCDS. None of the varieties carried *Lr67*. Therefore, an urgent need exists to increase the frequency of *Lr34* and find sources carrying *Lr67* for use in the SeedCo wheat breeding programmes.

Lastly, varieties were evaluated in MET trials for two seasons in Zimbabwe in order to identify ideotype varieties that are widely adapted with stable high yields, acceptable leaf rust resistance and that will meet the quality traits required in the wheat value chain. The AMMI and GGE-biplots effectively analysed the data from the MET trials collected over the two seasons showing significant differences in genotype performances due to variation among genotypes ($P < 0.001$), environments and GxE interaction. Analysis using biplots improved data interpretation and decision making compared to standard statistical analysis.

The GGE scatter plot grouped environments into two MEs, where site 2 (ART Farm) proved to be the ideal testing environment with varieties displaying higher yield potential results than site 1 (RARS) and site 3 (Sisal Farm) in ME1. Site 4 (Panmure) in ME2 was the least ideal environment with varieties displaying low yield potential. As a result, environment analysis presents an opportunity to reduce the number of environments where several environments provide similar data. This is important in potentially reducing the research costs of breeding programmes. The data shows ART Farm is a good representative of ME1 while Panmure represents ME2. Results showed that grain yield and quality traits were determined mainly by the environmental effects followed by genotypic influences.

Varieties SC001, SC002, SC004, SC027 and SC W9101 had outstanding yield performances and were stable across the tested environments. These varieties were the closest to the ideal variety demanded by the different stakeholders in the wheat value chain. However, a closer look at these selected five varieties shows that they either lack or carry a single APR *Lr* gene except for SC027. Thus, the most promising variety from a leaf rust resistance perspective is SC027 which has the APR genes, *Lr27* and *Lr46*, together with an unidentified ASR gene. Varieties SC002 and SC004 contain *Lr46* while SC W9101 contains *Lr46* and *Lr19*. Importantly, SC001 lack any known APR gene(s) making it the most vulnerable should the ASR gene, *Lr19*, predicted to be present, becomes ineffective. Therefore, despite good yield performance, SC001 is not considered an ideal candidate for commercial release.

Greenhouse seedling data combined with field responses in this study have demonstrated that two APR genes stacked in a variety do not confer complete resistance in the absence of an effective ASR gene. This is illustrated by the MR to MRMS field responses recorded at Greytown for the varieties SC Shungu (*Lr46* and *Lr68*), SC Nduna (*Lr46* and *Lr68*) and Peregrine (*Lr34* and *Lr46*) as they all lacked *Lr19*. Although at a much lower risk than the susceptible control PAN 3497 which had a 100S response, there is an urgent need to broaden the genetic base by introducing new commercial varieties that combine both effective ASR and APR *Lr* genes. It is therefore recommended that the Zimbabwean and SeedCo breeding

programmes should upscale the use of MAS and MAD. This will enable the introgression of more than one effective resistance gene and reveal the presence of resistance genes in wheat varieties with less well characterised parentage.

Grain quality is important as it defines commodity prices as well as the end-use of wheat grain and contributes significantly to profit margins across the wheat value chain. However, phenotyping for end-use quality traits such as grain protein content, wet gluten content, starch content, test weight densities, flour and loaf volume is usually delayed until advanced generations in wheat breeding owing to the associated costs, labour and number of seed required. With the recent technological advancement, GS is the approach adopted by most global plant breeding programmes. Genomic selection enhances the rate of genetic gain by estimating breeding values using genome-wide markers bypassing phenotyping. Previous studies have shown the advantages of multi-trait GS, machine and deep learning models to predict complex traits in wheat. This study showed a negative correlation between both protein and wet gluten content with low yielding varieties such as SC030, SC052 and MRI 834 having high protein content. Application of MAS and GS will aid to identify varieties with a combination of high yield, disease resistance and quality traits.

In conclusion, the research conducted and reported on in Chapters 2 to 4 answered the objectives set at the onset of this study. A predominantly resistance response was observed for the SeedCo wheat breeding lines to *Pt* race MCDS occurring in Zimbabwe and isolates from eight SA *Pt* control races. Field evaluations had similar responses at Greytown and Napier showing that currently the Zimbabwean wheat germplasm is not under immediate threat to the prevailing *Pt* race(s) in the region. The study, however, recommends continued research including frequent *Pt* surveillance and race analysis, the upscaling of MAS and MAD in the SeedCo wheat breeding programme, and adopting modern technologies such as GS to facilitate more efficient breeding for durable resistance to wheat leaf rust. The information generated in this study will contribute immensely to Zimbabwean wheat research in highlighting the status of *Pt* diversity, distribution, genetic relatedness to SA races and detection of *Lr* genes in Zimbabwean germplasm.

Appendices

Appendix 2.1: Field isolates of *Puccinia triticina* (*Pt*) collected across Zimbabwe.

No	*Pt* code	Location	Cultivar	Status	Incidence[1]	GPS coordinates[2]
1	1_1_2019	Africa University, Mutare	SC Nduna	Commercial	High	18°12'27"S; 30°23'21"E; 1193 masl
2	1_2_2019	Africa University, Mutare	SC Nduna	Commercial	High	18°12'27"S; 30°23'21"E; 1193 masl
3	2_1_2019	Africa University, Mutare	Morocco	Variety	High	18°12'27"S; 30°23'21"E; 1193 masl
4	2_2_2019	Africa University, Mutare	Morocco	Variety	High	18°12'27"S; 30°23'21"E; 1193 masl
5	3_1_2019	ART Farm, Harare	Avocet S	Variety	Medium	17°43'71"S; 31°03'64"E; 1528 masl
6	3_2_2019	ART Farm, Harare	Avocet S	Variety	Medium	17°43'71"S; 31°03'64"E; 1528 masl
7	4_1_2019	ART Farm, Harare	Avocet S	Variety	Medium	17°43'71"S; 31°03'64"E; 1528 masl
8	4_2_2019	ART Farm, Harare	Avocet S	Variety	Medium	17°43'71"S; 31°03'64"E; 1528 masl
9	5_1_2019	SRC, Harare	Morocco	Variety	High	17°42'31"S; 30°53'55"E; 1454 masl
10	5_2_2019	SRC, Harare	Morocco	Variety	High	17°42'31"S; 30°53'55"E; 1454 masl
11	6_1_2019	SRC, Harare	Morocco	Variety	High	17°42'31"S; 30°53'55"E; 1454 masl
12	6_2_2019	SRC, Harare	Morocco	Variety	High	17°42'31"S; 30°53'55"E; 1454 masl
13	7_1_2019	RARS, Harare	Line 3707	Variety	High	17°43'56"S; 31°13'1"E; 1358 masl
14	7_2_2019	RARS, Harare	Line 3707	Variety	High	17°43'56"S; 31°13'1"E; 1358 masl
15	8_1_2019	RARS, Harare	SC Nduna	Commercial	High	17°43'56"S; 31°13'1"E; 1358 masl
16	8_2_2019	RARS, Harare	SC Nduna	Commercial	High	17°43'56"S; 31°13'1"E; 1358 masl
17	9_1_2019	Lotham Farm, Acturus	SC Select	Commercial	Medium	17°43'22"S; 31°21'2"E; 1228 masl
18	9_2_2019	Lotham Farm, Acturus	SC Select	Commercial	Medium	17°43'22"S; 31°21'2"E; 1228 masl
19	10_1_2019	Chegutu	PAN 3402	Commercial	High	18°12'27"S; 30°23'21"E; 1193 masl
20	10_2_2019	Chegutu	PAN 3402	Commercial	High	18°12'27"S; 30°23'21"E; 1193 masl
21	11_1_2019	Sisal Farm, Mutare	SC Nduna	Commercial	High	18°54'56"S; 32°34'37"E; 1082 masl
22	11_2_2019	Sisal Farm, Mutare	SC Nduna	Commercial	High	18°54'56"S; 32°34'37"E; 1082 masl
23	12_1_2019	Concession	SC Nduna	Commercial	High	17°37'27"S; 30°95'03" E; 1270 masl
24	12_2_2019	Concession	SC Nduna	Commercial	High	17°37'27"S; 30°95'03" E; 1270 masl
25	13_1_2019	Chinhoyi University, Chinhoyi	SC Nduna	Commercial	High	17°21'57"S; 30°11'59"E; 1153 masl

Appendix 2.1 (cont.): Field isolates of *Puccinia triticina* (*Pt*) collected across Zimbabwe.

No	*Pt* code	Location	Cultivar	Status	Incidence	GPS coordinates
26	13_2_2019	Chinhoyi University, Chinhoyi	SC Nduna	Commercial	High	17°21'57"S; 30°11'59"E; 1153 masl
27	15_1_2019	Save Valley	SC Nduna	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
28	15_2_2019	Save Valley	SC Nduna	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
29	15_3_2019	Save Valley	SC Nduna	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
30	16_1_2019	Panmure, Bindura	MRI 834	Commercial	High	17°16'22"S; 31°37'14"E; 887 masl
31	16_2_2019	Panmure, Bindura	MRI 834	Commercial	High	17°16'22"S; 31°37'14"E; 887 masl
32	16_3_2019	Panmure, Bindura	MRI 834	Commercial	High	17°16'22"S; 31°37'14"E; 887 masl
33	18_1_2019	Inamo Agri, Glendale	SC Nduna	Commercial	Medium	17°20'57"S; 31°22'51"E; 1126 masl
34	18_2_2019	Inamo Agri, Glendale	SC Nduna	Commercial	Medium	17°20'57"S; 31°22'51"E; 1126 masl
35	19_1_2019	Pagejo Farm, Goromonzi	SC Nduna	Commercial	Low	17°51'22"S; 31°22' 47"E;1236 masl
36	19_2_2019	Pagejo Farm, Goromonzi	SC Nduna	Commercial	Low	17°51 22"S; 31°22'47"E;1236 masl
37	20_1_2019	Ivordale Farm, Goromonzi	SC Nduna	Commercial	High	17°43'22"S; 31°21'2"E; 1226 masl
38	20_2_2019	Ivordale Farm, Goromonzi	SC Nduna	Commercial	High	17°43'22"S; 31°21'2"E; 1226 masl
39	21_1_2019	Chiredzi Research Station	SC Nduna	Commercial	High	21°01'19"S; 31°33'91"E; 428 masl
40	21_2_2019	Chiredzi Research Station	SC Select	Commercial	Low	21°01'19"S; 31°33'91"E; 428 masl
41	21_3_2019	Chiredzi Research Station	Morocco	Variety	High	21°01'19"S; 31°33'91"E; 428 masl
42	22_1_2019	Pricabe Farm, Kwekwe	Peregrine	Commercial	Low	18°46'27"S; 29°46'47"E; 1170 masl
43	22_2_2019	Pricabe Farm, Kwekwe	Peregrine	Commercial	Low	18°46'27"S; 29°46'47"E; 1170 masl
44	23_1_2019	RARS, Harare	SC Serena	Commercial	Medium	17°43'56"S; 31°13'1"E; 1358 masl
45	23_2_2019	RARS, Harare	SC Serena	Commercial	Medium	17°43'56"S; 31°13'1"E; 1358 masl
46	24_1_2019	Chisumbanje	SC Nduna	Commercial	High	20°76'84"S; 32°22'85"E; 413 masl
47	24_2_2019	Chisumbanje	SC Nduna	Commercial	High	20°76'84"S; 32°22'85"E; 413 masl
48	24_3_2019	Chisumbanje	SC Nduna	Commercial	High	20°76'84" S; 32°22'85"E; 413 masl
49	25_1_2019	ART Farm, Harare	Morocco	Breeding line	High	17°43'71"S; 31°03'64"E; 1528 masl
50	25_2_2019	ART Farm, Harare	Morocco	Breeding line	High	17°43'71"S; 31°03'64"E; 1528 masl
51	3_1_2020	Midlands Acres, Kwekwe	SC Nduna	Commercial	Low	18°46'27"S; 29°46'47"E; 1170 masl
52	3_2_2020	Midlands Acres, Kwekwe	SC Nduna	Commercial	Low	18°46'27"S; 29°46'47"E; 1170 masl

Appendix 2.1 (cont.): Field isolates of *Puccinia triticina* (*Pt*) collected across Zimbabwe.

No	*Pt* code	Location	Cultivar	Status	Incidence	GPS coordinates
53	5_1_2020	Inamo Agri, Glendale	SC Nduna	Commercial	High	17°20'57"S; 31°2'51"E; 1126 masl
54	5_2_2020	Inamo Agri, Glendale	SC Nduna	Commercial	High	17°20'57"S; 31°2'51"E; 1126 masl
55	6_1_2020	SRC, Harare	SC Nduna	Commercial	High	17°42'31"S; 30°53'55"E; 1454 masl
56	6_2_2020	SRC, Harare	SC Nduna	Commercial	High	17°42'31"S; 30°53'55"E; 1454 masl
57	7_1_2020	Alpha farm, Matepatepa	SC Nduna	Commercial	High	17°0'8"S; 31°19'44"E; 1169 masl
58	8_1_2020	Cowley farm, Matepatepa	SC Select	Commercial	Medium	16°56'41"S; 31°21'11"E; 1122 masl
59	8_2_2020	Cowley farm, Matepatepa	SC Select	Commercial	Medium	16°56'41"S; 31°21'11"E; 1122 masl
60	9_1_2020	ART Farm, Harare	Morocco	Breeding line	Medium	17°43'71"S; 31°03'64"E; 1528 masl
61	13_1_2020	ART Farm, Harare	SC Nduna	Commercial	Low	17°43'71"S; 31°03'64"E; 1528 masl
62	13_2_2020	ART Farm, Harare	W9101	Commercial	Low	17°43'71"S; 31°03'64"E; 1528 masl
63	14_1_2020	Mbungo Estate, Masvingo	Peregrine	Commercial	High	16°56'41"S; 31°21'11"E; 1122 masl
64	14_2_2020	Mbungo Estate, Masvingo	Peregrine	Commercial	High	16°56'41"S; 31°21'11"E; 1122 masl
65	15_1_2020	SOS Farm, Bindura	SC Sahai	Commercial	High	17°21'15"S; 31°27'46"E; 989 masl
66	16_1_2020	RARS, Harare	WZ72-2017	Breeding line	Medium	17°43'56"S; 31°13'1"E; 1358 masl
67	16_2_2020	RARS, Harare	WZ72-2017	Breeding line	Medium	17°43'56"S; 31°13'1"E; 1358 masl
68	18_1_2020	RARS, Harare	SC Shungu	Commercial	High	17°43'56"S; 31°13'1"E; 1358 masl
69	18_2_2020	RARS, Harare	SC Shungu	Commercial	High	17°43'56"S; 31°13'1"E; 1358 masl
70	19_1_2020	RARS, Harare	WZ4263-6-15	Breeding line	Medium	17°43'56"S; 31°13'1"E; 1358 masl
71	19_2_2020	RARS, Harare	WZ4263-6-15	Breeding line	Medium	17°43'56"S; 31°13'1"E; 1358 masl
72	20_1_2020	RARS, Harare	WZ4477-6-4	Breeding line	Low	17°43'56"S; 31°13'1"E; 1358 masl
73	20_2_2020	RARS, Harare	WZ4477-6-4	Breeding line	Low	17°43'56"S; 31°13'1"E; 1358 masl
74	22_1_2020	RARS, Harare	SC Select	Commercial	Medium	17°43'56"S; 31°13'1"E; 1358 masl
75	22_2_2020	RARS, Harare	W9101	Commercial	Low	17°43'56"S; 31°13'1"E; 1358 masl
76	23_1_2020	SRC, Harare	Peregrine	Commercial	High	17°42'31"S; 30°53'55"E; 1454 masl
77	23_2_2020	SRC, Harare	Peregrine	Commercial	High	17°42'31"S; 30°53'55"E; 1454 masl
78	24_1_2020	Nyombwe Estate, Mazowe	SC Select	Commercial	Low	17°25'1"S; 31°2'16"E; 1208 masl
79	25_1_2020	Panmure, Bindura	WZ4194-6-12	Breeding line	High	17°16'22"S; 31°37'14"E; 887 masl

Appendix 2.1 (cont.): Field isolates of *Puccinia triticina* (*Pt*) collected across Zimbabwe.

No	*Pt* code	Location	Cultivar	Status	Incidence	GPS coordinates
80	25_2_2020	Panmure, Bindura	WZ4194-6-12	Breeding line	High	17°16'22"S; 31°37'14"E; 887 masl
81	26_1_2020	Save Valley, Save	MRI834	Commercial	Medium	20°23'11"S; 32°22'36"E; 466 masl
82	26_2_2020	Save Valley, Save	MRI834	Commercial	Medium	20°23'11"S; 32°22'36"E; 466 masl
83	27_1_2020	Save Valley, Save	Runde	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
84	27_2_2020	Save Valley, Save	Runde	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
85	28_1_2020	Hunyani Farm, Chinhoyi	Peregrine	Commercial	High	17°20'47"S; 30°15'2"E; 1165 masl
86	28_2_2020	Hunyani Farm, Chinhoyi	Peregrine	Commercial	High	17°20'47"S; 30°15'2"E; 1165 masl
87	29_1_2020	Chinhoyi University, Chinhoyi	SC Nduna	Commercial	Medium	17°21'57"S; 30°11'59"E; 1153 masl
88	29_2_2020	Chinhoyi University, Chinhoyi	SC Nduna	Commercial	Medium	17°21'57"S; 30°11'59"E; 1153 masl
89	30_1_2020	Chirinda Farm, Lion's Den	Peregrine	Commercial	Medium	17°16'12"S; 30°1'30"E; 1151 masl
90	30_2_2020	Chirinda Farm, Lion's Den	Peregrine	Commercial	Medium	17°16'12"S; 30°1'30"E; 1151 masl
91	31_1_2020	Sisal Farm, Mutare	K215W119	Commercial	Low	18°54'56"S; 32°34'37"E; 1082 masl
92	31_2_2020	Sisal Farm, Mutare	K215W119	Commercial	Low	18°54'56"S; 32°34'37"E; 1082 masl
93	32_1_2020	Sisal Farm, Mutare	WZ4263-6-15	Breeding line	Low	18°54'56"S; 32°34'37"E; 1082 masl
94	32_2_2020	Sisal Farm, Mutare	WZ4263-6-15	Breeding line	Low	18°54'56"S; 32°34'37"E; 1082 masl
95	1_1_2021	RARS, Harare	Peregrine	Commercial	High	17°43'56"S; 31°13'1"E; 1358 masl
96	2_1_2021	RARS, Harare	1C67-2015	Breeding line	High	17°43'56"S; 31°13'1"E; 1358 masl
97	5_1_2021	SRC, Harare	SC Sahai	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
98	5_2_2021	SRC, Harare	SC Sahai	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
99	5_3_2021	SRC, Harare	SC Sahai	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
100	5_4_2021	SRC, Harare	SC Sahai	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
101	5_5_2021	SRC, Harare	SC Sahai	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
102	5_6_2021	SRC, Harare	SC Sahai	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
103	7_1_2021	SRC, Harare	SC Shield	Commercial	Medium	17°42'31"S; 30°53'55"E; 1454 masl
104	8_1_2021	SRC, Harare	W11-2016	Breeding line	High	17°42'31"S; 30°53'55"E; 1454 masl

[1] High - ≥60%; Medium - 26 to 59%; Low - ≤25%; [2] masl = meters above sea level.

Appendix 2.2: Permit authorisation with phytosanitary conditions issued by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA for the transfer of *Puccinia triticina* field isolates from Zimbabwe.

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Page 1

Directorate Plant Health

Permit No. **P0095937**

PERMIT FOR THE IMPORTATION OF CONTROLLED GOODS

In terms of the provisions of section 3(1) of the Agricultural Pests Act, 1983 (Act 36 of 1983) and subject to the conditions stated here under, authorisation is hereby granted to-

DR WILLEM BOSHOFF **Tel No:**

P. O. BOX 339

BLOEMFONTEIN

9300

to import into the Republic the following controlled goods **ORGANISMS FOR LABORATORY ANALYSIS**

AS PER ATTACHED CONDITIONS **60** **SAMPLE (S)**

Name and address of foreign supplier **ZIMBABWE**

Conditions **1. AS ATTACHED**

Port of Entry : **O R TAMBO INTERNATIONAL AIRPORT**

Import authorized from **2019/07/23** TO **2020/07/23**

IMPORTANT : This permit does not exempt the holder from the provisions of any other Act, ordinance or agreement

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2019 -07- 23
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

Date

Executive Officer

Reference Number **9/19/38**

INQUIRIES : TEL.: (012)319 6102 (Christina Makgoba) **FAX: (012)319 6370**

Appendix 2.2 (cont.): Permit authorisation with phytosanitary conditions issued by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA for the transfer of *Puccinia triticina* field isolates from Zimbabwe.

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

PHYTOSANITARY REQUIREMENTS FOR THE IMPORTATION OF REGULATED ARTICLES FOR THE PURPOSE OF LABORATORY RESEARCH

1. The consignment must be inspected at the point of entry and found free from contaminants.

2. Quarantine label to be affixed to the parcel;

3. The imported **wheat leaves or stems infected by wheat leaf rust (*Puccinia triticina*), stripe rust (*P. striiformis* f. sp. *tritici*) and stem rust (*P. graminis* f. sp. *tritici*)** sample (s), shall only be handled in the at **University of Free State, Department of Plant Sciences** laboratory facility

4. Import sample(s) in a sealed container(s) shall be addressed to **W. Boshoff**

   Name of institution / company: **University of State**

   Postal address: **P.O. Box 339, Bloemfontein, 9300**

5. The importer shall be responsible for custom clearance;

6. The container(s) shall be opened and the material handled in the facility at : **University of State, Department of Plant Sciences**

7. Name of Laboratory facility: **University of State, Department of Plant Sciences**
   Physical address: **South of Faculty Road, Greenhouse and Rust Laboratory**

   Responsible laboratory technician: **Willem Boshoff**

   Tel nr: **051 401 2965**

8. Destroy all packing material and wrapping by incineration or autoclaving.

9. Due to the foreign status of the sample(s) all experimental material shall be marked as potentially dangerous to the South African agricultural industry.

10. Take precautions at all times to prevent the escape and introduction of any pest(s), which may be present in/on the samples into the RSA.

11. No cultures, sub-cultures or specimens of the imported sample(s) may be given to any other person or be used for any work outside the facility

DEPARTMENT OF AGRICULTURE
DIRECTORATE PLANT HEALTH
2015 -07- 23
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

Appendix 2.2 (cont.): Permit authorisation with phytosanitary conditions issued by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA for the transfer of *Puccinia triticina* field isolates from Zimbabwe.

mentioned in 3. of this condition for any reason whatsoever, without the written consent of the Director

12. **All imported sample(s) and organisms isolated from the samples must be destroyed by autoclaving/incineration after completion of the laboratory analysis and notify the Manager, (for attention: Ms Rorisang Mahlakoana: Fax 012 319 6101) immediately thereof in writing.**

13. If any of the above-mentioned conditions are not complied with or are violated, the material shall be destroyed, at the importer's expense.

THE FOLLOWING UNDERTAKING TO BE COMPLETED BY IMPORTER OR HIS AUTHORISED AGENT:

I, the undersigned, Willem Boshoff

I D No: 7101105007081

am fully aware of the above-mentioned conditions and understand that should I contravene or fail to comply with any of the above conditions I shall be guilty of an offence and be liable for prosecution under the Agricultural Pests Act, 1983 (Act No. 36 of 1983).

SIGNED: _____ DATE: 11/8/2019

PERMIT NO.: P0095937

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2019 -07- 23
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

Appendix 2.2 (cont.): Permit authorisation with phytosanitary conditions issued by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA for the transfer of *Puccinia triticina* field isolates from Zimbabwe.

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Page 1

Directorate Plant Health

Permit No. **P0100431**

PERMIT FOR THE IMPORTATION OF CONTROLLED GOODS

In terms of the provisions of section 3(1) of the Agricultural Pests Act, 1983 (Act 36 of 1983) and subject to the conditions stated here under, authorisation is hereby granted to-

UNIVERSITY OF THE FREE STATE,DR WILLEM HP **Tel No: 082 921 0521**

P O BOX /POSBUS 339
BLOEMFONTEIN
9300

to import into the Republic the following controlled goods **SAMPLES FOR ANALYSIS**

AS PER ATTACHED CONDITIONS **180** **LEAVES PER GENU**

Name and address of foreign supplier **ZIMBABWE**

Conditions **1. AS ATTACHED**

Port of Entry : **O R TAMBO INTERNATIONAL AIRPORT**

Import authorized from **2020/03/31** TO **2021/03/31**

IMPORTANT : This permit does not exempt the holder from the provisions of any other Act, ordinance or agreement

DEPARTMENT OF AGRICULTURE
FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2020 -03- 31
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

Date

.....
Executive Officer

Reference Number **9/19/277**

INQUIRIES : TEL.: (012)319 6102 (Christina Makgoba) **FAX: (012)319 6370**

Appendix 2.2 (cont.): Permit authorisation with phytosanitary conditions issued by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA for the transfer of *Puccinia triticina* field isolates from Zimbabwe.

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

PHYTOSANITARY REQUIREMENTS FOR THE IMPORTATION OF REGULATED ARTICLES FOR THE PURPOSE OF LABORATORY RESEARCH

1. The consignment must be inspected at the point of entry and found free from contaminants.

2. Quarantine label to be affixed to the parcel;

3. The imported **wheat leaves or stems infected by wheat leaf rust (*Puccinia triticina*), stripe rust (*P. striiformis* f. sp. *tritici*) and stem rust (*P. graminis* f. sp. *tritici*)** sample (s), shall only be handled in the at **University of Free State, Department of Plant Sciences** laboratory facility

4. Import sample(s) in a sealed container(s) shall be addressed to **W. Boshoff**

   Name of institution / company: **University of State**

   Postal address: **P.O. Box 339, Bloemfontein, 9300**

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2020 -03- 31
DIRECTORATE PLANT HEALTH
BOX 12624 ARCADIA 0007

5. The importer shall be responsible for custom clearance;

6. The container(s) shall be opened and the material handled in the facility at : **University of State, Department of Plant Sciences**

7. Name of Laboratory facility: **University of Free State, Department of Plant Sciences**
   Physical address: **South of Faculty Road, Greenhouse and Rust Laboratory**
   Responsible laboratory technician: **Willem Boshoff**
   Tel nr: **051 401 2965**

8. Destroy all packing material and wrapping by incineration or autoclaving.

9. Due to the foreign status of the sample(s) all experimental material shall be marked as potentially dangerous to the South African agricultural industry.

10. Take precautions at all times to prevent the escape and introduction of any pest(s), which may be present in/on the samples into the RSA.

11. No cultures, sub-cultures or specimens of the imported sample(s) may be given to any other person or be used for any work outside the facility

Appendix 2.2 (cont.): Permit authorisation with phytosanitary conditions issued by the Directorate Plant Health, Department of Agriculture, Forestry and Fisheries, Pretoria, SA for the transfer of *Puccinia triticina* field isolates from Zimbabwe.

mentioned in 3. of this condition for any reason whatsoever, without the written consent of the Director

12. **All imported sample(s) and organisms isolated from the samples must be destroyed by autoclaving/incineration after completion of the laboratory analysis and notify the Manager, (for attention: Ms Rorisang Mahlakoana: Fax 012 319 6101) immediately thereof in writing.**

13. If any of the above-mentioned conditions are not complied with or are violated, the material shall be destroyed, at the importer's expense.

THE FOLLOWING UNDERTAKING TO BE COMPLETED BY IMPORTER OR HIS AUTHORISED AGENT:

I, the undersigned, Willem Boshoff

I D No: 7101105007081

am fully aware of the above-mentioned conditions and understand that should I contravene or fail to comply with any of the above conditions I shall be guilty of an offence and be liable for prosecution under the Agricultural Pests Act, 1983 (Act No. 36 of 1983).

SIGNED: WHPBoshoff. DATE: 01/04/2020

PERMIT NO.: P0100431

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2020 -03- 31
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

Appendix 2.3: Seedling infection types recorded for Zimbabwean *Puccinia triticina* (*Pt*) single pustule and South African control race isolates to selected leaf rust (*Lr*) resistance genes.

***Lr* gene**[1]	**MCDS**[2]	**FBPT**[2]	***Pt* isolates**[3]																			
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
1	3+	;	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
2a	;	;1=	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
2c	;1=	3+X	;1=	;1=	;1	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1=	;1=	;1	;1=	;1=	;1=	;1=	;1	;1=	;1=
3a	3+	4	3+	4	3+	3+	3+	4	3+	3+	3+	3+	3+	4	3+	4	3+	3+	3+	3+	4	4	3+
9	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
16	2CN	12CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN
24	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
26	3	;	3	3	3+	3	3+	3	3	3	3	3	3+	3	3	3	3	3	3+	3	3	3	3+
3ka	;1	4	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1
11	1+	;1	1+	1+	1+	1	1+	1+	1+	1+	1	1+	1	1+	1+	1+	1	1+	1+	1+	1	1+	1+
17a	4	3X	3+	3+	4	4	4	3+	4	4	4	4	4	4	4	4	3+	4	4	4	4	4	3+
30	;1+	3+	;1+	1+	;1+	;1+	;1+	1+	;1+	;1+	;1+	1+	1+	;1+	1+	;1+	;1+	1+	1+	;1+	1+	;1+	;1+
B	3+	3X	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
10	3+	4	4	3+	3+	3+	3+	4	3+	3+	3+	3+	3+	4	3+	3+	3+	4	3+	4	3+	3+	4
14a	3+	4	4	3	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3+	3	3+	3	3+	3+	3+	3+
18	;1	3X	;1	;1+	;1	;1	;1	;1	;1+	;1+	;1+	;1	;1	;1	;1	;1+	;1	;1+	;1	;1	;1+	;1+	;1
2b	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
3bg	3+	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
15	3+	;	3+	3+	3+	3	3+	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3
17b	33+	12+X	33+	33+	3+	33+	33+	3+	33+	33+	33+	33+	33+	3+	33+	33+	33+	33+	33+	33+	33+	33+	3+
19	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
20	3+	;	3+	4	4	3+	3+	4	3+	3+	4	3+	3+	3+	4	3+	3+	3+	3+	4	3+	3+	3+
23	12+X	;1-	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X
28	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
27+31	3+	;1N	4	3+	4	4	4	4	3+	4	4	4	4	4	4	4	3+	4	4	4	4	4	4
32	;1	;1N	;1	;1	;1	;1	;1	;1	;1-	;1	;1	;1	;1-	;1	;1	;1	;1-	;1	;1	;1	;1	;1	;1
37	33-	;1	33-	3	33-	33-	33-	33-	33-	33-	3	33-	33-	33-	33-	33-	3	33-	33-	33-	33-	3	33-

Appendix 2.3 (cont.): Seedling infection types recorded for Zimbabwean *Puccinia triticina* (*Pt*) single pustule and South African control race isolates to selected leaf rust (*Lr*) resistance genes.

***Lr* gene**[1]	**MCDS**[2]	**FBPT**[2]	***Pt* isolates**[3]																			
			22	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**
1	3+	;	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
2a	;	;1=	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
2c	;1=	3+X	;1=	;1	;1	;1=	;1=	;1	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1	;1=	;1	;1=	;1=	;1
3a	3+	4	3+	4	3+	3+	4	3+	4	3+	3+	4	3+	3+	4	3+	3+	3+	4	4	3+	3+	3+
9	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
16	2CN	12CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN
24	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
26	3	;	3	3	3	3	3	3+	3	3	3	3	3+	3	3	3	3	3+	3	3	3	3+	3+
3ka	;1	4	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1
11	1+	;1	1	1+	1	1+	1+	1	1+	1	1+	1	1+	1+	1	1+	1+	1	1+	1+	1	1+	1
17a	4	3X	4	4	4	4	4	3+	4	4	4	4	4	4	4	3+	4	4	4	4	3+	4	4
30	;1+	3+	;1+	;1+	1+	1+	;1+	;1+	1+	;1+	;1+	1+	;1+	;1+	1+	;1+	;1+	1+	1+	;1+	;1+	1+	;1+
B	3+	3x	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
10	3+	4	3+	3+	3+	3+	3+	3+	4	3+	3+	3+	3+	4	3+	3+	3+	3+	3+	4	3+	3+	4
14a	3+	4	3+	3+	3	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3+	3+	3	3+	3+
18	;1	3X	;1	;1+	;1	;1	;1	;1+	;1	;1	;1	;1+	;1	;1	;1	;1	;1+	;1	;1	;1	;1+	;1	;1
2b	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
3bg	3+	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
15	3+	;	3+	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3	3+	3	3+	3+	3+	3+	3+	3
17b	33+	12+X	33+	33+	33+	33+	3+	33+	33+	33+	33+	3+	33+	33+	33+	33+	3+	3+	33+	33+	33+	33+	3+
19	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
20	3+	;	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	4	3+	3+	4	3+	3+
23	12+X	;1-	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X
28	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
27+31	3+	;1N	3+	4	4	4	4	4	4	3+	4	4	4	4	4	4	3+	4	4	4	4	4	3+
32	;1	;1N	;1	;1	;1	;1-	;1	;1	;1	;1	;1	;1-	;1	;1	;1	;1	;1-	;1	;1	;1	;1-	;1	;1
37	33-	;1	33-	33-	33-	3	33-	33-	33-	33-	33-	3	33-	33-	33-	33-	33-	3	33-	33-	3	33-	33-

Appendix 2.3 (cont.): Seedling infection types recorded for Zimbabwean *Puccinia triticina* (*Pt*) single pustule and South African control race isolates to selected leaf rust (*Lr*) resistance genes.

***Lr* gene**[1]	**MCDS**[2]	**FBPT**[2]	***Pt* isolates**[3]																			
			43	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**
1	3+	;	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
2a	;	;1=	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
2c	;1=	3+X	;1=	;1=	;1	;1=	;1=	;1=	;1	;1	;1=	;1=	;1	;1=	;1	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=
3a	3+	4	3+	3+	4	3+	3+	3+	4	3+	4	3+	4	3+	3+	3+	4	3+	3+	4	3+	4	3+
9	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
16	2CN	12CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN
24	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
26	3	;	3	3	3	3	3+	3	3	3+	3	3	3+	3	3	3+	3	3+	3	3	3+	3	3
3ka	;1	4	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1
11	1+	;1	1	1+	1+	1	1+	1+	1	1+	1+	1+	1+	1+	1	1+	1+	1+	1+	1	1+	1+	1+
17a	4	3X	4	4	4	3+	4	4	4	4	4	4	4	3+	4	4	4	4	3+	4	4	3+	4
30	;1+	3+	1+	;1+	;1+	1+	;1+	1+	;1+	;1+	;1	;1+	;1+	;1	;1+	;1+	;1+	;1+	;1+	;1+	;1+	;1+	;1
B	3+	3X	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
10	3+	4	3+	3+	3+	3+	3+	4	3+	4	3+	3+	4	3+	3+	4	3+	3+	3+	3+	3+	4	3+
14a	3+	4	3+	3+	3+	3+	3+	3+	3	3+	3	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3
18	;1	3X	;1	;1+	;1	;1	;1	;1+	;1	;1+	;1+	;1	;1+	;1	;1	;1	;1+	;1	;1+	;1	;1	;1	;1
2b	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
3bg	3+	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
15	3+	;	3+	3+	3+	3+	3+	3	3+	3+	3	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3	3+
17b	33+	12+X	33+	33+	33+	3+	33+	33+	33+	33+	3+	33+	3+	3+	33+	33+	3+	33+	33+	3+	33+	33+	3+
19	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
20	3+	;	3+	3+	4	3+	3+	3+	3+	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
23	12+X	;1-	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X
28	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
27+31	3+	;1N	4	4	4	4	3+	4	4	3+	4	4	3+	4	4	4	4	3+	4	4	4	3+	4
32	;1	;1N	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1-	;1	;1	;1	;1	;1	;1-	;1-	;1	;1	;1	;1
37	33-	;1	33-	33-	33-	33-	33-	3	33-	33-	3	33-	3	3	33-	33-	3	33-	33-	33-	3	33-	33-

Appendix 2.3 (cont.): Seedling infection types recorded for Zimbabwean *Puccinia triticina* (*Pt*) single pustule and South African control race isolates to selected leaf rust (*Lr*) resistance genes.

***Lr* gene**[1]	**MCDS**[2]	**FBPT**[2]	***Pt* isolates**[3]																			
			64	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**	**81**	**82**	**83**	**84**
1	3+	;	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
2a	;	;1=	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
2c	;1=	3+X	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1=
3a	3+	4	3+	4	3+	3+	4	3+	3+	4	3+	3+	4	3+	3+	4	3+	4	4	3+	4	3+	4
9	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
16	2CN	12CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN
24	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
26	3	;	3+	3	3	3+	3	3	3	3+	3	3	3+	3	3	3	3+	3	3	3+	3+	3+	3
3ka	;1	4	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1
11	1+	;1	1+	1+	1	1+	1+	1+	1+	1+	1	1+	1+	1+	1	1+	1+	1+	1	1+	1+	1+	1+
17a	4	3X	4	4	4	4	4	4	4	3+	4	4	4	4	4	3+	4	4	4	4	4	4	3+
30	;1+	3+	;1+	;1+	;1+	;1+	;1+	;1+	;1+	;1	;1+	;1+	;1+	;1+	;1+	;1	;1+	;1+	;1+	;1+	;1+	;1+	;1
B	3+	3X	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
10	3+	4	3+	3+	3+	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	3+	4	3+
14a	3+	4	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3	3+
18	;1	3X	;1+	;1	;1	;1	;1	;1	;1	;1	;1	;1+	;1+	;1+	;1	;1+	;1	;1	;1+	;1	;1	;1+	;1
2b	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
3bg	3+	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
15	3+	;	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3	3+	3+	3+
17b	33+	12+X	3+	33+	33+	3+	33+	33+	3+	33+	33+	3+	33+	33+	33+	3+	33+	33+	3+	33+	33+	3+	33+
19	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
20	3+	;	3+	3+	3+	3+	3+	3+	3+	4	3+	3+	3+	3+	3+	3+	3+	4	3+	3+	3+	3+	3+
23	12+X	;1-	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X
28	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
27+31	3+	;1N	4	4	4	4	4	4	3+	3+	4	4	4	3+	4	4	4	3+	4	4	3+	4	4
32	;1	;1N	;1-	;1	;1	;1	;1	;1-	;1	;1	;1	;1	;1-	;1	;1	;1	;1-	;1	;1	;1-	;1	;1	;1
37	33-	;1	33-	3	33-	33-	33-	3	33-	3	33-	33-	3	3	33-	33-	3	33-	33-	33-	3	33-	33-

Appendix 2.3 (cont.): Seedling infection types recorded for Zimbabwean *Puccinia triticina* (*Pt*) single pustule and South African control race isolates to selected leaf rust (*Lr*) resistance genes.

***Lr* gene**[1]	**MCDS**[2]	**FBPT**[2]	***Pt* isolates**[3]																			
			85	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**93**	**94**	**95**	**96**	**97**	**98**	**99**	**100**	**101**	**102**	**103**	**104**
1	3+	;	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
2a	;	;1=	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
2c	;1=	3+X	;1=	;1	;1=	;1	;1	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1=	;1	;1=	;1=	;1=	;1
3a	3+	4	3+	4	3+	4	3+	4	3+	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	3+	3+
9	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
16	2CN	12CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN	2CN
24	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
26	3	;	3+	3	3+	3	3	3	3+	3	3	3+	3+	3	3	3+	3+	3	3	3+	3+	3
3ka	;1	4	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1
11	1+	;1	1+	1+	1	1+	1+	1+	1+	1+	1	1+	1+	1+	1	1+	1+	1+	1	1+	1+	1+
17a	4	3X	4	4	4	4	3+	4	4	4	4	3+	4	4	4	3+	4	4	4	3+	4	4
30	;1+	3+	;1+	;1+	;1+	;1+	;1	;1+	;1+	;1+	;1	;1+	;1+	;1+	;1	;1+	;1+	;1+	;1	;1+	;1+	;1+
B	3+	3X	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
10	3+	4	3+	3+	4	3+	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	3+	3+	4	3+	3+
14a	3+	4	3+	3	3+	3+	3+	3	3+	3+	3+	3	3+	3+	3+	3	3+	3+	3+	3	3+	3+
18	;1	3X	;1	;1+	;1	;1	;1	;1+	;1	;1	;1+	;1	;1	;1	;1+	;1+	;1	;1	;1+	;1+	;1	;1
2b	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
3bg	3+	4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
15	3+	;	3+	3+	3+	3+	3+	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
17b	33+	12+X	33+	3+	33+	33+	3+	33+	33+	33+	3+	33+	33+	33+	3+	33+	33+	33+	3+	33+	33+	33+
19	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
20	3+	;	3+	4	3+	3+	3+	3+	3+	4	3+	4	3+	4	3+	4	3+	4	3+	4	3+	4
23	12+X	;1-	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X	12+X
28	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;	;
27+31	3+	;1N	4	4	3+	4	4	3+	4	4	3+	4	4	4	3+	3+	4	4	3+	3+	4	4
32	;1	;1N	;1	;1-	;1	;1	;1-	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1	;1
37	33-	;1N	33-	3	33-	33-	33-	3	33-	33-	33-	33-	33-	33-	33-	33-	33-	33-	33-	33-	33-	33-

[1] Differential lines used are listed in Table 2.2; [2] Isolates UVPt21_75 and UVPt22_1 of *Puccinia triticina* (*Pt*) race MCDS and FBPT originally collected in South Africa were used as controls; [3] Isolates of *Pt* as listed in Appendix 1.

Appendix 2.4: Representative field isolates of *Puccinia triticina* (*Pt*) collected across Zimbabwe that were used for microsatellite genotyping.

No	*Pt* code	Location	Cultivar	Status	Incidence[1]	GPS coordinates[2]
1	1_1_2019	Africa University, Mutare	SC Nduna	Commercial	High	18°12'27"S; 30°23'21"E; 1193 masl
2	2_1_2019	Africa University, Mutare	Morocco	Breeding line	High	18°12'27"S; 30°23'21"E; 1193 masl
3	3_2_2019	ART Farm, Harare	Avocet S	Breeding line	Medium	17°43'71"S; 31°03'64"E; 1528 masl
4	4_2_2019	ART Farm, Harare	Avocet S	Breeding line	Medium	17°43'71"S; 31°03'64"E; 1528 masl
5	5_1_2019	SRC, Harare	Morocco	Breeding line	High	17°42'31"S; 30°53'55"E; 1454 masl
6	7_2_2019	RARS, Harare	Line 3707	Breeding line	High	17°43'56"S; 31°13'1"E; 1358 masl
7	9_2_2019	Lotham Farm, Acturus	SC Select	Commercial	Medium	17°43'22"S; 31°21'2"E; 1228 masl
8	10_2_2019	Chegutu	PAN 3402	Commercial	High	18°12'27"S; 30°23'21"E; 1193 masl
9	12_1_2019	Concession	SC Nduna	Commercial	High	17°37'27"S; 30°95'03" E; 1270 masl
10	13_2_2019	Chinhoyi University, Chinhoyi	SC Nduna	Commercial	High	17°21'57"S; 30°11'59"E; 1153 masl
11	15_2_2019	Save Valley	SC Nduna	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
12	16_2_2019	Panmure, Bindura	MRI 834	Commercial	High	17°16'22"S; 31°37'14"E; 887 masl
13	18_1_2019	Inamo Agri, Glendale	SC Nduna	Commercial	Medium	17°20'57"S; 31°22'51"E; 1126 masl
14	19_1_2019	Pagejo Farm, Goromonzi	SC Nduna	Commercial	Low	17°51'22"S; 31°22' 47"E;1236 masl
15	20_2_2019	Ivordale Farm, Goromonzi	SC Nduna	Commercial	High	17°43'22"S; 31°21'2"E; 1226 masl
16	23_2_2019	RARS, Harare	SC Serena	Commercial	Medium	17°43'56"S; 31°13'1"E; 1358 masl
17	24_3_2019	Chisumbanje	SC Nduna	Commercial	High	20°76'84" S; 32°22'85"E; 413 masl
18	25_1_2019	ART Farm, Harare	Morocco	Breeding line	High	17°43'71"S; 31°03'64"E; 1528 masl
19	3_2_2020	Midlands Acres, Kwekwe	SC Nduna	Commercial	Low	18°46'27"S; 29°46'47"E; 1170 masl
20	5_1_2020	Inamo Agri, Glendale	SC Nduna	Commercial	High	17°20'57"S; 31°2'51"E; 1126 masl

Appendix 2.4 (cont.): Representative field isolates of *Puccinia triticina* (*Pt*) collected across Zimbabwe that were used for microsatellite genotyping.

No	*Pt* code	Location	Cultivar	Status	Incidence[1]	GPS coordinates[2]
21	6_2_2020	SRC, Harare	SC Nduna	Commercial	High	17°42'31"S; 30°53'55"E; 1454 masl
22	7_1_2020	Alpha Farm, Matepatepa	SC Nduna	Commercial	High	17°0'8"S; 31°19'44"E; 1169 masl
23	8_2_2020	Cowley Farm, Matepatepa	SC Select	Commercial	Medium	16°56'41"S; 31°21'11"E; 1122 masl
24	9_1_2020	ART Farm, Harare	Morocco	Breeding line	Medium	17°43'71"S; 31°03'64"E; 1528 masl
25	13_1_2020	ART Farm, Harare	SC Nduna	Commercial	Low	17°43'71"S; 31°03'64"E; 1528 masl
26	14_1_2020	Mbungo Estate, Masvingo	Peregrine	Commercial	High	16°56'41"S; 31°21'11"E; 1122 masl
27	15_1_2020	SOS Farm, Bindura	SC Sahai	Commercial	High	17°21'15"S; 31°27'46"E; 989 masl
28	16_1_2020	RARS, Harare	WZ72-2017	Breeding line	Medium	17°43'56"S; 31°13'1"E; 1358 masl
29	18_1_2020	RARS, Harare	SC Shungu	Commercial	High	17°43'56"S; 31°13'1"E; 1358 masl
30	19_2_2020	RARS, Harare	WZ4263-6-15	Breeding line	Medium	17°43'56"S; 31°13'1"E; 1358 masl
31	20_1_2020	RARS, Harare	WZ4477-6-4	Breeding line	Low	17°43'56"S; 31°13'1"E; 1358 masl
32	23_2_2020	SRC, Harare	Peregrine	Commercial	High	17°42'31"S; 30°53'55"E; 1454 masl
33	24_1_2020	Nyombwe Estate, Mazowe	SC Select	Commercial	Low	17°25'1"S; 31°2'16"E; 1208 masl
34	25_1_2020	Panmure, Bindura	WZ4194-6-12	Breeding line	High	17°16'22"S; 31°37'14"E; 887 masl
35	26_2_2020	Save Valley, Save	MRI834	Commercial	Medium	20°23'11"S; 32°22'36"E; 466 masl
36	27_2_2020	Save Valley, Save	Runde	Commercial	High	20°23'11"S; 32°22'36"E; 466 masl
37	28_2_2020	Hunyani Farm, Chinhoyi	Peregrine	Commercial	High	17°20'47"S; 30°15'2"E; 1165 masl
38	30_1_2020	Chirinda Farm, Lion's Den	Peregrine	Commercial	Medium	17°16'12"S; 30°1'30"E; 1151 masl
39	31_1_2020	Sisal Farm, Mutare	K215W119	Commercial	Low	18°54'56"S; 32°34'37"E; 1082 masl
40	32_1_2020	Sisal Farm, Mutare	WZ4263-6-15	Breeding line	Low	18°54'56"S; 32°34'37"E; 1082 masl

[1] High = ≥60% incidence; Medium = 26 to 59% incidence; Low = ≤25% incidence; [2] Masl = meters above sea level.

Appendix 2.5: Dissimilarities between representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African control races as calculated by DARwin analysis.

***Pt* isolate**	**1_1_2019 (MCDS)**	**2_1_2019 (MCDS)**	**3_2_2019 (MCDS)**	**4_2_2019 (MCDS)**	**5_1_2019 (MCDS)**	**7_2_2019 (MCDS)**	**9_2_2019 (MCDS)**	**10_2_2019 (MCDS)**	**12_1_2019 (MCDS)**	**13_2_2019 (MCDS)**
2_1_2019 (MCDS)	0.21									
3_2_2019 (MCDS)	0.00	0.21								
4_2_2019 (MCDS)	0.00	0.21	0.00							
5_1_2019 (MCDS)	0.00	0.21	0.00	0.00						
7_2_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00					
9_2_2019 (MCDS)	0.08	0.26	0.08	0.08	0.08	0.08				
10_2_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08			
12_1_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00		
13_2_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	
15_2_2019 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
16_2_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
18_1_2019 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
19_1_2019 (MCDS)	0.11	0.29	0.11	0.11	0.11	0.11	0.18	0.11	0.11	0.11
20_2_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
23_2_2019 (MCDS)	0.05	0.16	0.05	0.05	0.05	0.05	0.11	0.05	0.05	0.05
24_3_2019 (MCDS)	0.05	0.24	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
25_1_2019 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
3_2_2020 (MCDS)	0.03	0.21	0.03	0.03	0.03	0.03	0.11	0.03	0.03	0.03
5_1_2020 (MCDS)	0.13	0.26	0.13	0.13	0.13	0.13	0.21	0.13	0.13	0.13
6_2_2020 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
7_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
8_2_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
9_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
13_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
14_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
15_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
16_1_2020 (MCDS)	0.11	0.21	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
18_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
19_2_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
20_1_2020 (MCDS)	0.13	0.32	0.13	0.13	0.13	0.13	0.21	0.13	0.13	0.13
23_2_2020 (MCDS)	0.11	0.29	0.11	0.11	0.11	0.11	0.18	0.11	0.11	0.11
24_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
25_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
26_2_2020 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
27_2_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
28_2_2020 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
30_1_2020 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
31_1_2020 (MCDS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
32_1_2020 (MCDS)	0.03	0.24	0.03	0.03	0.03	0.03	0.08	0.03	0.03	0.03
3SA137 (SCDS)	0.29	0.42	0.29	0.29	0.29	0.29	0.37	0.29	0.29	0.29
3SA144 (SDDN)	0.29	0.45	0.29	0.29	0.29	0.29	0.37	0.29	0.29	0.29
3SA146 (MCDS)	0.05	0.26	0.05	0.05	0.05	0.05	0.13	0.05	0.05	0.05
3SA147 (FBPT)	0.50	0.58	0.50	0.50	0.50	0.50	0.55	0.50	0.50	0.50
3SA248 (CFPS+*Lr20*)	0.24	0.40	0.24	0.24	0.24	0.24	0.29	0.24	0.24	0.24
3SA10 (CFPS)	0.26	0.42	0.26	0.26	0.26	0.26	0.32	0.26	0.26	0.26
3SA127 (MCPS)	0.00	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
3SA100 (MFPS)	0.03	0.24	0.03	0.03	0.03	0.03	0.11	0.03	0.03	0.03

Appendix 2.5 (cont.): Dissimilarities between representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African control races as calculated by DARwin analysis.

***Pt* isolate**	**15_2_2019 (MCDS)**	**16_2_2019 (MCDS)**	**18_1_2019 (MCDS)**	**19_1_2019 (MCDS)**	**20_2_2019 (MCDS)**	**23_2_2019 (MCDS)**	**24_3_2019 (MCDS)**	**25_1_2019 (MCDS)**	**3_2_2020 (MCDS)**	**5_1_2020 (MCDS)**
16_2_2019 (MCDS)	0.05									
18_1_2019 (MCDS)	0.11	0.05								
19_1_2019 (MCDS)	0.16	0.11	0.16							
20_2_2019 (MCDS)	0.05	0.00	0.05	0.11						
23_2_2019 (MCDS)	0.11	0.05	0.11	0.16	0.05					
24_3_2019 (MCDS)	0.11	0.05	0.11	0.05	0.05	0.11				
25_1_2019 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05			
3_2_2020 (MCDS)	0.08	0.03	0.08	0.13	0.03	0.05	0.08	0.03		
5_1_2020 (MCDS)	0.18	0.13	0.18	0.13	0.13	0.18	0.08	0.13	0.16	
6_2_2020 (MCDS)	0.11	0.05	0.08	0.16	0.05	0.11	0.11	0.05	0.08	0.18
7_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
8_2_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
9_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
13_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
14_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
15_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
16_1_2020 (MCDS)	0.16	0.11	0.16	0.21	0.11	0.05	0.16	0.11	0.11	0.24
18_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
19_2_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
20_1_2020 (MCDS)	0.18	0.13	0.18	0.13	0.13	0.18	0.08	0.13	0.16	0.13
23_2_2020 (MCDS)	0.16	0.11	0.16	0.11	0.11	0.16	0.05	0.11	0.13	0.11
24_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
25_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
26_2_2020 (MCDS)	0.11	0.05	0.08	0.16	0.05	0.11	0.11	0.05	0.08	0.18
27_2_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
28_2_2020 (MCDS)	0.11	0.05	0.11	0.16	0.05	0.11	0.11	0.05	0.08	0.18
30_1_2020 (MCDS)	0.11	0.05	0.11	0.05	0.05	0.11	0.11	0.05	0.08	0.18
31_1_2020 (MCDS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
32_1_2020 (MCDS)	0.08	0.03	0.08	0.13	0.03	0.08	0.08	0.03	0.05	0.16
3SA137 (SCDS)	0.34	0.29	0.32	0.29	0.29	0.34	0.24	0.29	0.32	0.21
3SA144 (SDDN)	0.34	0.29	0.32	0.29	0.29	0.34	0.24	0.29	0.32	0.26
3SA146 (MCDS)	0.11	0.05	0.11	0.16	0.05	0.11	0.11	0.05	0.08	0.18
3SA147 (FBPT)	0.53	0.50	0.53	0.55	0.50	0.55	0.53	0.50	0.53	0.58
3SA248 (CFPS+*Lr20*)	0.26	0.24	0.29	0.32	0.24	0.29	0.29	0.24	0.26	0.32
3SA10 (CFPS)	0.29	0.26	0.32	0.34	0.26	0.32	0.32	0.26	0.29	0.34
3SA127 (MCPS)	0.05	0.00	0.05	0.11	0.00	0.05	0.05	0.00	0.03	0.13
3SA100 (MFPS)	0.08	0.03	0.08	0.13	0.03	0.08	0.08	0.03	0.05	0.16

Appendix 2.5 (cont.): Dissimilarities between representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African control races as calculated by DARwin analysis.

***Pt* isolate**	**6_2_2020 (MCDS)**	**7_1_2020 (MCDS)**	**8_2_2020 (MCDS)**	**9_1_2020 (MCDS)**	**13_1_2020 (MCDS)**	**14_1_2020 (MCDS)**	**15_1_2020 (MCDS)**	**16_1_2020 (MCDS)**	**18_1_2020 (MCDS)**	**19_2_2020 (MCDS)**
7_1_2020 (MCDS)	0.05									
8_2_2020 (MCDS)	0.05	0.00								
9_1_2020 (MCDS)	0.05	0.00	0.00							
13_1_2020 (MCDS)	0.05	0.00	0.00	0.00						
14_1_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00					
15_1_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00				
16_1_2020 (MCDS)	0.16	0.11	0.11	0.11	0.11	0.11	0.11			
18_1_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11		
19_2_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	
20_1_2020 (MCDS)	0.18	0.13	0.13	0.13	0.13	0.13	0.13	0.24	0.13	0.13
23_2_2020 (MCDS)	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.21	0.11	0.11
24_1_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
25_1_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
26_2_2020 (MCDS)	0.00	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05
27_2_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
28_2_2020 (MCDS)	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05
30_1_2020 (MCDS)	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05
31_1_2020 (MCDS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
32_1_2020 (MCDS)	0.08	0.03	0.03	0.03	0.03	0.03	0.03	0.11	0.03	0.03
3SA137 (SCDS)	0.26	0.29	0.29	0.29	0.29	0.29	0.29	0.40	0.29	0.29
3SA144 (SDDN)	0.26	0.29	0.29	0.29	0.29	0.29	0.29	0.40	0.29	0.29
3SA146 (MCDS)	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05
3SA147 (FBPT)	0.45	0.50	0.50	0.50	0.50	0.50	0.50	0.58	0.50	0.50
3SA248 (CFPS+*Lr20*)	0.29	0.24	0.24	0.24	0.24	0.24	0.24	0.32	0.24	0.24
3SA10 (CFPS)	0.32	0.26	0.26	0.26	0.26	0.26	0.26	0.34	0.26	0.26
3SA127 (MCPS)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
3SA100 (MFPS)	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.13	0.03	0.03

Appendix 2.5 (cont.): Dissimilarities between representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African control races as calculated by DARwin analysis.

***Pt* isolate**	**20_1_2020 (MCDS)**	**23_2_2020 (MCDS)**	**24_1_2020 (MCDS)**	**25_1_2020 (MCDS)**	**26_2_2020 (MCDS)**	**27_2_2020 (MCDS)**	**28_2_2020 (MCDS)**	**30_1_2020 (MCDS)**	**31_1_2020 (MCDS)**	**32_1_2020 (MCDS)**
23_2_2020 (MCDS)	0.08									
24_1_2020 (MCDS)	0.13	0.11								
25_1_2020 (MCDS)	0.13	0.11	0.00							
26_2_2020 (MCDS)	0.18	0.11	0.05	0.05						
27_2_2020 (MCDS)	0.13	0.11	0.00	0.00	0.05					
28_2_2020 (MCDS)	0.18	0.16	0.05	0.05	0.11	0.05				
30_1_2020 (MCDS)	0.18	0.16	0.05	0.05	0.11	0.05	0.11			
31_1_2020 (MCDS)	0.13	0.11	0.00	0.00	0.05	0.00	0.05	0.05		
32_1_2020 (MCDS)	0.16	0.13	0.03	0.03	0.08	0.03	0.08	0.08	0.03	
3SA137 (SCDS)	0.26	0.18	0.29	0.29	0.26	0.29	0.34	0.34	0.29	0.32
3SA144 (SDDN)	0.29	0.21	0.29	0.29	0.26	0.29	0.32	0.34	0.29	0.32
3SA146 (MCDS)	0.18	0.16	0.05	0.05	0.11	0.05	0.05	0.11	0.05	0.08
3SA147 (FBPT)	0.47	0.50	0.50	0.50	0.45	0.50	0.53	0.53	0.50	0.47
3SA248 (CFPS+*Lr20*)	0.34	0.32	0.24	0.24	0.29	0.24	0.29	0.26	0.24	0.21
3SA10 (CFPS)	0.37	0.34	0.26	0.26	0.32	0.26	0.32	0.29	0.26	0.24
3SA127 (MCPS)	0.13	0.11	0.00	0.00	0.05	0.00	0.05	0.05	0.00	0.03
3SA100 (MFPS)	0.16	0.11	0.03	0.03	0.05	0.03	0.08	0.08	0.03	0.05

Appendix 2.5 (cont): Dissimilarities between representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and eight South African control races as calculated by DARwin analysis.

***Pt* isolate**	**3SA137 (SCDS)**	**3SA144 (SDDN)**	**3SA146 (MCDS)**	**3SA147 (FBPT)**	**3SA248 (CFPS+*Lr20*)**	**3SA10 (CFPS)**	**3SA127 (MCPS)**	**3SA100 (MFPS)**
3SA144 (SDDN)	0.132							
3SA146 (MCDS)	0.342	0.316						
3SA147 (FBPT)	0.526	0.5	0.5					
3SA248 (CFPS+*Lr20*)	0.421	0.447	0.289	0.395				
3SA10 (CFPS)	0.395	0.421	0.316	0.368	0.026			
3SA127 (MCPS)	0.289	0.289	0.053	0.5	0.237	0.263		
3SA100 (MFPS)	0.289	0.289	0.079	0.5	0.263	0.289	0.026	

Appendix 2.6: Avirulence/virulence profiles of representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and South African *Pt* control races to leaf rust resistance genes in 27 differential lines.

	Leaf rust gene													
***Pt* isolate**	***Lr1***	***Lr2a***	***Lr2b***	***Lr2c***	***Lr3a***	***Lr3bg***	***Lr3ka***	***Lr10***	***Lr11***	***Lr14a***	***Lr15***	***Lr16***	***Lr17a***	***Lr20***
3SA147 (FBPT)	0[1]	0	0	1[2]	1	1	1	1	0	1	0	0	1	0
3SA248 (CFPS+*Lr20*)	0	0	0	0	1	1	1	1	0	1	1	0	1	1
3SA10 (CFPS)	0	0	0	0	1	1	1	1	0	1	1	0	1	0
3SA146 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
3SA127 (MCPS)	1	0	0	0	1	1	1	1	0	1	1	0	1	1
3SA100 (MFPS)	1	0	0	0	1	1	1	1	0	1	1	0	1	1
3_2_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
6_2_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
16_1_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
23_2_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
28_2_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
30_1_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
32_1_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
1_1_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
2_1_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
9_2_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
15_2_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
18_1_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
20_1_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
5_1_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
19_1_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
23_2_2020 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
24_3_2019 (MCDS)	1	0	0	0	1	1	0	1	0	1	1	0	1	1
3SA137 (SCDS)	1	1	1	1	0	0	0	1	1	1	1	0	1	0
3SA144 (SDDN)	1	1	1	1	0	0	0	0	0	1	1	0	1	1

Appendix 2.6 (cont.): Avirulence/virulence profiles of representative Zimbabwean *Puccinia triticina* (*Pt*) isolates and South African *Pt* control races to leaf rust resistance genes in 27 differential lines.

	Leaf rust gene												
***Pt* isolate**	***Lr24***	***Lr26***	***Lr30***	***LrB***	***Lr18***	***Lr19***	***Lr23***	***Lr28***	***Lr17b***	***Lr37***[3]	***Lr9***	***Lr27+31***	***Lr32***
3SA147 (FBPT)	0[1]	0	1[2]	1	1	0	0	0	0	0	0	0	0
3SA248 (CFPS+*Lr20*)	1	1	1	1	0	0	0	0	1	0	0	1	0
3SA10 (CFPS)	1	1	1	1	0	0	0	0	1	0	0	1	0
3SA146 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
3SA127(MCPS)	0	1	1	1	0	0	1	0	1	1	0	1	0
3SA100 (MFPS)	1	1	1	1	0	0	0	0	1	1	0	1	0
3_2_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
6_2_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
16_1_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
23_2_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
28_2_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
30_1_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
32_1_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
1_1_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
2_1_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
9_2_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
15_2_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
18_1_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
20_1_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
5_1_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
19_1_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
23_2_2020 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
24_3_2019 (MCDS)	0	1	0	1	0	0	0	0	1	1	0	1	0
3SA137 (SCDS)	0	0	0	0	0	0	1	1	1	0	0	0	0
3SA144 (SDDN)	1	0	0	1	0	0	0	1	1	0	0	0	1

[1] Avirulent; [2] Virulent; [3] Based on seedling infection types for Trident.

Appendix 3.1: List of 72 Zimbabwean wheat varieties screened for all-stage and adult plant resistance genes to *Puccinia triticina* through multi-pathotyping and molecular genotyping.

Name	Group[1]	Status	Source
SC001	3	Breeding line	SeedCo
SC002	3	Breeding line	SeedCo
SC003	2	Breeding line	SeedCo
SC004	1	Breeding line	SeedCo
SC005	1	Breeding line	SeedCo
SC006	1	Breeding line	SeedCo
SC W9101	4	Commercial cultivar	SeedCo
SC008	1	Breeding line	SeedCo
SC009	1	Breeding line	SeedCo
SC010	3	Breeding line	SeedCo
SC011	1	Breeding line	SeedCo
SC012	1	Breeding line	SeedCo
SC013	1	Breeding line	SeedCo
SC014	2	Breeding line	SeedCo
SC015	1	Breeding line	SeedCo
SC016	1	Breeding line	SeedCo
SC017	1	Breeding line	SeedCo
SC018	1	Breeding line	SeedCo
SC019	2	Breeding line	SeedCo
SC020	1	Breeding line	SeedCo
SC Select	3	Commercial cultivar	SeedCo
SC Nduna	2	Commercial cultivar	SeedCo
SC Serena	1	Commercial cultivar	SeedCo
K215W119	5	Commercial cultivar	Klein Karoo
Runde	5	Commercial cultivar	Crop Breeding Institute
SC026	3	Breeding line	SeedCo
SC027	1	Breeding line	SeedCo
SC028	4	Breeding line	SeedCo
SC029	4	Breeding line	SeedCo
SC030	3	Breeding line	SeedCo
SC031	1	Breeding line	SeedCo
SC032	1	Breeding line	SeedCo
SC033	1	Breeding line	SeedCo
SC034	1	Breeding line	SeedCo
SC035	4	Breeding line	SeedCo
SC036	3	Breeding line	SeedCo
SC037	1	Breeding line	SeedCo
SC038	4	Breeding line	SeedCo
SC039	1	Breeding line	SeedCo
SC040	3	Breeding line	SeedCo

Appendix 3.1 (cont.): List of 72 Zimbabwean wheat varieties screened for all-stage and adult plant resistance genes to *Puccinia triticina* through multi-pathotyping and molecular genotyping.

Name	Group[1]	Status	Source
SC041	1	Breeding line	SeedCo
SC042	1	Breeding line	SeedCo
SC043	1	Breeding line	SeedCo
SC044	1	Breeding line	SeedCo
SC045	1	Breeding line	SeedCo
SC046	4	Breeding line	SeedCo
SC047	4	Breeding line	SeedCo
SC048	2	Breeding line	SeedCo
SC049	3	Breeding line	SeedCo
SC050	1	Breeding line	SeedCo
SC051	1	Breeding line	SeedCo
SC052	2	Breeding line	SeedCo
SC053	3	Breeding line	SeedCo
SC054	3	Breeding line	SeedCo
SC055	4	Breeding line	SeedCo
SC056	2	Breeding line	SeedCo
SC057	1	Breeding line	SeedCo
SC058	1	Breeding line	SeedCo
SC059	4	Breeding line	SeedCo
SC060	4	Breeding line	SeedCo
SC061	3	Breeding line	SeedCo
SC062	1	Breeding line	SeedCo
SC063	1	Breeding line	SeedCo
SC064	4	Breeding line	SeedCo
SC065	4	Breeding line	SeedCo
SST 875	5	Commercial cultivar	Sensako
PAN 3494	5	Commercial cultivar	Corteva
Peregrine	5	Commercial cultivar	Klein Karoo
PAN 3402	5	Commercial cultivar	Corteva
SC Sicho	1	Commercial cultivar	SeedCo
SC Shungu	2	Commercial cultivar	SeedCo
MRI 834	5	Commercial cultivar	Synergy

[1]SeedCo breeding programme pedigree groups