Stem rust resistance and yield performance of irrigated Zimbabwean spring wheat

By

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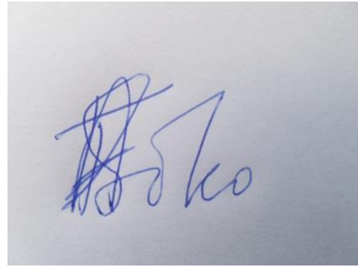
Declaration

I hereby declare that the thesis submitted by me for the degree, Doctor of Philosophy in Interdisciplinary Plant Pathology and Plant Breeding at the University of the Free State is my own independent work and has not been previously submitted by me at any other University or Faculty. I further relinquish copyright of the thesis in favour of the University of the Free State.

.

Tegwe Soko 22 January 2018

Dedication

To my wife (Mavis), daughter (Charlotte), two sons (Tafadzwa and Tanaka) and late parents (Adam and Rudia).

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Acronyms

A2	classification of small scale commercial farmers
AFLP	amplified fragment length polymorphism
AMMI	additive main effects and multiplicative interaction
ANOVA	analysis of variance
APR	adult plant resistance
ARC-SA	Agriculture Research Council of South Africa
ARDA	Agricultural Rural Development Authority
ART	Agricultural Research Trust farm
ASR	all stage resistance
AUDPC	area under disease progression curve
BC	backcross
BGRI	Borlaug Global Rust Initiative
BIL	backcross inbred line
bp	base pair
CAPS	cleaved amplified polymorphic sequence
CBI	Crop Breeding Institute
cDNA	complementary DNA
CHZ	Chiredzi research station
CI	coefficient of infection
CIMMYT	Maize and wheat improvement centre
CISRO	Commonwealth Scientific and Industrial Research Office

CGIAR	Consultative Group for International Agricultural Research
cM	centimorgan
COMESA	Common market for Eastern and Southern Africa
CTAB	Cetyltrimethylammonium bromide
DArT	Diversity array technology
DGGW	Delivering genetic gain in wheat
DNA	deoxyribonucleic acid
DP	donor parent
DR&SS	Department of Research and Specialist Services
E	environment
EB	ethidium bromide
EDTA	ethylenediaminetetraacetic acid
EST	Expressed sequence tags
EtOH	ethyl alcohol
FAM™	Specific FRET cassette used in KASP
F_n	filial generation
FRET	fluorescence resonance energy transfer
G	genotype
g	gram
gDNA	genomic DNA
GEI	genotype-environment interactions
GGE	genotype and genotype-environment model
GMB	Grain Marketing Board

GP	grain protein
GRRC	Global Rust Reference Centre
GS	genomic selection
GWS	genome wide selection
HEX^{TM}	Specific FRET cassette used in KASP
HIT	High infection type
HLM	hectolitre mass
HMWGS	high molecular weight glutenin subunits
H_O	null hypothesis
HTISC	high temperature induced seedling chlorosis
i	selection intensity/pressure
ICAR	Indian Council of Agricultural Research
ICARDA	International Centre for Agricultural Research in Dry Areas
IPCA	Interaction Principle Component Axes
IT	infection type
KALRO	Kenya Agriculture and Livestock Research Organization
KASP	Kompetitive Allele Specific PCR
K2	Klein Karoo
kg	kilogram
kg/ha	kilogram per hectare
KRC	Kadoma Research Center
LGC	Laboratory of Government Chemist (UK)
LIMS	laboratory information management systems

LIT	low infection type
Lr	leaf rust resistance gene
LR	linear regression
Lr	low reaction
LSD	least significant difference
Ltd	Limited
m^2	square meters
MAB	marker assisted breeding
MABC	marker assisted backcross
MAGIC	multi-parent advanced generation inter-cross
MARS	marker assisted recurrent selection
MAS	marker assisted selection
masl	metres above sea level
MASWheat	marker assisted selection in wheat
mb	moisture basis
ME	mega environment
MET	multiple environment testing
mHz/s	Megahertz per second
MR	moderate resistance
MS	mean square
MS	moderate susceptible
NH_4OAc	ammonium acetate
NIAB	National Institute of Agricultural Botany

NID	normally and independently distributed
NPK	Nitrogen, Phosphorous and Potassium
NR	natural regions
NTC	non-treated check
O	observed
^{O}C	degree Celsius
PAN	Panmure Experiment Station
PBC	pseudo black chaff
PC	principle component
PCA	principal component analysis
PCR	polymerase chain reaction
Pgt	*Puccinia graminis* f. sp. *tritici*
P_i	cultivar superiority index
Pm	powdery mildew
P_n	parent
Pty	proprietary
QTL	quantitative trait loci
R	replication
R	resistance
r	selection accuracy/precision
R^2	regression coefficient
RAPD	random amplified polymorphic DNA
RARS	Rattray Arnold Research Station

RC	recurrent parent
RCF	relative centrifugal force
RFLP	restriction fragment length polymorphism
RIL	recombinant inbred line
R_t	genetic gain over time
RT-PCR	real time PCR
S	susceptible
SA	South Africa
SADC	Southern Africa Development Community
SAVE	Save valley experiment station
SC	Seed-Co
SCAR	sequence characterized amplied region
SDS	sodium dodecyl sulphate
SE	standard error
SED	standard deviation
SNP	single nucleotide polymorphism
Sr	stem rust resistance gene
SRC	Stapleford Research Center
SRCI	stem rust coefficient of infection
SS	sum of squares
SSA	Sub-Saharan Africa
SSR	simple sequence repeat
STS	sequence-tagged-site

SVD	singular value decomposition
T	trace
TAE	Tris-acetate
TBE	Tris-borate
TKW	thousand kernel weight
$T^{O}C$	Temperature
TPE	Tris-phosphate
Tris-Cl	Tris chloride
Tris-HCl	Tris hydrochloride
UFS	University of the Free State
Ug99	original Ugandan isolate of stem rust race TTKSK
UN-FAO	United Nations Agency-Food and Agriculture Organization
USDA-ARS	United States Department of Agriculture-Agricultural Research Services
V_g	genetic variance
VS	very susceptible
WISP	wheat improvement strategic programme
y	year
Yr	yellow (stripe) rust resistance gene
χ^2	Chi-square
#	number
% w/v	percent weight per volume
µL	microliter

Summary

The strategic importance of wheat in Sub-Saharan Africa and Zimbabwe is under threat of the Ug99 stem rust race group. Since first detection of Ug99 (TTKSK according to the North American nomenclature system using five sets of four gene differential lines (www.fao.org, accessed 27/3/2018)) in 1998/9, 13 races have been detected in 13 countries by 2016. PTKST, TTKSF and TTKSF+*Sr9h* have been confirmed in Zimbabwe since 2009 while South Africa has TTKSP as a fourth race. Ug99 is virulent to a broad spectrum of resistance genes including *Sr9h*, *Sr24*, *Sr31*, *Sr36* and *SrTmp* that are found in Southern Africa wheat germplasm. Genotyping of Zimbabwean lines by CenGen (2012) and Limagrain (2015) showed a frequency of 38.7-44.4% of lines possessing *Sr2* alone or in combination with *Sr31*. *Sr24* alone or in combination with unknown *Sr* genes constituted 10.2-11.1% whereas *Sr31* alone or in combination constituted 53.1%. *Sr36* had a frequency of 2% and *Lr19/Sr25* alone or in combination with *Sr2* was recorded in 39.9% entries. Continuous genotyping, pathotyping and annual rust surveys are important components of an updated wheat database. For example, the lack of early confirmation of stem rust occurrence led to deregistration of a new variety "Busi" in 2002.

Breeding for durable resistance is the most effective, efficient, environmentally friendly and sustainable way of managing the threat of stem rust. Resistance breeding can aim for adult plant resistance (APR) or all stage resistance (ASR) or a combination of the two. Four APR genes have been catalogued and these confer resistance to stem, leaf and stripe rust and powdery mildew. Any one of *Sr2/Yr30/Lr27*, *Lr34/Yr18/Sr57*, *Lr46/Yr29/Sr58* and *Lr67/Yr46/Sr55* must be used as the foundation for gene pyramiding. A successful disease breeding programme requires investment in effective breeding procedures, marker assisted breeding, gene stewardship, pathogenicity surveys and analysis to keep track of pathogen dynamics. Although global initiatives offer a platform for germplasm and knowledge sharing, in-country expertise and ongoing rust programmes are required in at-risk regions.

Results from this study indicate PTKST with virulence on *Sr31* is a major threat to wheat production in Zimbabwe with 63.3% of germplasm containing either *Sr31* or *Sr24*. Compared to TTKSF, TTKSF+*Sr9h* and TTKSP, PTKST was the most virulent race with 40.8% of lines being susceptible in the greenhouse. At seedling stage, 59.2% of lines were resistant to all four Southern Africa Ug99 races. At adult stage, 42.9% of the lines were resistant or moderately

resistant to PTKST in the greenhouse and a 55.1% resistance frequency was recorded in the field. PTKST resulted in 29% and 21% mean yield loss in susceptible varieties (SC1 and SC3, respectively) at Greytown, South Africa over two seasons. ASR offers better protection against Ug99 with SC8 recording the lowest yield loss of 6.4%. This was lower than losses in the APR lines Kingbird (10.1%), W1406 (19.5%) and W6979 (15.4%). Molecular markers can be used to complement phenotypic markers in characterizing wheat lines. A total of 89.5% of lines genotyped to have *Sr2* also showed *Sr2* flecking at adult stage when inoculated with PTKST. SC2, SC8, SC30, SC35 and SC36 had best protection as measured by infection type at seedling stage and modified Cobb field scores at adult stage against PTKST showing the value of gene pyramids. These lines have *Sr2*, *Sr31* and other *Sr* genes. SC1 confirmed to have only *Sr31* was susceptible to PTKST in both seedling and adult stages and needs to be replaced as a commercial variety. To test the viability of using marker assisted backcrossing, *Sr25*, *Sr26* and *Sr39* were successfully transferred to, and tracked in the development of adapted genotypes.

Generally, it is difficult to predict phenotypic responses of varieties due to complexity in gene interactions and environmental influences. In the current project an AMMI model and GGE biplots identified desirable and stable genotypes and demarcated most favourable environments, important considerations for strategic decision making in wheat breeding and variety release in Zimbabwe.

Key words: breeding, *Puccinia graminis*, resistance genes, stem rust, Ug99, wheat, yield loss, Zimbabwe.

Overview and objectives

Although there are several institutions involved in wheat research and development in Zimbabwe, very little information was available on the status of wheat stem rust. Seed-Co (SC), one of the private seed companies involved in wheat research, is dependent on conventional breeding and currently has eleven wheat varieties on the Zimbabwe national variety catalogue maintained by Seed Services of the Ministry of Agriculture. The national variety catalogue, referred to as the second schedule, is a list of all registered crop varieties that contain information on year of release and variety owner (responsible for maintaining breeders' seed). Some of the SC wheat varieties have been registered in Zambia (five varieties) and Malawi (three). In 2017, three commercial varieties namely SC1, SC12 and SC4 have been applied for inclusion on the Common market for Eastern and Southern Africa (COMESA) variety catalogue. Therefore, lack of information on the genetic composition, especially on stem rust resistance genes in these varieties, was an area of concern. Seedling and adult plant responses of SC wheat varieties to the prevalent Ug99 stem rust pathotypes (TTKSF, TTKSF+*Sr9h* and PTKST) were unknown. The programme relies on natural infections for disease screening. This might be ineffective given that the level of natural inoculum varies from season to season and the identified Ug99 strains were all observed in the southern lowveld of Zimbabwe while most of the breeding work is done in the northern part of the country. Thus, a need was identified to carry out seedling and adult plant infection studies to establish the response of SC germplasm to the identified Ug99 strains.

The study was divided into nine chapters, designed to give a global picture on stem rust (Chapter 1: General overview) and the current Zimbabwean stem rust situation (Chapter 2). Repeated study protocols and materials used were summarized in Chapter 3. Some methods cut across study projects while some of the wheat germplasm was used in more than one project thus the need to give a summary of these materials and methods in Chapter 3. Chapter 4 covers the greenhouse seedling response and field adult plant responses of forty-nine Zimbabwean wheat varieties after being inoculated with the four Ug99 races that are prevalent in Southern Africa. Races TTKSF, TTKSF+*Sr9h*, PTKST are common in both Zimbabwe and South Africa. TTKSP is prevalent in South Africa only. The same material was also evaluated at adult stage for their response to Ug99 race PTKST. This was a continuation of work on characterization of the forty-nine Zimbabwean varieties. Chapter 5 further characterizes the most commonly grown wheat variety in Zimbabwe. SC1, a high yielding wheat variety that is

widely grown in Zimbabwe (67.3% of SC Zimbabwe wheat seed sales in the period 2009 to 2017) and in other parts of Southern Africa (excluding South Africa). The mode of resistance for SC1 to current Ug99-related strains in Southern Africa was unknown. This study established the inheritance of resistance in SC1 using a SC1 by SC20 segregating population. The characterization work in Chapters 4 and 5 would be part of a Zimbabwean database on wheat unlike the current situation where even parentage for SC1 is unknown despite being the most commonly grown commercial variety. Confirmed virulence for *Sr31* to PTKST in Zimbabwe added new geographical records for Ug99-related races, as a result Southern African cultivars with 1B.1R (*Sr31*) resistance are at risk (Mukoyi *et al.*, 2011). The same race was also detected in South Africa in 2009 (Pretorius *et al.*, 2010a; Visser *et al.*, 2011). Characterization of SC1 was therefore a priority.

The 2012 genotyping results (Chapter 2) showed that the SC wheat breeding programme was over-dependent on a few *Sr* genes such as *Sr31, Sr24* and *Sr36* that were now ineffective against Ug99 (TTKSK) and related races. This demanded urgent introgression of new *Sr* resistant genes. Globally, effective stem rust resistant genes have already been identified, as described in Chapter 1, and it was important for the SC wheat breeding programme to introgress such genes into its breeding programme. This will enable the release of resistant varieties in particular regions where the constituent genes are effective against existing pathotypes. Introgressing these identified genes into SC germplasm would allow SC to manage the gene deployment and management strategy as called for by the wheat global community in the control of rust diseases. The introgression of new resistance genes will be part of gene pyramiding onto *Sr2* background, already in existence in SC germplasm. Such introgression is time consuming and complicated if only conventional breeding methods are used. Marker Assisted Breeding offers a solution to such complexities though this can only be done for *Sr* genes whose markers are available. Chapter 6 of this study therefore describes the use of molecular markers to track Ug99 resistant *Sr25*, *Sr26* and *Sr39* genes introgressed into SC1, SC8 and SC15 using a backcrossing method.

No work has been done using Southern African wheat varieties to quantify the level of protection offered by adult plant resistance genes against Ug99 races on yield, yield components and industrial quality. Chapter 7 describes work done at Greytown using three wheat varieties known to be susceptible to Ug99 (SC1, SC3 and Line 37-07), three varieties with adult plant resistance (W1406, W6979 and Kingbird) and one variety (SC8) known to

have all stage host resistance. Field trials were inoculated with Ug99 race PTKST to assess the level of protection in the three types of varieties over two seasons. Information generated from the trial will be useful not to researchers only but also to farmers and extension workers since it will quantify the yield losses caused by Ug99 on susceptible and resistant varieties. It will also show if use of fungicides can protect susceptible varieties against Ug99.

Wheat is grown under irrigation across all the five agro-ecological zones of Zimbabwe and varieties that show wide adaptation and stability such as SC1 are more ideal. Multi-location testing of the current wheat experimental lines was done for two seasons to identify other potential wheat varieties that were stable for possible commercialization. Chapter 8 covers multi-environmental trials that were done over two seasons in Zimbabwe to identify better performing wheat varieties.

Lastly, Chapter 9 gives a summary of all five study projects, outlining important findings and giving recommendations where they are due. The five study projects addressed the following objectives:

(a) To determine the all-stage resistance (ASR, formerly seedling) and adult plant resistance (APR) responses of SC varieties and lines to four Ug99 stem rust related strains namely TTKSF, TTKSF+*Sr9h*, PTKST (common in both Zimbabwe and South Africa) and TTKSP (prevalent in South Africa) by artificial inoculation in the greenhouse;

(b) To study the inheritance of stem rust resistance in SC1 to determine number and characterize genes responsible for conferring resistance to *Sr31* avirulent pathotype, using an SC1 by SC20 segregating population;

(c) To introgress three effective stem rust (*Puccinia graminis f. sp. tritici)* (*Pgt*) resistance genes (*Sr25*, *Sr26* and *Sr39*) into the SC Spring wheat breeding programme using marker assisted backcrossing (MABC);

(d) To quantify yield losses caused by Ug99, using the related PTKST race, on seven wheat varieties namely; SC1, SC3, Line 37-07 (all known to be susceptible to some Ug99 pathotypes), SC8 (an ASR variety), W6979, W1406 and Kingbird (all known to possess stem rust APR). This will provide information on the impact of the Ug99 related strains

on wheat production in Southern Africa and at the same time would allow researchers to determine the amount of protection offered by APR genes against Ug99 races in Southern Africa; and

(e) To identify SC varieties and advanced lines that are stable, for wide or narrow adaptation using an additive main effects and multiplicative interaction (AMMI) model and genotype and genotype-environment interaction (GGE) biplots.

1. General introduction

1.1. Wheat

Wheat (*Triticum aestivum* L.) is a monocotyledonous plant, which belongs to the Poaceae family. Bread wheat is now a strategic commodity for achieving food security and political stability in Sub-Saharan Africa (SSA) due to increased demand as a result of income growth and rapid urbanization. However, SSA countries produce only about 30% of their domestic requirements (Negassa *et al*., 2013). Wheat provides 21% of total energy and 20% of the protein to more than 4.5 billion people in 94 developing countries (Singh *et al*., 2011a; Crespo-Herrera *et al*., 2016). Wheat covers 15.4% of world arable land (Goutam *et al*., 2015) stretching from the equator to latitudes 60°N and 44°S and at altitudes ranging from sea level to more than 3000 meters above sea level. In 2016, 722 million tonnes of wheat were produced in the world, Africa contributing 26 million tonnes and SSA only 6 million tonnes, though consumption in SSA stood at 22 million tonnes (http://www.agri-outlook.org 20/6/17). Therefore, SSA relies on imports to meet its consumption demand. Only seven countries in SSA (South Africa, Ethiopia, Sudan, Kenya, Zimbabwe, Tanzania and Zambia) produce 97% of wheat grown in the region (Heisey and Lantican, 2000).

Bread wheat (*T. aestivum* L.) is an allohexaploid (genome AABBDD, 2n=42) hybrid of emmer wheat (*Triticum turgidum,* AABB) with goat grass (*Aegilops tauschii*, DD) (Dubcovsky and Dvorak, 2007) and is important because of its ecological range of cultivation, cultivar diversity, and extent to which it has become inseparable to the cultures and religions of diverse societies worldwide (Macharia and Ngina, 2017). Wheat production constraints include abiotic and biotic factors. Among the biotic constraints, rust diseases are widespread and economically important in cereal crops worldwide. Race Ug99, or TTKSK (North American nomenclature), of the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*), causes stem or black rust on wheat, was first observed in Uganda in 1998 and has been recognized to be a major threat to wheat production (Hiebert *et al*., 2010; Singh *et al*., 2011a). Isolates of the Ug99 race group have rendered most of the world wheat germplasm susceptible due to their virulence to a broad spectrum of resistance genes. About one billion people reside in the anticipated path of Ug99 and most of them consume wheat produced within their borders (Wanyera *et al*., 2016). Disease resistance, especially resistance to at least one of the three rust diseases of wheat, must be a component of the genotype package that a breeder has to offer to farmers (CIMMYT, 1988). To meet future

demands for wheat, a 2% annual yield increase till 2020, must be achieved with the aid of current technologies (Reynolds *et al*., 2008). This demands resource investment into wheat research and development by both governments and private companies. Such global investment has been focusing on the control of stem rust, especially Ug99 races since the discovery in Uganda.

1.2. Stem rust

Stem rust (black rust) of wheat caused by the fungus *Pgt* has plagued mankind for thousands of years. Race Ug99 (TTKSK) of *Pgt*, first identified in Uganda in 1998 and described in 1999, has been recognized as a major threat to wheat production (Hiebert *et al.*, 2010; Singh *et al*., 2011a). Stem rust was the worst disease during the first half of the 20th century destroying approximately a fifth of America's harvest in periodic epidemics. *Pgt* is very aggressive, spreads rapidly over large distances by wind or accidental human transmission. This can be through contaminated clothing or infected plant material. *Pgt* affects stems, leaves, occasionally heads, glumes and awns, and has potential to cause significant yield losses especially on susceptible cultivars. A small chlorotic fleck appears a few days after initial infection and mature pustules produce brownish red urediniospores that rupture the epidermis resulting in a rusty appearance (Singh *et al*., 2012). Host maturity results in uredinia changing into telia that are initially dark brown in colour, but turn to black for over-seasoning, thus the name "black rust". *Pgt* infection under favourable conditions can (a) cause death of tillers or entire plants at seedling stage; (b) retard plant growth or even kill adult plants by reducing photosynthetic area; (c) cause nutrient and water loss by disrupting the plant transport system due to ruptured cells; (d) increase respiration rate; (e) decrease transportation of carbohydrates to the grain (carbohydrates are directed to infected areas for growth) resulting in shrivelled grain and; (f) weaken stems that may break or lodge resulting in yield losses due to failure to combine (Roelfs, 1985; Roelfs *et al*., 1992; Dubin and Brennan, 2009). Quarantine regulations can only delay the spread of, for example Ug99, but it will not stop the pathogen from moving from infected areas to new areas due to the airborne nature of the disease.

1.2.1. Epidemiology of the stem rust pathogen

The pathogen has a complicated life cycle that requires a primary host (*T. aestivum* [bread wheat] or *T. durum* [durum wheat]) and an alternate host (common barberry). Warmer

conditions of 15-35°C and dew are two important factors favouring crop infection (Roelfs *et al*., 1992; Goutam *et al*., 2015). All rust diseases are promoted by unusually favourable environmental conditions such as climate change, presence of susceptible varieties, alterations in cultural practices and a combination of the three. Environmental conditions conducive for the various developmental stages of the stem rust fungus are shown in Table 1.1.

Table 1.1: Environmental conditions for different stages of stem rust development

	Temperature (°C)				
Stage	Minimum	Optimum	Maximum	Light	Free Water
Germination	2	15 – 24	30	Low	Necessary
Germling		20		Low	Necessary
Appressorium		16 – 27		None	Necessary
Penetration	15	29	35	High	Necessary
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None

Source: Extracted from Roelfs *et al*. (1992)

1.2.2. World distribution and virulence of Ug99 stem rust

Ug99 or TTKSK, an African strain of stem rust caused by *Pgt* virulent for the widely used *Sr31* resistance gene in wheat, was first observed in Uganda (1998) and characterized in 1999 (Pretorius *et al*., 2000; Singh *et al*., 2008a; Hiebert *et al*., 2010). Ug99 was the first known *Pgt* race with virulence for *Sr31* located in the rye (*Secale cereale*) translocation, 1BL.1RS (Singh *et al*., 2006a; 2008a; 2008b). Isolates of *Pgt* of the Ug99 race group are virulent to a broad spectrum of resistance genes, and they rendered 90% of global wheat varieties susceptible to stem rust by 2011 (Singh *et al*., 2011a; 2015). The USDA-ARS Cereal Disease Laboratory reported 80% of hard red spring wheat grown in the Northern Great Plains to be susceptible in 2007 (maswheat.ucdavis.edu 23/6/17). Since first detection of the original Ug99 isolate, 13 races belonging to this lineage have been identified by February 2016, and Ug99 is present in 13 countries namely; Uganda, Kenya, South Africa, Ethiopia, Sudan, Yemen, Iran, Tanzania, Zimbabwe, Eritrea, Mozambique, Rwanda and most recently Egypt (http://rusttracker.cimmyt.org 16/6/17). The continued emergence of new Ug99 races will persist to render once effective *Sr* genes ineffective. Jin *et al*. (2008; 2009), Bhavani *et al*.

(2011) and Njau *et al.* (2010) reported new strains of Ug99, with added virulence to *Sr24* and *Sr36*, and these rendered more than half of TTKSK resistant lines susceptible.

Epidemics of stem rust due to races not related to Ug99 have recently been recorded. Olivera *et al.* (2015) reported a non-Ug99 strain, TKTTF that caused extensive damage on "Digalu" wheat variety in Ethiopia in 2013-14. A similar race was also reported in Turkey, Germany and Sicily. Olivera *et al.* (2017) reported six non-Ug99 races including TKTTF in Germany in 2013. Researchers in Southern Africa should not only concentrate on Ug99 but also on other important biotic stresses. Experience with historical disease pathosystems such as soybean rust (*Phakospora pachyrhizi*), first identified in Uganda and subsequently in South Africa via Kenya, Rwanda, Zimbabwe and Zambia between 1996 and 2001 (Jarvie, 2009; Pretorius *et al.*, 2015), implies that the new generation of stem rust races may also invade the region. Figure 1.1 shows a list of countries where the 13 Ug99 stem rust races have been identified as of February 2016 while Table 1.2 indicates the virulences and when the races were first detected in the various countries.

1.2.3. Economic importance of stem rust

Stem rust has been a recurrent threat to wheat production historically, causing famines and ruining economies (Dean *et al*., 2012). The bible mentions several curses that relate to crops "smitten by mildew" (mildew was an old name for mainly stem rust) and Romans considered rust to be a numen (a spirit of deity) that needs to be feared, appeased with processions, sacrifices and feasts during the Robigalia festival, otherwise crops could be destroyed (Roelfs, 1982; Roelfs *et al*., 1992; Dubin and Brennan, 2009). Furthermore, commercial losses due to stem rust have impacted on national and global policies on disease management. Carleton (1905) as cited by Hodson (2011) reported a loss of one million tonnes of wheat due to stem rust in 1904 in North America. This was followed by a loss of 5.67 million hectares of wheat due to stem rust in 1916 (Roelfs, 1982; Hodson, 2011). The 1916 epidemic rendered thousands of farmers bankrupt (Dubin and Brennan, 2009) and resulted in the barberry (alternate host to *Pgt*) eradication policy across American states between 1916 and 1955 (Roelfs, 1982). Further losses in North America were recorded in 1954 with 2.1 million tonnes lost due to a virulent *Pgt* strain, 15B and 350 000 tonnes were lost in 1962 (Leonard, 2001).

Elsewhere, in Australia, the 1973 epidemic resulted in 25-30% losses (Hodson, 2011), worth more than AU$200 million, and was described as "the most severe in the Australian wheat industry" by Watson and Butler as cited by Park (2007). This led to the birth of a highly successful, nationally co-ordinated rust control approach in 1974 (Hodson, 2011). Dubin and Brennan (2009) reported crop losses between 5-20% in Eastern Europe including Russia in 1932 and 40% losses in 1951 in Chile. In Africa, an average yield loss of 42% was suffered in Ethiopia during the 1993-94 season when a modern semi-dwarf variety 'Enkoy' succumbed to stem rust (Dubin and Brennan, 2009). A wheat cultivar called 'Digalu' became susceptible to a non-Ug99 stem rust race during November 2013 to December 2014 and resulted in almost 100% yield loss in Ethiopia when fields were infected (Olivera *et al*., 2015).

The economic importance of Ug99 emanates from its impact on loss of wheat yields that vary from region to region depending on susceptibility of varieties grown and virulence of the Ug99 strain. Roelfs *et al*. (1992) referred to stem rust as the worst of the three rusts with potential to cause losses up to 50% within a month if conditions are conducive for development and up to 100% losses in susceptible varieties. Average grain yield losses of 35% have been recorded around the world and in South Africa (Pretorius *et al*., 2007a; Figlan *et al*., 2014; Terefe *et al*., 2016) but losses can reach 70% when conditions are conducive and susceptible varieties are grown (www.fao.org 20/6/17). In Kenya, stem rust epidemics caused approximately 70% grain losses in experimental plots and in farmers' fields with non-sprayed crops reaching 100% in 2007 (Wanyera, 2008). The broad virulence of Ug99 is a major concern especially in SSA countries, where bread wheat is the staple crop characterized by a widening gap between production and consumption. Negassa *et al*. (2013) reported that this rapid growth in wheat demand and dependency on imports may increase the vulnerability of countries in SSA to political instability as food prices escalate.

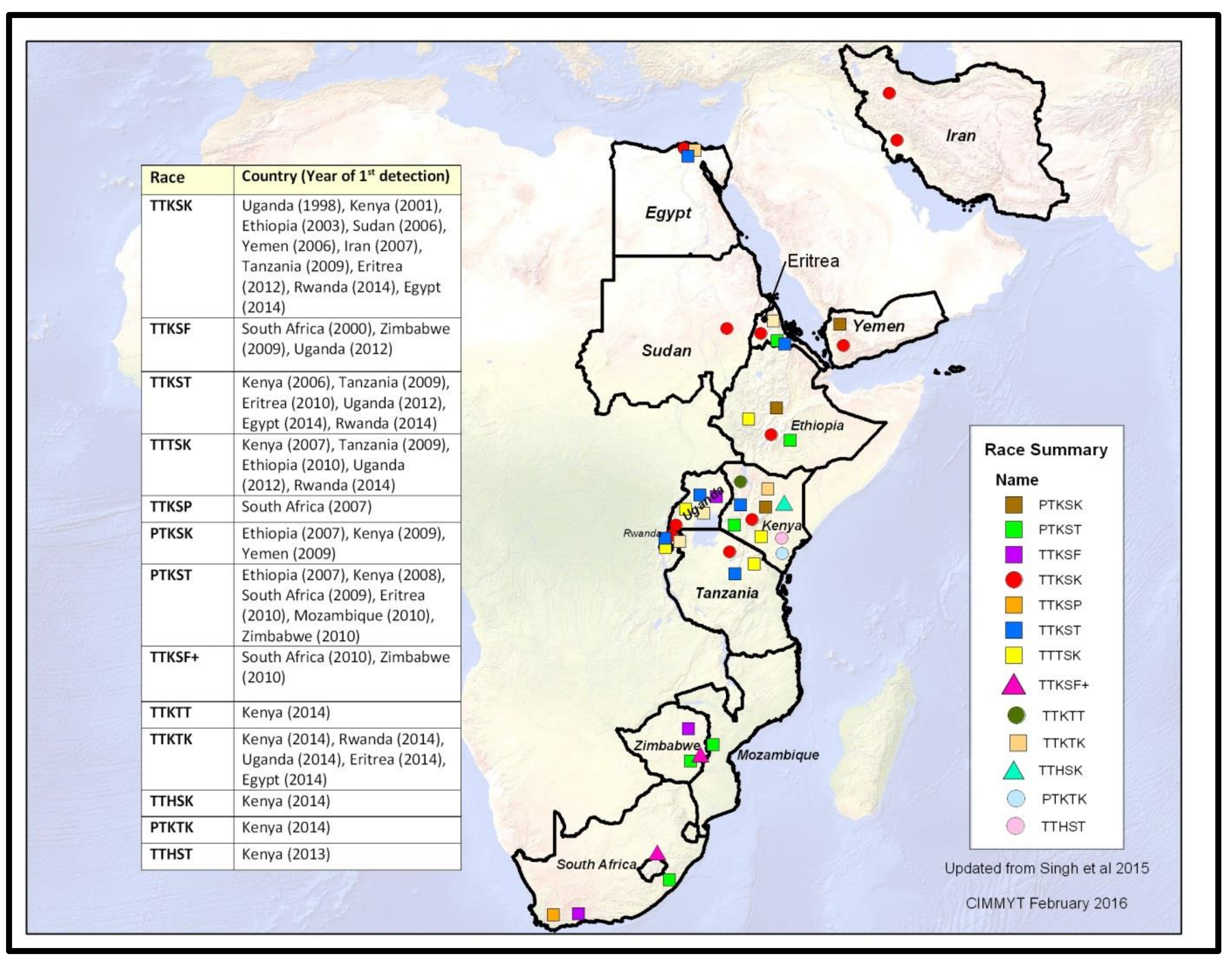

Figure 1-1: Global distribution of Ug99 races.

Source: http://www.rusttracker.cimmyt.org(16/6/17)

1.2.4. Stem rust resistance genes effective to Ug99

A total of 62 genes/alleles located at 55 different loci that confer resistance to *Pgt* have been catalogued (Pretorius *et al*., 2017). Four (*Sr1*, *Sr3*, *Sr4* and *Sr9c*) of these have been abandoned for various reasons. These catalogued genes have different origins that range from cultivated wheat to wild relatives and some of these genes are already ineffective against Ug99. According to http://www.rusttracker.cimmyt.org (accessed on 16/6/17) the following genes are ineffective against Ug99; *Sr5, 6, 7a, 7b, 8a, 9a, 9b, 9d, 9e, 9f, 9h, 10, 11, 12, 16, 17, 18, 20, 21, 23, 24, 30, 31, 41, 49, 54, McN, Tmp* (*Sha7*) and *Wld-1*. Prins *et al*. (2016) listed 41 genes that they referred to be effective to at least one pathotype within the Ug99 race group (including *9h*, *21*, *24*, *36* and *Tmp* with some known Ug99 virulences). Table 1.3 gives a summary of these genes indicating their origin.

Table 1.2: Summary of Ug99 lineage races identified in affected countries, year of detection and key virulence (+) and avirulence (-) genes

Race [a]	Key virulence (+) or avirulence (-) *	Year identified	Confirmed countries (year)
TTKSK	+*Sr31*	1999	Uganda (1998/9), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014)
TTKSF	-*Sr31*	2000	South Africa (2000), Zimbabwe (2009), Uganda (2012)
TTKST	+*Sr31*, +*Sr24*	2006	Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012), Egypt (2014), Rwanda (2014)
TTTSK	+*Sr31*, +*Sr36*	2007	Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014)
TTKSP	-*Sr31*, +*Sr24*	2007	South Africa (2007)
PTKSK	+*Sr31*, -*Sr21*	2007	Uganda (1998/9), Kenya (2009), Ethiopia (2007), Yemen (2009)
PTKST	+*Sr31*, +*Sr24*, -*Sr21*	2008	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+*Sr9h*	-*Sr31*, +*Sr9h*	2012	South Africa (2010), Zimbabwe (2010)
TTKTT	+*Sr31*, +*Sr24*, +*SrTmp*	2015	Kenya (2014)
TTKTK	+*Sr31*, +*SrTmp*	2015	Kenya (2014), Egypt (2014), Eritrea (2014), Rwanda (2014), Uganda (2014)
TTHSK	+*Sr31*, -*Sr30*	2015	Kenya (2014)
PTKTK	+*Sr31*, -*Sr21*, +*SrTmp*	2015	Kenya (2014)
TTHST	+*Sr31*, -*Sr30*, +*Sr24*	2015	Kenya (2013)

[a] Some uncertainty exists over reaction of *Sr21* gene (this influences the initial code letter being "T" (+*Sr21*) or "P" (-*Sr21*). Current table presents most plausible races. * Only key *Sr* genes are indicated, not the complete virulence/avirulence profile.
Source: http://rusttracker.cimmyt.org. (Accessed on 16/6/17)

Table 1.3: Stem rust genes that are effective to at least one Ug99 race

Sr gene source	*Sr* gene
Triticum aestivum	*15*[abf], *9h*[a], *28*[a], *29*[bc], *42*[ab], *55*[bd], *57*[bd], *58*[bd], *Huw234*[ab], *ND643*[b], *Yaye*[b], *Tmp*[a]
Triticum turgidum	*2*[bd], *13*[ab], *14*[ab]
Triticum monococcum	*21*[a], *22*, *35*[a]
Triticum timopheevii	*36*[a], *37*[c]
Aegilops speltoides	*32*[c], *39*[c], *47*[e]
Aegilops tauschii	*33*[b], *45*[ab], *46*[ae], *TA10171*[e], *TA1662*[ae], *TA10187*[ae]
Aegilops searsii	*51*
Aegilops geniculata	*53*
Dasypyrum villosum	*52*
Triticum araraticum	*40*[c]
Thinopyrum elongatum	*24*[a], *25*[a], *26*, *43*[ac]
Thinopyrum intermedium	*44*[ac]
Secale cereale	*27*[a], *50*[a], *1RS(Amigo)*[ab]

[a]Genes with known virulent Ug99 races or other *Pgt* races. [b]Genes that confer inadequate resistance under high disease pressure in the field. [c]Only mutant sources of these genes without undesirable traits in the translocation must be used. [d]APR genes that confer slow rusting. [e]Field response of these genes to Ug99 still to be tested. [f]Data verification needed for this gene.

Source of data (modified): Singh *et al*. 2015.

As part of this study (Chapter 5) three genes namely *Sr25*, *Sr26* and *Sr39* that are still effective against current Ug99 pathotypes in SADC region, were introgressed into Zimbabwean wheat germplasm using Marker Assisted Backcross (MABC) method.

1.2.4.1. *Sr25*

The *Sr25* gene, closely linked to *Lr19* originated from *Thinopyrum ponticum* and was transferred to the long arm of wheat chromosome 7D (Sharma and Knott, 1966). Initially, the original translocated segment was not commercially used because of genetic drag that resulted

in undesirable yellow flour (maswheat.ucdavis.edu/protocols/sr25/index.htm 21/6/17). A mutant line "Agatha-28" containing *Sr25/Lr19* with reduced yellow colour was developed (Knott, 1980), then backcrossed into Australian wheat and the mutant has been used by CIMMYT in the variety "Wheatear". The *Sr25/Lr19* gene resulted in 10-15% yield increase in CIMMYT germplasm (Singh *et al.*, 2006b). Such germplasm containing *Sr25/Lr19*, in combination with *Sr2* was recently released in Afghanistan (Muqawim 09), Egypt (Misr 1, Misr 2), and Pakistan (NR356) (Singh *et al.*, 2011a; Pumphrey, 2012).

1.2.4.2. *Sr26*

Sr26 is effective against race TTKSK and its *Sr24* virulent derivative, TTKST (maswheat.ucdavis.edu/protocols/sr26/index.htm, 21/6/17). The gene has a low infection type of 0; to 2-. *Sr26* was introgressed into the long arm of hexaploid wheat chromosome 6A from *Thinopyrum ponticum* (Knott, 1961). It is an ideal gene for breeding purposes because of its effectiveness against the TTKSK family of races, has a low frequency among modern cultivars, availability of donor lines with reduced alien segments and existence of molecular markers (Singh *et al.*, 2011a). It has been used in Australia as a source of resistance since release of the variety Eagle in 1971 (www.globalrust/gene/sr26, 21/6/17).

1.2.4.3. *Sr39*

Sr39 is resistant to current Ug99 races (maswheat.ucdavis.edu/protocols/sr39/index.htm, 21/6/17). Resistance is incompletely dominant and the gene has low infection type of 1 to 2 (www.globalrust/gene/sr39, 21/6/17). The gene was transferred to chromosome 2B of the hexaploid wheat variety "Marquis" from *Aegilops speltoides* (Kerber and Dyck, 1990). *Sr39* is linked to an adult plant hypersensitive leaf rust resistance gene *Lr35* though the gene has not been adequately assessed for use in agriculture (www.globalrust/gene/sr39, 21/6/17). A South African line Karee*6/RL6082 (with both *Sr39* and *Lr35*) recorded a significant increase in flour water absorption compared to the recurrent parent Karee (Labuschagne *et al.*, 2002), a trait that is ideal for baking.

1.3. Global approaches in the control of Ug99 stem rust

The global threat caused by Ug99 resulted in multi-sectorial approaches being implemented to reduce the effects of stem rust. Management methods for *Pgt* include cultural, chemical and

genetic resistance. Key to implementation of these various control methods are collaborative partnerships at community, national and international levels. Coordination, information and sharing of resistant germplasm are the corner stones of these partnerships.

1.3.1. Cultural control

Use of early maturing varieties, early planting, eradication of stem rust alternate host such as barberry (*Berberis vulgaris),* destruction of volunteer plants and mixed cropping are some of the cultural methods that can be practiced. Early maturing varieties and early planting result in wheat reaching the reproductive stage and maturing during times when the disease pressure is low resulting in disease escape. Destruction of volunteer plants and barberry eradication deprives the pathogen of alternative hosts for over-seasoning, breaking the disease cycle and preventing completion of the sexual cycle, in case of the barberry host. These practices delayed disease onset, reduced initial inoculum level, reduced number and stabilized pathogen phenotypes (Roelfs, 1985; Nayar *et al*., 2002). New infections rely on exogenous inoculum that may arrive late in the season, weather conditions have to be favourable for disease increase in the source area, for spore transportation, deposition, infection in the target area and spores must have virulence for the cultivar in the target area (Roelfs, 1985). All these requirements may not be met in most cases. Continuous cropping is not ideal in stem rust management because it creates a "green-bridge" that results in build-up of endogenous inoculum. Use of more than one variety with diverse genetic backgrounds is encouraged to spread the risk of crop failure. In India, mixed cropping of wheat with other species like chickpea, lentil, pea, rapeseed, mustard, linseed and sunflower had been practiced to reduce infection but at the same time the farmer is assured of a harvest even upon failure of the main wheat crop (Nayar *et al*., 2002). Quarantine measures can only delay the disease invasion given the airborne nature of the pathogen. However, it is always good practice to wash clothes after visiting a wheat field to avoid unknowingly trans-border transportation of the pathogen. Cultural control methods are environmentally friendly but may be rendered useless by exogenous inoculum.

1.3.2. Chemical control

Fungicides can be used as foliar or seed treatments in integrated management of stem rust especially when resistant varieties are not available (Wanyera, 2008). This method of rust control was successful in Europe in the past, permitting yields of between 6-7 t/ha (Roelfs *et al*., 1992). Use of chemicals increases production costs, is not environmentally friendly and is not economically viable for resource-poor farmers in the developing world, especially in

Africa. Registered chemicals have to be applied at the right time under optimum weather conditions and farmers have to be skilled in all aspects associated with their use and application. In most cases when an outbreak occurs, industry and farmers will not have adequate quantities in stock thereby delaying the control of the disease.

1.3.3. Smart collaborative Private-Public Partnerships: BGRI

The Borlaug Global Rust Initiative (BGRI) is a good example of partnerships targeted towards the control of stem rust. It is an international consortium initiated in 2008 by ICAR, CIMMYT, ICARDA, UN-FAO and Cornell University. More than 1000 scientists from many institutions have worked towards rust control through "(i) the reduction of the world's vulnerability to leaf, stem and stripe rusts, (ii) facilitation of sustainable international partnerships to contain the threat of wheat rusts and (iii) enhancing world productivity to withstand global threats to wheat security" (www.globalrust.org, 21/6/17). BGRI strategies include monitoring the spread of Ug99, development of Ug99 resistant wheat germplasm and global distribution of resistance sources for use by various stakeholders both in public and private sectors. BGRI projects incorporate Delivering Genetic Gain in Wheat (DGGW), Global Rust Reference Centre (GRRC), Marker Assisted Selection in Wheat (MASWheat), RustTracker and WheatAtlas. Through these various projects BGRI is assisting in monitoring occurrence and frequency of *Sr* genes in the global market place.

1.3.4. Use of resistant varieties

Historically, this has been the most effective way of controlling stem rust. It is cost effective and sustainable to both the farmer and the environment since disease control is managed through the distributed seed, costs (for variety development) are spread to all stakeholders and most of the resistance can last during the lifespan of the variety if chosen sensibly and supported by research on pathogen variability. Variety breakdown is a result of inadequate knowledge of the existing virulences in the pathogen population, mutations or new recombinants in the existing pathogen population and inadequate disease screening protocols to allow identification of resistant varieties (Roelfs *et al.*, 1992). Lack of knowledge on how to utilize existing resistant genes in the gene-pool and inadequate tools to utilize these can also negatively affect the release of resistant varieties. The resistance has to be "durable" and this can only be possible if there is a sound and well managed programme on disease resistance breeding. The larger the area of cultivation of a single variety, the greater the probability that a virulent

pathotype will evolve. Variety release and distribution (to include distribution of specific R genes) must be monitored and intercepted when necessary. Durable resistance was defined by Johnson (1984) as “resistance that remains effective during prolonged and widespread use of a cultivar in an environment favourable for the disease”.

1.4. Breeding for disease resistance

McIntosh *et al*. (1988) stated that a strategy for resistance breeding encompasses monitoring pathogen variability (surveys), searching and utilization of resistance sources, breeding and commercialization of cultivars and post release monitoring of resistance. It is the understanding of the host-pathogen relationship that is critical to successfully breed for disease resistance.

1.4.1. Host-pathogen genetic studies

According to McIntosh *et al*. (1995), incompatibility between a host and a pathogen involves corresponding genes in each organism (Flor`s gene-for-gene relationship). This led to two fundamental rules which parallel the basic rules of genetics formulated by Mendel in the 19th century:

First rule: relates to single gene interactions between the host genotype and the pathogen genotype. Incompatibility occurs when a resistant host genotype interacts with an avirulent pathogen genotype resulting in a low disease response (low infection type) resulting in no disease and vice versa. Table 1.4 shows possible responses when a pathogen infects the host.

Table 1.4: Host-pathogen interaction disease responses between two homozygous host and pathogen genotypes

	Pathogen genotype	
Host genotype	AA	aa
RR	Low	High
rr	High	High

RR: resistant host genotype. rr: susceptible host genotype. AA: Avirulent pathogen genotype. aa: virulent pathogen genotype. Low: Low infection type (resistant response) and High: High infection type (susceptible).

Second Rule: relates to host-pathogen interactions involving more than one gene in both genotypes where interactions are expected.

(a) When there is complete dominance, the resultant disease response (observed phenotype) is conferred by the most effective host gene. For instance if *Sr36* has a low infection type of zero and *Sr25* with infection type 2, the *Sr36* infection type will be observed (IT of 0).

(b) When there is incomplete dominance and the response is due to additive or complementary gene effects, a whole range of responses including intermediate responses will occur.

There are two types of resistances that can be utilized by breeders namely all stage resistance (ASR, formerly seedling resistance) and adult plant resistance (APR).

1.4.1.1. All stage resistance

This type of resistance is race specific and expressed in both seedling and adult plants. Specificity derives from a gene-gene relationship (rule one) between host plant resistance gene and corresponding avirulence genes in the pathogen (Singh *et al.*, 2011b). The majority of race specific genes remain effective only for a few years when deployed at large scale due to rapid adaptation of the pathogen. This demands development of new varieties with different resistance genes to continuously replace susceptible varieties (Herrera-Foessel *et al.*, 2008; Lagudah *et al.*, 2009). Seedling host response is either susceptible or resistant depending on the infection type produced by a specific isolate. The infection type is dependent on environmental conditions, host age, host nutrition, host tissue, inoculum density and time (Roelfs *et al.*, 1992). Known gene carrying checks (known as differential sets) should be included and conditions under which the infection type was observed should be noted. According to Roelfs *et al.* (1992), seedling infection types 3 and 4 indicate susceptibility while infection types 2, X, Y and Z may provide inadequate levels of protection if disease pressure is high. Y and Z represent heterogeneous (variable-sized uredinia) infection type responses with larger uredinia at the leaf tip (Y) or at leaf base (Z), respectively. Small-to-medium-sized uredinia (with limited sporulation) and surrounded by chlorosis when inoculated with avirulent races, are characteristic of most race specific stem rust genes (Singh *et al.*, 2011a). Table 1.5 shows the seedling infection type scores for stem rust according to Roelfs *et al.* (1992) with some modifications.

Disadvantages of seedling studies include failure to measure low receptivity (reduced number of infections) and latent period duration, all useful resistances which are not expressed in seedling leaves and no established relationship between infection type and usefulness of resistance in the breeding programme (Roelfs *et al*., 1992). Ug99 (TTKSK), carries virulence for several widely used race specific genes and variants with virulence against *Sr24* (TTKST), *Sr36* (TTTSK), *Sr9h* (TTKSF+*Sr9h*) and *SrTmp* (TTKTK) have also been detected (Mago *et al*., 2009; Bhavani *et al*., 2010; Pretorius *et al*., 2012; Patpour *et al*., 2016 and Terefe *et al*., 2016). It is because of these shortcomings that adult plant infection studies are important.

Table 1.5: Stem rust host response and infection type descriptions used in this study according to Roelfs *et al*. (1992) with some modifications

Host response (class)	Infection type[a]	Description of disease symptoms
Immune	0	No visible disease symptoms
Nearly immune	;	Hypersensitive symptoms seen as necrotic/chlorotic or chlorotic flecks
Resistant	1	Small uredinia surrounded by necrosis
Moderately resistant	2	Small to medium uredinia often surrounded by chlorosis or necrosis; green island may be surrounded by chlorotic/necrotic border
Heterogeneous	X	Random distribution of variable-sized uredinia on single leaf
Moderately Susceptible	3	Medium-sized uredinia that may be associated with chlorosis
Susceptible	4	Large uredinia without chlorosis

a: Other possible modifications of infection type: = uredinia at lower size limit; - uredinia somewhat smaller than normal ; +, uredinia somewhat larger than normal ; ++, uredinia at the upper size limit; C, more chlorosis than normal; and N, more necrosis than normal. Discrete infection types on a single leaf when infected with a single biotype are separated by a comma.

1.4.1.2. Adult plant resistance (APR)

Nonspecific interactions have been referred to as adult-plant, horizontal, generalized, slow rusting, partial or minor gene or durable resistances (Roelfs *et al*., 1992; Singh *et al*., 2009). Reduction in number of pustules, or disease severity resulting in "slow rusting" is used as a measure of resistance for which *Sr2* is a good example (Singh *et al*., 2011b). Several minor genes pyramided in one variety can confer adequate levels of APR to stem rust as the "*Sr2* complex". The *Sr2* complex comprises of *Sr2*, derived from the cultivar Hope, and unknown slow rusting resistant genes from tetraploid wheat "Iumillo" (durum) transferred into hexaploid wheat varieties Thatcher and Chris (Roelfs *et al*., 1992; Njau *et al*., 2010; Singh *et al*., 2008a; 2011b). APR is governed by the second rule, as explained above. Besides *Sr2/Yr30/Lr27*, other APR genes include *Lr34/Yr18/Sr57*, *Lr46/Yr29/Sr58* and *Lr67/Yr46/Sr55* and confer slow rusting/mildewing to leaf rust, stripe rust and powdery mildew (Singh *et al*., 2011b; 2015; Li *et al*., 2016; Prins *et al*., 2016; Pretorius *et al*., 2017; Yu *et al*., 2017). Yu *et al*. (2012) observed the *csSr2* marker for *Sr2* interacting with *wPt4930* on 6BS and *wPt729773* in an unknown location suggesting gene complexity in wheat APR.

APR studies are normally field based with disease intensity notes taken at the end of the season. Field disease scores are based on two scores that are normally combined (Roelfs *et al*., 1992):

(i) Modified Cobb scale that is used to determine the percentage of tissue rusted (disease severity); and

(ii) Host response to field infection where R = Resistance, MR = Moderate Resistance, MS = Moderate Susceptible, S = Susceptible and T = Trace.

Coefficient of Infection (CI) normally used for analysis, is a value derived by multiplying disease severity and a constant for host response: where immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8 and S = 1.0 (Roelfs *et al*., 1992). Stem rust is scored on the stem leaf sheaths and true stem and disease severity on the stem is related to yield loss (Roelfs *et al*., 1992).

Most breeding programmes are focusing on durable resistance breeding in order to release resistant wheat varieties against Ug99 races. According to Singh *et al*. (1998) CIMMYT uses the following steps to generate different combinations of slow-rusting genes;

(a) Use of diverse sources of resistance in the crossing block. These sources include elite commercial varieties that have shown durable resistance and wide adaptation. Wild

relatives can be used for gene exploration in pre-breeding programmes. The objective is to limit the use of currently effective hypersensitive genes alone unless they are in combination with an adult plant resistance gene. *Sr2* in combination with these minor genes still offers protection against stem rust especially when the disease pressure is low.

(b) Wide crosses so as to increase gene diversity by introgressing other APR genes from wheat wild relatives. Resultant parents that carry new sources of resistance can be used in crosses as sources of APR genes. The objective is to retard disease progress rates by accumulating four to five minor genes into one variety resulting in negligible disease levels that are close to "complete immunity" (Singh *et al*., 2008a).

(c) Artificial inoculation of segregating populations with a prescribed pathogen. Accumulating such complex resistance is made easy by the high disease pressure that might not exist under natural conditions at some breeding sites. This phenotyping work enables identification of lines that show high levels of resistance for further evaluation.

(d) Identification and advancement of lines with low disease severity towards the end of the season. These plants will have high levels of tolerance against the existing rust races.

(e) Conducting multi-locational testing with the objective of assessing level of resistance and adaptability of the lines across environments. CIMMYT's global shuttle breeding programme between Mexico (Toluca or El Batan) and Kenya (Njoro) has been effective in transferring APR genes (minor genes) to adapted backgrounds and allowing wide adaptation of the identified material (Singh *et al*., 2015).

(f) Use existing molecular markers to track desired genes in the selected lines. Markers make it easy to simultaneously track several genes that are pyramided in a single line. For instance, the phenotypic marker of pseudo black chaff (PBC) exhibited by *Sr2* and the presence of known molecular markers for *Sr2* permits selection of plants that have the *Sr2* background.

1.5. Molecular plant breeding

Xu (2010) referred to molecular plant breeding as a "multidisciplinary field of modern plant breeding that combines molecular tools and methodologies with conventional approaches for improvement of crop plants". Conventional plant breeding involves observations, evaluation of multiple genotypes, selection of various segregating material, testing widely and data analysis. Molecular plant breeding complements these activities by availing allelic

information, DNA sequences, elucidating factors governing gene operation and associated genetic markers. These technologies have allowed breeders to be quick in increasing genetic gains, simplified some processes, improved effectiveness and efficiency of the breeding work. Molecular plant breeding is usually referred to as marker assisted breeding (MAB) and different terms are used depending on what markers are being used for. When markers are being used for line selection then it is marker assisted selection (MAS) and marker assisted backcrossing (MABC) when used in a backcross programme. MAB can be used to track introgression of disease resistance especially in breeding for APR where several minor genes have to be tracked simultaneously (Singh *et al*., 1998) including traits controlled by recessive genes such *Sr2*.

Varshney *et al*. (2009) indicated that MAB increases genetic gains in breeding programmes through (i) early seedling screening of segregants even for traits normally exhibited at adult stage (e.g. quality), (ii) screening "complex traits" such as water and heat stress and root systems, that are usually expensive, time consuming and difficult to score, and (iii) several traits can be selected simultaneously which is difficult with conventional breeding and allelic status is easily distinguished (heterozygotes/homozygotes) without progeny tests thereby saving time, effort and resources. Gupta *et al*. (1999) indicated that molecular markers can be applied in the development of genetic and physical chromosome maps, increase breeding efficiency by permitting indirect selection for some traits and they can be used in germplasm characterization, recombinant characterization, genetic diagnostics, study the whole genome of an organism and diversity studies. MAB is therefore ideal for gene pyramiding that is fundamental in APR breeding.

1.5.1. Types of genetic markers

1.5.1.1. Classical markers

These can be subdivided into three marker groups (Xu, 2010) as follows:

(i) ***Morphological markers***: visible traits that represent genetic polymorphism which can easily be seen and manipulated. Most of the morphological markers are associated with agronomic traits and can be useful for selection. Wheat examples include PBC linked to *Sr2* gene in wheat, leaf shape, leaf tip necrosis, plant height and presence or

absence of awns. Morphological markers are limited and not linked with economic traits such as yield and quality.

(ii) ***Cytological markers***: These are differences in chromosomal structural features that can be used to detect normal and mutant chromosomes.

(iii) ***Biochemical/Protein markers***: Use of isozymes (alternative forms of enzymes) in seed genetic purity is a good example. These are not frequently used because there are very few forms in most crops and most of them are stain specific for identification (Jiang, 2013). Evaluation of wheat quality based on high molecular weight glutenin subunit (HMWGS) is an example where a biochemical marker is used in plant breeding.

1.5.1.2. DNA markers

These are fragments of DNA revealing mutations/variations which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool (Jiang, 2013). These are small regions of DNA sequence that are due to base deletion, insertion and substitution, that show polymorphism between individuals. Molecular marker technology has evolved rapidly due to technological advancements. This has seen probe-based techniques such as random amplified polymorphic DNA (RAPD) becoming out-dated. Discovery of more cost effective, user friendly, flexible, reliable, high throughput and scalable molecular marker techniques such as sequence tagged site (STS), sequence characterized amplified region (SCAR), simple sequence repeat (SSR), single nucleotide polymorphism (SNPs), Diversity array technology (DArT), Expressed sequence tags (EST) and Kompetative Allele Specific PCR (KASP) has seen more wheat breeding programmes adopting marker assisted selection (MAS). The marker assisted wheat (MASWheat) website (www.maswheat.ucdavis.edu 18/8/17) shows continued use of various marker types in wheat genotyping such as KASP for *Sr2* (KASP marker *wMAS000005*), *Sr28* (*KASP_1WB1208*), SSR for *SrND643* (*gwm350*), *Sr43* (*Xcfa2040*); EST markers for *Sr25* (*BF145935*), *Sr33* (*BE405778*); CAPS marker for *Sr2* (*csSr2*) and DArT derived marker for *Sr28* (*wPt-7004-PCR*). Among the current markers in use, SNPs have been widely used with different SNP mining and detecting platforms in existence. Next generation sequencing technologies have been developed by several companies such as LGC and DArT, taking advantage of SNP markers which has taken agrigenomics to a higher level. These technologies together with wheat genome sequencing exercise have opened new avenues for crop research.

The cereals data base (CerealsDB) website (www.cerealsdb.uk.net 7/8/17) contains four SNP marker detecting platforms namely; KASP, Axiom®, iSelect and TaqMan®. DArT markers and wheat genome sequence data are also contained on the website. The KASP platform by LGC genomics has developed 8 700 markers with 7 228 of these having been validated for SNP genotyping. There are two types of SNP Axiom® platforms namely Axiom® Wheat HD Genotyping array and Breeders` Axiom® array. The former platform, contains 819 571 SNPs derived from different cultivated wheat accessions, wheat relatives, diploid and tetraploid wheat. It can be used to characterize various wheat related species and is therefore less likely to be used by breeders because of this diversity. The latter Axiom® array (breeders' array) has 35 143 SNPs that are very informative when characterizing a wide range of cultivated wheat and has been used by the Wheat Improvement Strategic Programme (WISP). The two Axiom® arrays are offered under the Affymetrix platform. SNPs iSelect by Illumina (https://www.illumina.com 17/8/17) has 81 587 markers on the array with 41 704 having been validated. ThermoFisher Scientific (www.thermofisher.com 17/8/17), formerly Life Technologies, offers the SNPs Taq®Man platform that has 4 800 markers currently available. The Australian based DArT private limited company (www.diversityarrays.com 19/8/17) offers next generation sequencing services with the objective of making these services affordable. Table 1.6 gives a summary of some of the platforms that are utilizing SNPs.

The amount of data generated by these technologies is so immense thereby presenting data management challenges to the platforms. This resulted in development of special software for molecular data analysis, viewing, storage and management. Companies have developed tailor made laboratory information management systems (LIMS) and software such as Kraken LIMS by LGC (www.lgcgroup.com 17/8/17). Axiom® `s Affymetrix software suite has user friendly consoles that allows clients to download various softwares for different uses such as Axiom analysis suite 3.0.1 (www.affymetrix.com 17/8/17). Affymetrix is a subsidiary of ThermoFisher Scientific. The urgent need to address the dwindling genetic diversity in cultivated wheat varieties as a result of hybridization, domestication (that resulted in targeted selection) and inbreeding (Winfield *et al*., 2016; Allen *et al*., 2017) has also allowed these platforms to avail their databases to the public with the aim of facilitating breeders and other researchers to make strides in developing wheat varieties that will meet future global wheat demands.

1.5.1.2.1. Characteristics of ideal DNA markers and marker detecting systems

Choice of marker system is determined by study objectives, requirements of the application, accessible facilities and skills available (Gupta *et al.*, 1999). Molecular markers that are essential in a plant breeding programme must meet most of the following requirements:

(a) High level of polymorphism to enable tracking of genes and allow the recombinant differentiation. A number of multi-parent advanced generation inter-cross (MAGIC) populations have been created by various institutions such as National Institute of Agricultural Botany (NIAB) in United Kingdom and Commonwealth Scientific and Industrial Research Office (CSIRO) in Australia that have high levels of polymorphism (Bentley and Mackay, 2017). These populations result in increased polymorphism ultimately improving mapping precision and allowing detection of more quantitative trait loci (QTL) than bi-parental populations.

(b) Polymorphism must be scattered throughout the genome for ease of identification rather than being congested in a small part of the genome.

(c) Genotyping systems must be in a position to identify both homozygotes and heterozygotes (co-dominant markers) such that recessive alleles can easily be tracked especially when dealing with recessive traits. The genotyping platform must result in easy analysis of results by having features that differentiate different alleles.

(d) Markers must be specific for certain genes rather than having pleiotropic effects.

(e) Genotyping costs are a key determining factor in fostering adoption of the technology and thus enhancement of genetic gains. The costs have to be affordable and sustainable as compared to phenotypic costs that demand multi-environment testing during a season thereby limiting the number of generations per year. Bentley and Mackay (2017) citing several researchers suggest genotypic costs to be 25% of the costs of maintaining a yield plot in order to realise genetic gains through genomic selection.

(f) Marker systems must allow detection of polymorphisms and most platforms have developed fluorescent probes that can easily be detected.

(g) Genomic marker detecting systems must be automated so that they allow many samples to be processed within a short time to enhance decision making. Automation will enhance genotyping throughput such as the use of robots to perform most of the laboratory processes that were supposed to be done manually.

(h) Genotyping must be easily accessible, flexible, scalable and suitable for multiplexing. The system must handle different sizes of assays (94, 384, 1536, cards or chips), handle

moderate to high numbers of genotyping samples and be able to accommodate several genotyping studies within the same run. ThermoFisher's Quant™ Studio 12K Flex Real-Time PCR system is a good example, handling from one to 12 000 data points in a run using different assay sizes and is compatible with a diverse range of assays from SNP genotyping to pathogen detection (www.thermofisher.com 20/8/17).

(i) The screening technique should have high reproducibility across laboratories so that protocols and marker information can be repeated and shared among users.

(j) The screening techniques must be economical and user friendly. Real-Time PCR, touch screen equipment, data analysis software, data viewing software and LIMS software all allow for handling the enormous amount of data generated during molecular marker work.

(k) Molecular markers must be genome-specific and capable of tracking genes of interest in polyploidy crops such as wheat where polymorphism can be present in homoeologous and paralogous genes in a very large genome, ≈ 17 300Mb (Allen *et al*., 2011). This complication has resulted in wheat lagging behind in utilization of MAS as compared to maize and rice.

1.5.1.2.2. Summary of the advantages of marker assisted plant breeding

(a) Early plant characterization: Presence or absence of gene of interest is determined during early stages of a breeding programme, usually at seedling stage and only plants with the desired gene are advanced to the next stage. This saves on time and costs that were supposed to be incurred during the whole season waiting for plants to express these traits at an adult stage or phenotypic multi-environment testing of material that would not be released for commercial production.

(b) Gene presence is not influenced by the environment, unlike with some morphological markers whose expression may be influenced by environmental conditions such as temperature or light.

(c) Exposure of plants to high disease pressure in order to induce disease resistance responses is eliminated. Molecular markers rely on gene presence resulting in selection being done for diseases that are not prevalent in certain areas facilitating anticipatory breeding.

(d) Increases genetic gains. As long as markers co-segregate with the desired genes (closely linked to the trait of interest), a high throughput system for marker identification is in place and is cost effective, several breeding cycles can be managed

within a single year and only plants with desired traits are advanced thereby improving selection accuracy. All these positive factors allow molecular marker assisted breeding to enhance genetic gain. The selection intensity is high under MAS because it is done on an individual plant basis rather than in multiple trial plots (Bentley and Mackay, 2017).

(e) Molecular markers allow tracking of several genes at the same time in a gene pyramiding programme. Breeders wishing to stack several genes of interest within one variety using MAB can be easily assisted in such endeavours as long as tracked genes have robust molecular markers available.

(f) Molecular markers can be used for high economic value traits that are sometimes difficult to characterize in the field. These include traits such as yield, quality and drought resistance. MAS can increase the selection response on these traits that usually have low heritability (Bentley and Mackay, 2017) by indirectly selecting for closely associated markers.

MAS must be used to complement conventional plant breeding given that genotype-environment interactions (GEI) are observed in farmers' fields that cannot be identified in the laboratory. As a result field verification will always continue to be a pre-requisite in plant breeding but chances of success are enhanced given that only MAS products are tested. The other challenge of MAS is the ability to handle and utilize the huge amount of data that is generated, at both phenotypic and genotypic levels. The quality of phenotypic data needs to be very high so that the two can complement each other in decision making. This is the reason why a considerable investment is going into automated phenotyping technologies.

Table 1.6: Summary or examples of next generation sequencing platforms utilizing SNP markers

	KASP™	Axiom®	iSelect	TaqMan®
Proprietor	LGC	Affymetrix	Illumina	ThermoFisher
Species	Any	Any	Any	Any
Utility	Whole genome	Whole genome	Whole genome	Whole genome
Probes	Fluorophore-quencher		Beads: Infinium I & II	TaqMan MGB
Assays	96, 384, 1536	96, 384	24-Sample HD & HTS	96, 384, card, plates
Multiplexing	YES	YES	YES	YES
Shelf life (Storage)	Indefinite (-80°C)		12 months	±5 yrs (-15 to -20, dark)
Genotypes[1]	SNP/Indels	SNP/Indel/OTV/CNV	SNP/Indels/CNV/SV/GV	SNP/Indels
DNA quantity	3 - 10ng	150ng	200ng	1 - 100ng
DNA quality	High	High	High	High
Mapped SNPs[2]: A	1675		15 342	1635
B	1986		21 166	1931
D	1331		5 196	1169
Softwares[3]	KlusterCaller™ SNPviewer Kraken (LIMS)	Affymetrix™ software suite Analysis Suite 3.0.1 (Genotyping console)	GenomeStudio Beeline Illumina (LIMS)	TaqMan™ GenoTyper
Diversity/genetics	YES	YES	YES	YES
Genotyping	YES	YES	YES	YES
Wheat library	> 8 000 markers	35K & 820K SNPs	3072 - 700K bead type	
Website	www.lgcgroup.com	www.affymetrix.com	www.illumina.com	www.thermofisher.com

[1]: SNP – single nucleotide polymorphism; Indels – insertion and deletions; OTV – off target variants; CNV – copy number variants; SV – structural variants; GV – germline variants. [2]: Hexaploid wheat chromosomes A, B and D. [3]: LIMS: Laboratory information management system

Source of data: proprietor websites and www.cerealsdb.uk.net (accessed on 18/7/17)

2. Current Ug99 status in Zimbabwe

2.1. Location of Zimbabwe and general land use

Zimbabwe is a land locked country in Southern Africa, 390 000 km^2 in size, situated between 15° and 22° South (latitude), 26° and 34° East (longitude) with one rainy season from November - March (www.moa.gov.zw, 6/8/17). Zimbabwe is bordered by Zambia, Mozambique, South Africa and Botswana and divided into ten administrative provinces (Harare Metropolitan, Bulawayo Metropolitan, Mashonaland East, Mashonaland Central, Mashonaland West, Manicaland, Midlands, Masvingo, Matebeleland North and Matebeleland South). There are five agro-ecological zones which are referred to as natural regions (NR I – NR V; Figure 2.1) and classification was based on rainfall, soil quality and vegetation. The quality of land resource declines from NR I to NR V (Nyamadzawo *et al.,* 2012; www.fao.org/docrep/009/a0395e/a0395e06.htm 6/8/17).

2.2. Wheat breeding and production in Zimbabwe

SC Zimbabwe Limited, Crop Breeding Institute (CBI) in the Department of Research and Specialist Services (DR&SS) of the Ministry of Agriculture, Pannar Seed and Klein Karoo (K2) are the only wheat breeding institutions in Zimbabwe. SC, a private seed company involved in breeding, evaluation and commercialization of improved varieties of major field crops including wheat, across the African continent has about 90% market share of the Zimbabwean wheat market. The company is focusing on the development of widely adapted, durable disease resistant, high yielding, excellent industrial quality and stable varieties with emphasis on maintenance of genetic diversity to counter the effects of genetic vulnerability.

Zimbabwean wheat varieties and lines are not screened regularly to assess their responses to existing stem rust pathotypes. It is important to note that long term success of breeding for disease resistance is influenced by the nature of the pathogen and diversity of virulence in the population, available diversity and type of genetic resistance, screening methodology and selection environment for tracking resistance (Jiankang *et al*., 2003). Currently none of the four institutions has the capacity to carry out regular in-house germplasm phenotyping against existing or future pathotypes due to lack of infrastructure for controlled inoculations and screening. There is an urgent need for annual rust surveys so as to keep track of changes in

rust pathotypes. SC's wheat breeding programme has been working with the University of the Free State and Agricultural Research Council (ARC)-Small Grains of South Africa on rust race analysis as part of regional rust surveillance. Variety genotyping, rust surveillance through annual surveys, rust race analysis, release of resistant varieties based on "adult plant resistance" with *Sr2* as the foundation and incorporation of marker assisted breeding (MAB) should be key elements of Zimbabwean wheat breeding programmes. These activities will minimize the severe effects of Ug99-related races (TTKSK) and other stem rust strains.

Wheat is the second, after maize, most important cereal in Zimbabwe with production being done under irrigation during the dry months from May to October when harvesting is done before the early summer rains to avoid pre-harvest sprouting. Wheat is produced across all five agro-ecological zones of the country but most of the wheat area is concentrated in NR I, II and III where A2 (small scale commercial farmers) and large scale commercial farmers are the main players. In NR IV and V there are patches of wheat production mainly in irrigation schemes and on estates owned by Agricultural Rural Development Authority (ARDA), a parastatal institution responsible for food security. Crop production is financed through individuals, contract farming schemes by agro-dealers (farmer groupings), agro-industry (millers and bakers) and government sponsored programmes such as the "2017 Command Agriculture" programme.

Wheat production in Zimbabwe, in the last ten years (2004 to 2014), has generally declined with 2009 experiencing the lowest production of 12 000 metric tonnes (www.wheatatlas.org, 13/7/17; Figure 2.2). The production decline has been attributed to several factors including unreliable power supplies for irrigating the crop, dilapidated irrigation infrastructure, late payments by the Grain Marketing Board (GMB) and lack of long term financial support services to farmers (disinvestment) and general decline in national economic activities (Mutambara *et al*., 2013). On the other hand, there is a steady increase in wheat grain demand estimated to be 300 000 to 400 000 metric tonnes/year, as a result of urbanization and change in consumer tastes. Zimbabwe is currently a net importer of wheat grain due to the widening gap between demand and production.

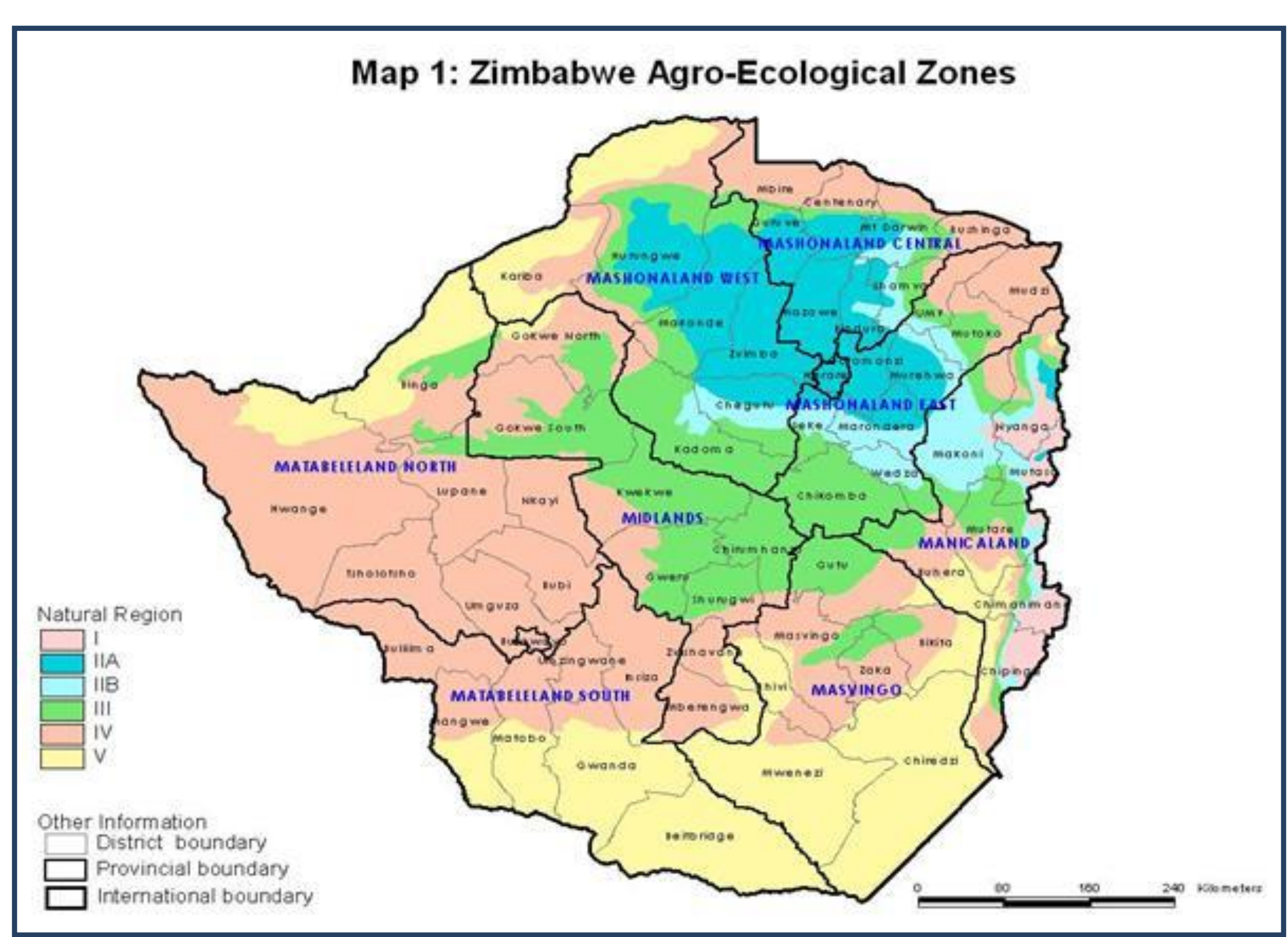

Figure 2.1: Five natural regions and ten administrative provinces of Zimbabwe

Source: www.fao.org/docrep/011/ai483e/ai483e00.htm, (Accessed on 6/8/17)

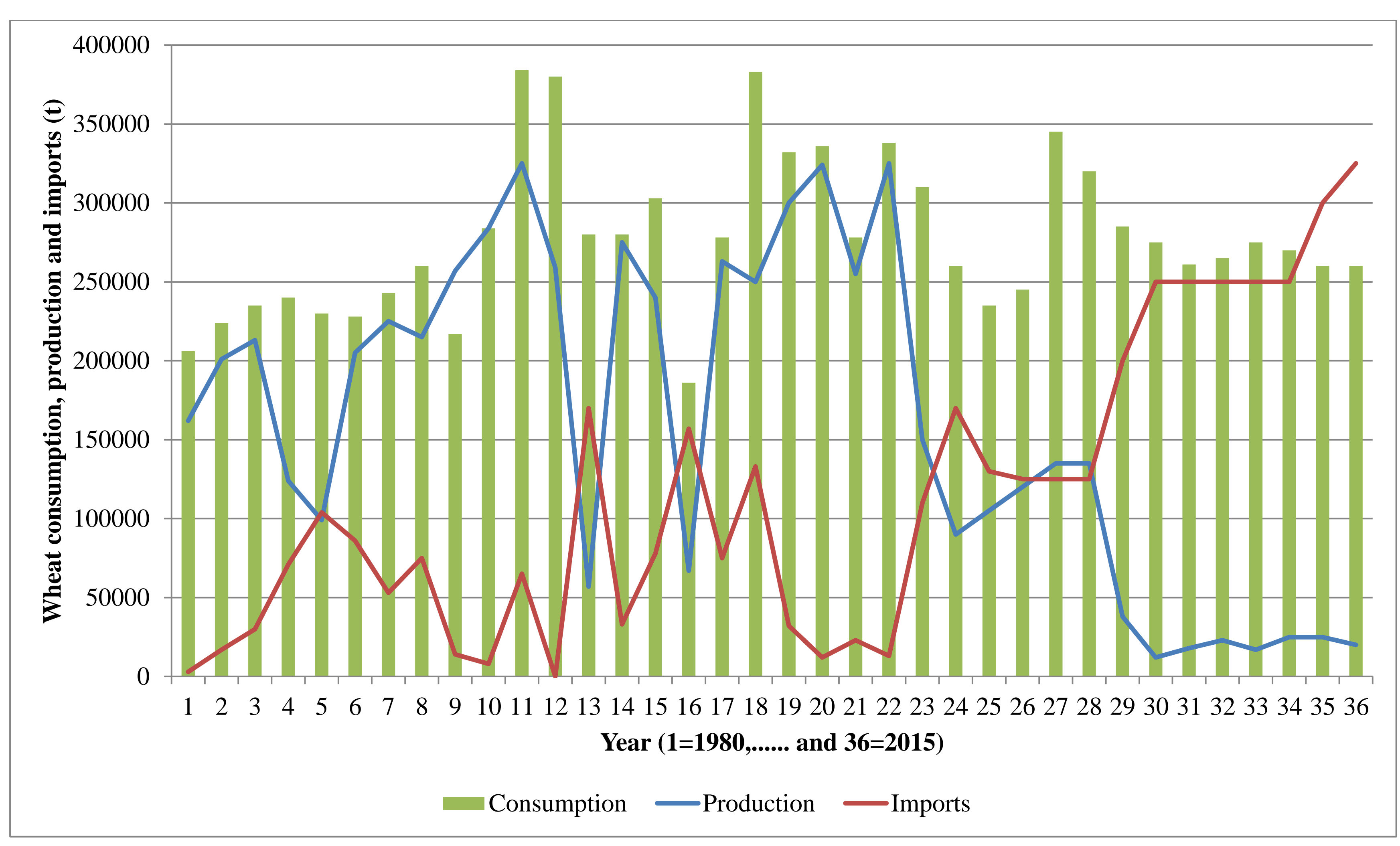

Figure 2.2: Zimbabwe wheat production, consumption and importation statistics

Source: www.wheatatlas.org/country/consumption/ZWE/0 (Accessed on 13/7/2017).

2.3. Prevalence of Ug99 stem rust in Zimbabwe

Three Ug99 race related strains, TTKSF, TTKSF+*Sr9h* and PTKST have been identified in Zimbabwe since 2009 (Chapter 1, Figure 1.1). TTKSF (avirulent on *Sr31*) was first detected in 2009 and is also the most prevalent race in South Africa since its detection in 2000 (Pretorius *et al*., 2007a). PTKST (virulent on both *Sr31* and *Sr24*) was first detected in 2010 (Mukoyi *et al.*, 2011) and puts Southern African wheat varieties with 1B.1R translocation to risk of an epidemic. Race TTKSF+*Sr9h* (avirulent on *Sr31*) was also detected in 2010 from a sample collected at Birchenough Bridge Irrigation scheme in Zimbabwe and was the eighth Ug99 race after it also severely affected the South African winter wheat variety Matlabas (Pretorius *et al.*, 2012). The presence of these three Ug99 variants in Zimbabwe emphasizes the variability within this internationally important group and their similarity to the South African races indicates the role played by wind movements between Zimbabwe and South Africa.

According to Pretorius *et al*. (2007a) South Africa initiated stem rust pathotyping in the 1920s, but regular surveillance was discontinued until 1960s when it resumed. Standard *Pgt* races identified between 1922 and 1973 included races 11, 13, 15-17, 21, 29, 34, 38, 95, 98-100, 192, 194, 222, 232 and 279. Races 21, 34, 194, 222 and 279 were also found in Zimbabwe during the 1960s whereas races 21, 34, 194 and 222 were reported in Mozambique in 1970. Therefore the rust information on responses of Zimbabwean wheat varieties to the four Ug99 strains will aid in the regional strategy of deploying Ug99 resistant genes into wheat varieties in order to curb the disease scourge.

2.4. Genotyping work by CenGen (Pty) Ltd in 2012

Prior to this study, molecular analysis was done in 2012 by CenGen using 49 Zimbabwean wheat lines. The objective was to determine critical rust genes (both leaf and stem rust genes) that were present in the germplasm. CAPS marker *csSr2* (Table 2.1) was used for *Sr2* with Pavon 76 as the check variety, *iag95* marker and Federation*4/Kavkaz were used for *Sr31*, markers *wmc477* and *stm773-2* with SrTt1 as the check variety were used for *Sr36* and for *Sr24* marker *Sr24#50* was used with Palmiet as the check variety. A total of 49 wheat genotypes were used in the genotyping work (Table 2.2).

Table 2.1: Markers, primers and their sequences used by CenGen during 2012 genotyping work for rust genes in Zimbabwean lines

Gene	Marker (Technique)	Primer name	Sequence (5`…….3`)	Reference(s)
Sr2	CAPS	*csSr2*-F	CAAGGTTGCTAGGATTGGAAAAC	Mago *et al*. (2011);
		csSr2-R	AGATAACTCTTATGATCTTACATTTTTCTG	maswheat.ucdavis.edu 28/6/17
Sr24	STS	*Sr24#50*-F	CCCAGCTCGGTGAAAGAA	Mago *et al*. (2005);
		Sr24#50-R	ATGCGGAGCCTTCACATTTT	maswheat.ucdavis.edu 7/8/17
Sr31	STS	*iag95*	CTCTGTGGATAGTTACTTGATCGA	Mago *et al*. (2002)
			CCTAGAACATGCATGGCTGTTACA	
Sr36	Microsatellites	*wmc477*-F	CGTCGAAAACCGTACACTCTCC	maswheat.ucdavis.edu 7/8/17
		wmc477-R	GCGAAACAGAATAGCCCTGATG	Hayden and Sharp (2001)
		stm773-2F	ATGGTTTGTTGTGTTGTGTGTAGG	
		stm773-2R	AAACGCCCCAACCACCTCTCTC	

F: Forward primer. R: Reverse primer. *Sr*: stem rust gene designation.. CAPS: Cleaved amplified polymorphic sequence. STS: Sequence-tagged-site. DNA strand sequence from 5` to 3` end.

Table 2.2: List of 49 Zimbabwean wheat lines genotyped by CenGen in 2012 and 45 genotyped by Limagrain in 2015 (excluding those with *)

No	Entry	Status	Source
1	SC1	Commercial	Seed-Co
2	SC2	Commercial	Seed-Co
3	SC3	Commercial	Seed-Co
4	SC4	Commercial	Seed-Co
5	SC5	Commercial	Seed-Co
6	SC6	Commercial	Seed-Co
7	SC7	Commercial	Seed-Co
8	SC8	Commercial	Seed-Co
9	SC9	Commercial	Seed-Co
10	SC10	Commercial	Seed-Co
11	SC11	Commercial	Seed-Co
12	SC12	Commercial	Seed-Co
13	SC13	Old	Seed-Co
14	CBI1	Old	CBI
15	CBI2	Old	CBI
16	CBI3	Old	CBI
17	CBI4	Old	CBI
18	SC14	Experiment	Seed-Co
19	SC15	Experiment	Seed-Co
20	SC16	Experiment	Seed-Co
21	SC17	Experiment	Seed-Co
22	SC18	Experiment	Seed-Co
23	SC19*	Experiment	Seed-Co
24	CBI5	Commercial	CBI
25	CBI6	Commercial	CBI
26	CBI7	Commercial	CBI
27	CBI8	Commercial	CBI
28	PAN1	Commercial	Pannar
29	SC20	Old	Seed-Co
30	SC21	Experiment	Seed-Co

No	Entry	Status	Source
31	SC22	Experiment	Seed-Co
32	SC23	Experiment	Seed-Co
33	SC24	Experiment	Seed-Co
34	SC25	Experiment	Seed-Co
35	SC26	Experiment	Seed-Co
36	SC27	Experiment	Seed-Co
37	SC28	Experiment	Seed-Co
38	SC29	Experiment	Seed-Co
39	SC30	Experiment	Seed-Co
40	SC31*	Experiment	Seed-Co
41	G1	Commercial	Zambia
42	SC32	Experiment	Seed-Co
43	SC33	Experiment	Seed-Co
44	SC34	Experiment	Seed-Co
45	SC35	Experiment	Seed-Co
46	SC36	Experiment	Seed-Co
47	SC37*	Experiment	Seed-Co
48	SC38*	Experiment	Seed-Co
49	SC39	Experiment	Seed-Co

2.4.1. CenGen genotyping results

The CenGen results revealed presence of *Sr2*, *Sr24*, *Sr31*, *Sr 36* and some unknown *Sr* genes within the gene-pool. *Sr2* in combination with *Sr31* and/or other unknown minor *Sr* genes are responsible for resistance shown by some commercial varieties such as SC8 and SC2 against current Ug99 pathotypes in Zimbabwe. Figure 2.3 summarizes the distribution of *Sr* genes within the analyzed Zimbabwean germplasm, and Figure 2.4 presents the status of Zimbabwean stem rust genes in each variety. For genes occurring singly in varieties, *Sr2* and *Sr36* had a frequency of 2%, with SC13 and CBI1 being the sole candidates for each gene. *Sr31* and *Sr24* alone had frequencies of 14.3% and 4.1%, respectively. Unknown genes had the highest frequency of 20.4% of the germplasm. A total frequency of 57.1% represented lines with various combinations of the three genes (*Sr2*, *Sr24* and *Sr31*) and unknown stem rust genes that were not identified by markers used (Figure 2.3 and Table 2.3).

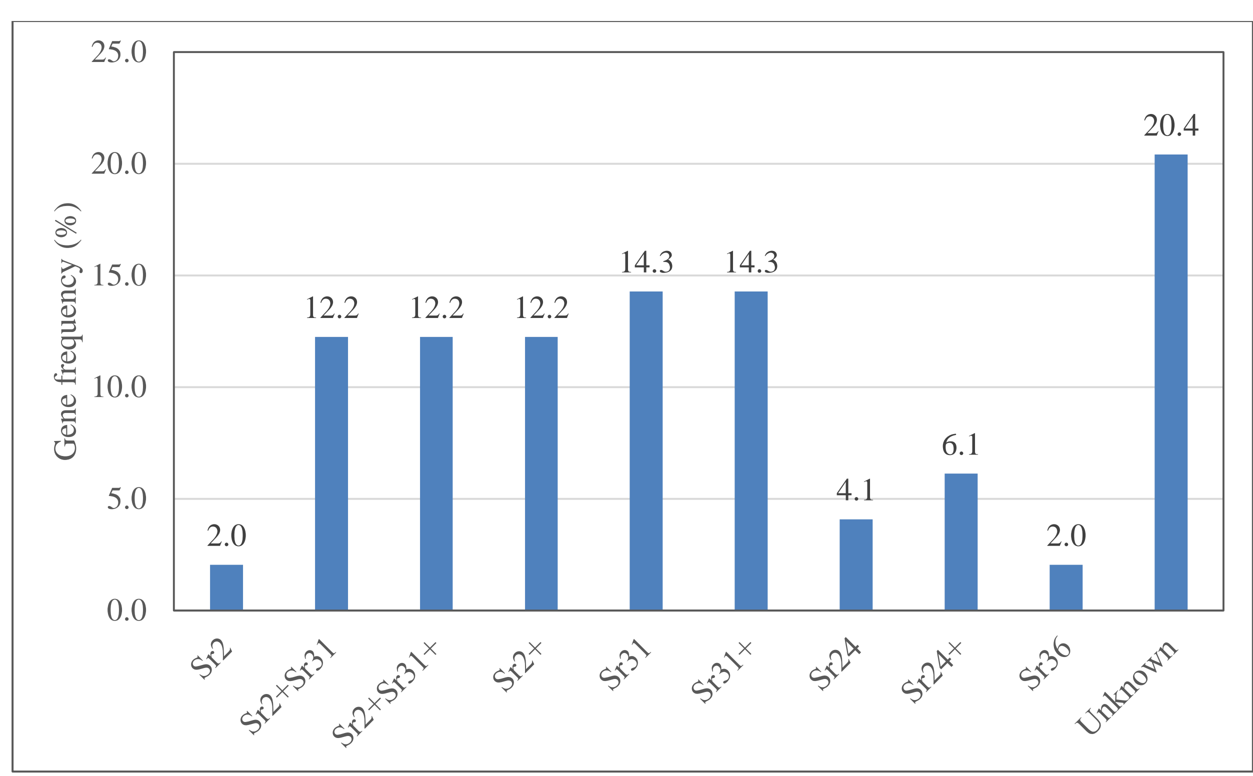

Figure 2.3: Distribution (%) of stem rust resistance genes among 49 Zimbabwean wheat lines as genotyped by CenGen in 2012

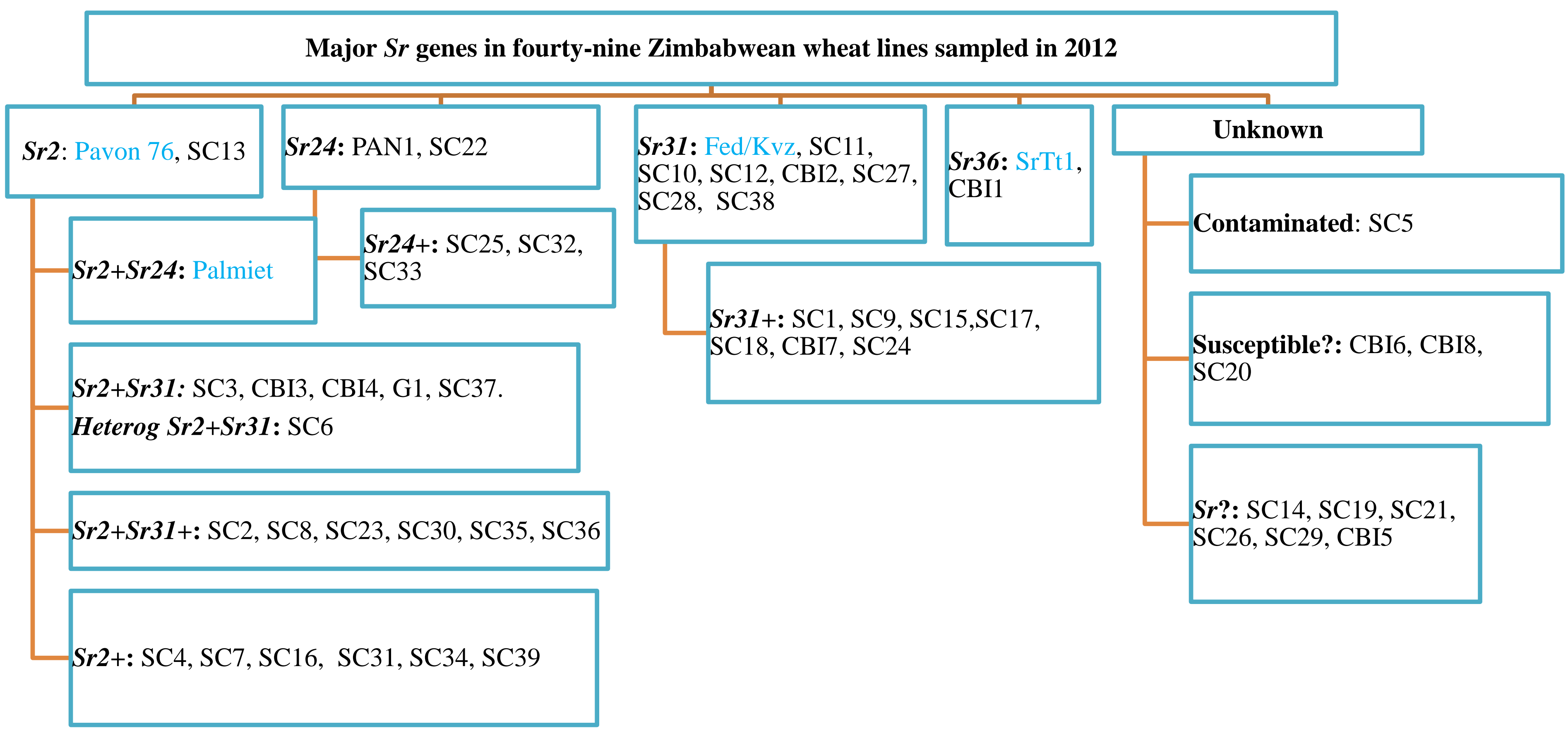

Figure 2.4: Status of stem rust resistance genes within the 49 Zimbabwean wheat lines as genotyped by CenGen in 2012

Molecular markers used during genotyping: *csSr2* (*Sr2*), *Sr24#50* (*Sr24*), *iag95* (*Sr31*), *wmc477* and *stm773-2* (*Sr36*)

2.4.1.1. *Sr2*

Sr2 has been used in breeding as a source of durable and broad-spectrum adult plant resistance (APR) including against Ug99 related isolates since the release of a wheat variety called Hope by McFadden (www.globalrust.org/gene/sr2, 21/6/17). Hope was a product of a stem rust resistant tetraploid emmer wheat (*Triticum turgidum*) Yaroslav and Marquis (susceptible hexaploid wheat). *Sr2* was mapped onto chromosome 3BS (Knott 1971; Hare and McIntosh, 1979) and the gene has two important properties of conferring partial resistance when alone (especially when infection occurs later in the season) and boosting levels of resistance of other resistance genes (R-genes) when in combination (Ellis *et al*., 2014). Mode of resistance for *Sr2* was initially thought to be recessive but recent work has described it as "partially hemizygous effective" because of its ability to positively enhance resistance levels of other genes (Ellis *et al*., 2014). It is closely linked with a gene that confers pseudo-black chaff (PBC), which involves melanin pigmentation of the glumes and stem particularly below the uppermost node (McIntosh, 1988; Singh *et al*., 2011a). The PBC trait linked to *Sr2* is still being used as a morphological marker in breeding. Pavon 76 (Figure 2.4) is one of the varieties that carry the gene. SC13, an old Zimbabwean commercial variety also carries *Sr2*. Pyramiding several minor APR genes onto *Sr2* background tends to give durable resistance against most stem rust pathotypes and this has been the global push for breeding programmes to implement. Rajaram and Hettel (1995) reported "*Sr2* gene complexes" that consisted of *Sr2* plus eight to ten genes pyramided in three-to four-gene combinations with *Sr2* serving as the "backbone" gene. Singh *et al*. (2011a) reported that *Sr2* conferred inadequate protection on its own in field evaluations in Kenya. *Sr2*, *Sr7b*, *Sr17* and *Sr9b* were the basis of resistance in Hope, *Sr2* and *Sr17* provided protection against stem rust in Australia, CIMMYT's green revolution cultivars (post 1940) often had *Sr2* and *Sr31* in combination (before emergence of Ug99) while post Ug99 period has seen *Sr2* being stacked with four to five APR genes or other effective R genes (Ellis *et al*., 2014; Singh *et al*,. 2008a). It was the objective of this study to introgress effective *Sr* genes into SC *Sr2* varieties. Zimbabwean wheat germplasm had a 38.7% frequency for *Sr2* alone or in combination with *Sr31* and other unknown genes.

2.4.1.2. *Sr24*

Sr24 is effective against the original Ug99 (TTKSK) strain but ineffective against TTKST (www.globalrust.org/gene/*sr24* 21/6/17) or some of the other members of this race group. It is found on chromosome 3DL within a translocation of the 3Ag chromosome from *Agropyron*

elongatum. Originally, *Sr24* was linked to a red grain colour gene until 1973 when Sears developed lines with a smaller *A. elongatum* translocation segment and broke this linkage. *Sr24* with a shorter arm could now be used for introgression into white grained wheats. In the variety Amigo, *Sr24* is located on chromosome 1BS instead of 3D. The two recombinant types of *Sr24* are both linked to *Lr24* which is found in *A. elongatum*. *Sr24* was reported in 10.2% of Zimbabwean varieties either alone or in combination with unknown *Sr* genes. The gene is associated with a typical low infection type as described by McIntosh *et al*. (1995).

2.4.1.3. *Sr31*

Much of the world resistance to stem rust in wheat was based at least on the *Sr31* gene (Pretorius *et al*., 2000) until the emergence of Ug99 (TTKSK) in 1998 when all entries known to carry only the 1BL.1RS chromosome translocation containing the *Sr31*, *Lr26* and *Yr9* genes of rust resistance were defeated. The gene was derived from rye (*Secale cereale*) cv. Petkus (Jin and Singh, 2006). *Sr31* is located on 1BS chromosome (1BL.1RS translocations) or 1R (1B substitution) (McIntosh *et al*., 1995). Newcomb *et al.* (2016) reported high infection types that were above 3 for *Sr31* lines inoculated with Ug99 strains TTKSK (3+), TTKST (3+), TTTSK (4), TTKTK (3+), TTKTT (3+) and TTHST (3+). *Sr31* is susceptible to 11 current Ug99 pathotypes and only resistant to two (TTKSF+*Sr9h* and TTKSP) (www.globalrust.org/gene/*sr31* 16/6/17). The presence of *Sr31* is readily confirmed by the concurrent presence of *Lr26* and *Yr9* (www.globalrust.org/gene/*sr31*). CIMMYT varieties such as Bacanora 81, Bobwhite, Genaro 81 (=Veery#3), Glennson 81 (=Veery#1) and Seri 82 (=Veery#5) contain *Sr31* (McIntosh *et al*., 1995). A South African variety, Gamtoos (=Veery#3) also has *Sr31*. Zimbabwean varieties with *Sr31* (Figure 2.4) include SC1, SC12, SC11, CBI2, SC9, CBI7, SC10 and SC15. Purnhauser *et al*. (2011) observed that 24.1% of 156 varieties tested in Hungary contained *Sr31*. *Sr31* has been used in spring, winter and facultative wheats through the widespread use of the Russian and other East European wheat varieties Kavkaz, Aurora and Loverin that originally carried the 1BL.1RS wheat rye translocation. The international, widespread use of *Sr31* wheat material is accredited to their wide agronomic adaptability and high yield rather than the unique contribution of stem rust resistance (www.globarust.org/gene/*sr31*). About 90% of wheat varieties grown worldwide were susceptible to the Ug99 group of races (Singh *et al*., 2011a). Baking quality problems associated with *Sr31* have been reported before and the gene was not widely used commercially in Australia where only two varieties ("Grebe" for biscuits and "Warbler" for feed wheat) were

released (McIntosh *et al*., 1995). *Sr31* alone or in combination with other *Sr* genes constituted 53.1% of Zimbabwean wheat varieties.

2.4.1.4. *Sr36*

The gene is located on chromosome 2BS and was derived from *Triticum timopheevii*. Newcomb *et al.* (2016) recorded low infection types (0) during the seedling stages when entries with *Sr36* were inoculated with TTKSK, TTKST, TTKTK, TTKTT and TTHST while TTTSK resulted in a high infection type score of 4. *Sr36* seedling infection type may be influenced by environmental conditions and wheat lines with the gene were described as having low receptivity to pathotypes normally considered virulent (www.globalrust.org/gene/*sr36*). *Sr36* has been an important gene for Australian wheat production while resistance in soft winter wheat in the USA is primarily due to *Sr36* (Olson *et al*., 2010). Examples of *Sr36* varieties include an Australian variety Mendos; South African varieties Dipka, Flamink, Gouritz, SST101 and SST107 and Mexican variety Zaragoza 75 (McIntosh *et al*., 1995). CBI1 (2%) is the only variety with *Sr36* in the Zimbabwean genepool tested (Figure 2.4).

Table 2.3: Summary of genotyping results by CenGen using 49 Zimbabwean wheat lines in 2012

Entry	**Status**	**Source**	***Sr2***	***Sr24***	***Sr31***	***Sr36***	***Lr34/Yr18***	***Sr* genes**
SC1	Commercial	SC	-	-	+	-	-	*Sr31+*
SC2	Commercial	SC	+	-	+	-	-	*Sr2+31+*, mixed for *Sr*
SC3	Commercial	SC	+	-	+	-	-	*Sr2+31*
SC4	Commercial	SC	+	-	-	-	-	*Sr2+*
SC5	Commercial	SC	-	-	-	-	-	Unknown
SC6	Commercial	SC	+/-	-	+	-	-	*Heterog Sr2,* also *Sr31*
SC7	Commercial	SC	+	-	-	-	-	*Sr2+*,
SC8	Commercial	SC	+	-	+	-	-	*Sr2+31+*, mixed for *sr*
SC9	Commercial	SC	-	-	+	-	-	*Sr31+*
SC10	Commercial	SC	-	-	+	-	+	*Sr31*
SC11	Commercial	SC	-	-	+	-	+	*Sr31*
SC12	Commercial	SC	-	-	+	-	-	*Sr31*
SC13	Old	CBI	+	-	-	-	+	*Sr2*
CBI1	Old	CBI	-	-	-	+	-	*Sr36*
CBI2	Old	CBI	-	-	+	-	-	*Sr31*
CBI3	Old	CBI	+	-	+	-	-	*Sr2+31*
CBI4	Old	CBI	+	-	+	-	-	*Sr2+31*
SC14	Experiment	SC	-	-	-	-	-	Unknown
SC15	Experiment	SC	-	-	+	-	-	*Sr31+*

Entry	**Status**	**Source**	***Sr2***	***Sr24***	***Sr31***	***Sr36***	***Lr34/Yr18***	***Sr* genes**
SC16	Experiment	SC	+	-	-	-	-	*Sr2+*
SC17	Experiment	SC	-	-	+	-	-	*Sr31+*
SC18	Experiment	SC	-	-	+	-	-	*Sr31+*
SC19	Experiment	SC	-	-	-	-	-	Unknown
CBI5	Commercial	CBI	-	-	-	-	-	Unknown
CBI6	Commercial	CBI	-	-	-	-	-	Unknown
CBI7	Commercial	CBI	-	-	-/+	-	-	mixed for *Sr31+*
CBI8	Commercial	CBI	-	-	-	-	-	Unknown
PAN1	Commercial	Pannar	-	+	-	-	-	*Sr24*
SC20	Old	SC	-	-	-	-	-	Unknown
SC21	Experiment	SC	-	-	-	-	-	Unknown
SC22	Experiment	SC	-	+	-	-	-	*Sr24*
SC23	Experiment	SC	+	-	+	-	-	*Sr2+31+*
SC24	Experiment	SC	-	-	+	-	+	*Sr31+*
SC25	Experiment	SC	-	+	-	-	-	*Sr24+*
SC26	Experiment	SC	-	-	-	-	-	Unknown
SC27	Experiment	SC	-	-	+	-	-	*Sr31*
SC28	Experiment	SC	-	-	+	-	-	*Sr31*
SC29	Experiment	SC	-	-	-	-	-	Unknown
SC30	Experiment	SC	+	-	+	-	-	*Sr2+31+*

Entry	Status	Source	*Sr2*	*Sr24*	*Sr31*	*Sr36*	*Lr34/Yr18*	*Sr* genes
SC31	Experiment	SC	+	-	-	-	-	*Sr2*+
G1	Commercial	Zam	+	-	+	-	-	*Sr2+31*, mixed
SC32	Experiment	SC	-	+	-	-	-	*Sr24*+
SC33	Experiment	SC	-	+	-	-	-	*Sr24*+,
SC34	Experiment	SC	+	-	-	-	-	*Sr2*+
SC35	Experiment	SC	+	-	+	-	-	*Sr2+31*+
SC36	Experiment	SC	+	-	+	-	-	*Sr2+31*+
SC37	Experiment	SC	+	-	+	-	-	*Sr2+31*
SC38	Experiment	SC	-	-	+	-	-	*Sr31*
SC39	Experiment	SC	+	-	-	-	-	*Sr2*+

SC: Seed-Co line; CBI: Crop Breeding Institute; Zam: Variety once grown in Zambia; Checks (Not included) : Palmiet (*Sr2, 24*); Federation*4/Kavkaz (*Sr31*); SrTt1 (*Sr36*); Pavon 76 (*Sr2*); Kariega (*Lr34/Yr18*); +/-: Presence/Absence; *Sr24*+: Stem rust gene 24 and unknown genes; *Sr*: Stem rust gene designation; *Lr*: Leaf rust gene designation and *Yr*: Yellow (stripe) rust gene

2.5. Limagrain genotyping work in 2015

SC was over reliant on conventional breeding without data on molecular characterization of wheat germplasm. As part of molecular characterization to complement work done by CenGen in 2012, 94 wheat lines (including 45 genotyped by CenGen) were genotyped by the Limagrain laboratory in Chappes, France in 2015. Missing from the list that was genotyped by CenGen in 2012 were SC19, SC31, SC37 and SC38. CenGen did not genotype for *Lr19/Sr25* in 2012. The lines were genotyped for five groups of traits namely (a) quality (b) plant height and development (c) disease resistance, (d) insect resistance and (e) other traits. Among disease resistance genotyping platforms were leaf rust (*Lr34/Yr18*, *Lr24/Sr24* and *Lr19/Sr25*), stripe rust (*Yr15*, *Yr7*, *Yr10*, *Yr26* and *Yrorigin1*) and stem rust (*Sr2/Lr27*). Stripe rust results were not presented in this study. KASP markers were used for all leaf rust genes and a real time marker was used for *Sr2*/*Lr27* gene (Table 2.4).

Table 2.4: Stem and leaf rust genes genotyped by Limagrain in 2015

Gene	Number of markers	Marker type	Marker code
Sr2/Lr27	1	Real time	W-0099226
Lr19/Sr25	1	KASP	WC-0212347
Lr24/Sr24	1	KASP	WC-0176459
Lr34/Yr18	2	KASP	WC-0099155, WC-0099156

Sr=stem rust gene designation, Lr=leaf rust gene designation, Yr=yellow rust gene designation, KASP=kompetitive allele specific PCR genotyping system

2.5.1. Results of genotyping work by Limagrain

Results revealed the existence of *Lr19/Sr25* within the Zimbabwean wheat germplasm. This agreed with what was reported by Pretorius *et al*. (2015) when 18 out of 49 Zimbabwean wheat lines that were resistant to SFDS, CCPS, MCDS, FBPT, SCDS and TCPS leaf rust strains, tested positive for $Lr19STS_{130}$ marker for *Lr19*. Mtisi and Mashiringwani (1988) reported breakdown of *Lr9* and *Lr19* against leaf rust races in Zimbabwe when they tested the resistance of 16 wheat lines that had a total of 25 leaf rust single genes at three locations from 1984 to 1986. This indicates the continued presence of *Lr19* within the Zimbabwean germplasm. *Sr25* as described in Chapter 1 is still effective against Ug99 races that are prevalent in Southern Africa. A non-Ug99 race virulent for *Sr25*, PKTSC, has been detected in India's Nilgiri Hills in 2007 (Singh *et al*. 2011a). *Lr19/Sr25* alone had a frequency of 6.6% and in combination with

Sr2 occurred in 33.3% of Zimbabwean germplasm. *Sr2* alone was postulated to be in 44.4% of the germplasm while in combination with *Sr24* it was in 11.1% of the lines. Wheat lines whose *Sr* genes were not amplified by used markers constituted 4.4%. Leaf rust gene *Lr34/Yr18* was also found within the Zimbabwean wheat germplasm. Figure 2.5 shows the distribution of the stem rust genes within the Zimbabwean germplasm according to Limagrain genotyping work while Table 2.5 summarizes the genotyping results for the different lines.

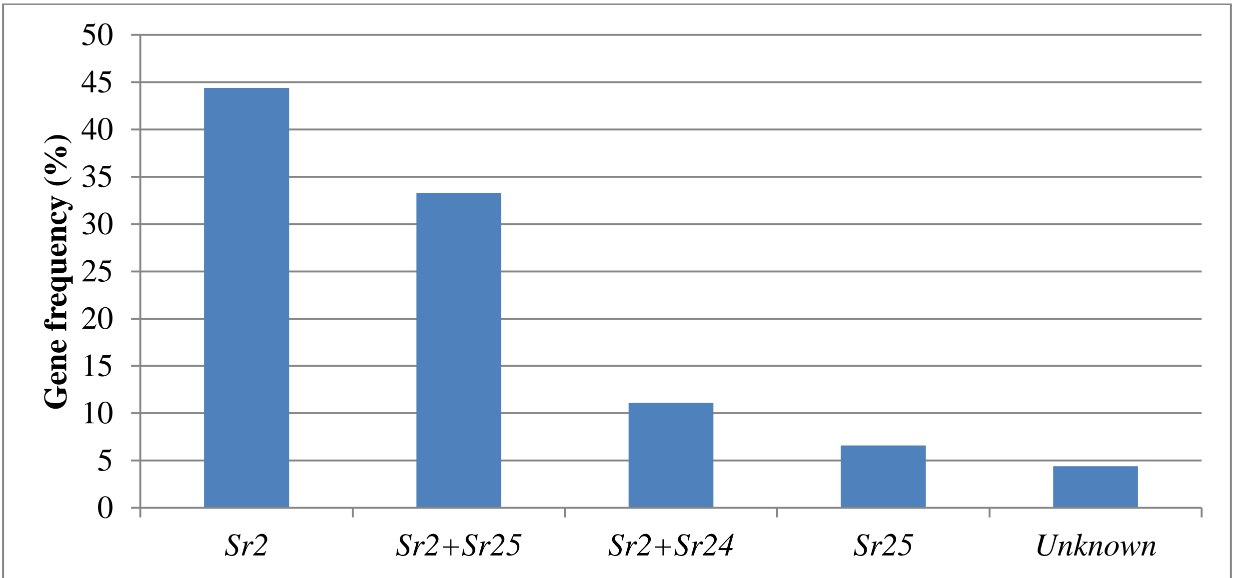

Figure 2.5: Distribution (%) of stem rust resistance genes among the 45 Zimbabwean wheat lines as genotyped by Limagrain in 2015

Unknown=no gene amplification was reported

Table 2.5: Summary of genotyping results by Limagrain in 2015 using 45 Zimbabwean wheat lines

Entry	***Lr34/Yr18***	***Lr24/Sr24***	***Lr19/Sr25***	***Sr2/Lr27***
SC1	-	-	-	+
SC2	-	-	+	+
SC3	-	-	-	+
SC4	-	-	+	+
SC5	-	-	-	+
SC6	-	-	-	+
SC7	-	-	-	+
SC8	-	-	+	+
SC9	-	-	-	+
SC10	+	-	-	-
SC11	+	-	-	+
SC12	-	-	-	+
SC13	+	-	-	+
CBI1	-	-	-	+
CBI2	-	-	-	-
CBI3	-	-	-	+
CBI4	-	-	-	Het
SC14	-	-	+	-
SC15	-	-	+	+
SC16	-	-	+	+
SC17	-	-	+	+
SC18	-	-	+	+
CBI5	-	-	-	+
CBI6	-	-	-	+
CBI7	Het	-	-	+
CBI8	-	-	-	+
PAN1	Het	Het	-	Het
SC20	-	-	-	+
SC21	-	-	+	-
SC22	-	Het	-	+

Entry	*Lr34/Yr18*	*Lr24/Sr24*	*Lr19/Sr25*	*Sr2/Lr27*
SC23	+	-	Het	-
SC24	+	-	Het	Het
SC25	-	Het	-	+
SC26	-	-	-	+
SC27	-	-	+	+
SC28	-	-	-	+
SC29	-	-	+	+
SC30	-	-	+	Het
G1	-	-	-	Het
SC32	-	Het	-	+
SC33	-	Het	-	+
SC34	-	-	+	+
SC35	-	-	+	+
SC36	-	-	+	+
SC39	-	-	+	Het
W6979	+	-	-	-
Agatha/9#LMPg6*	-	-	+	-
*WBC08 Row 2**	-	Nr	-	-
25#2/163*	-	Nr	-	+

* Control lines: Agatha/9#LMPg6 (*Sr25*); WBC08 Row *2* (*Sr26*), 25#2/163 (*Sr39*). Real time marker *W-0099226* used for *Sr2* gene; +/- presence or absence of gene; Het: Heterozygotes; *Sr*: Stem rust gene designation; *Lr*: Leaf rust gene designation and *Yr*: Yellow (stripe) rust gene designation. Nr: not reported.

2.6. Summary on effective Ug99 stem rust genes in Zimbabwe

Lack of information on stem rust has been a major challenge in Zimbabwe. Official confirmation of the three Ug99 races in Zimbabwe are 2009 (TTKSF) and 2010 (PTKST and TTKSF+*Sr9h*) (http://www.rusttracker.cimmyt.org 16/6/17) but Ug99 could have been there by 2002. A newly released spring wheat line by CBI called "Busi" that was under foundation seed production by ARDA Seeds at ARDA Sisi estate in Raffingora was written off due to stem rust (unpublished). This prompted Seed Services to deregister the variety before it could reach the market.

The presence of TTKSF (*Sr31* avirulent), TTKSF+*Sr9h* (*Sr31* avirulent, *Sr9h* virulent), PTKST (*Sr31* and *Sr24* virulent, *Sr21* avirulent) in both Zimbabwe and South Africa and TTKSP (*Sr31* avirulent and *Sr24* virulent) in South Africa alone, exposes the Zimbabwean wheat industry. TTTSK virulent to *Sr36* was also detected in Kenya in 2007 (Singh *et al*. 2008a). Currently *Sr2, Sr24, Sr25, Sr31* and other unknown *Sr* genes are present in the Zimbabwean wheat genepool. It is the presence of *Sr25* combined with *Sr2* that has conferred resistance responses in varieties like SC8, SC2 and SC4. *Sr2* offers a good foundation for gene pyramiding in response to Ug99. The discrepancy in *Sr2* frequency by Cengen in 2012 and Limagrain results in 2015 might be due to the use of different seed sources. The seed used in 2015 by Limagrain was produced by cycles of selfing the 2012 source and contamination could have occurred in the process. The pace at which Ug99 is mutating into more virulent races makes it necessary for Zimbabwean wheat breeding programmes to introgress more stem rust resistant genes in order to widen the genetic base. These sources of resistance are globally and publicly available through collaborative partnerships.

The purpose of this chapter is to give an outline of common protocols that were repeated in the five study projects.

3.1. Seedling infection type studies

This procedure was followed to establish the seedling host responses after being inoculated by various Ug99 stem rust pathotypes. Plastic pots (10 cm diameter) were filled with a steam-sterilized soil-peat mixture. Five entries of experimental lines were planted in each pot. Five to ten seeds per entry were planted at each planting station in the pot. Four pots were placed per 30 cm diameter plastic tray to allow watering from below. The potting mix was kept moist throughout each experiment. After sowing, seeds were germinated at 25°C in a growth chamber for three days before placement in a rust-free greenhouse cubicle at 18-25°C. Seven days after sowing, when plants had a fully extended primary leaf, seedlings were inoculated with a suspension of fresh urediniospores recently bulked from the University of the Free State's stem rust culture collection. During inoculation, freshly collected urediniospores were suspended in light mineral oil, Soltrol 130® (~5 mg/ml), in gelatin capsules and sprayed onto plants using the system developed for applying rust spores to seedlings (Browder, 1971). Urediniospores are lipophilic and are readily suspended by oils and other non-polar fluids. Inoculation was performed by placing a tray with four pots on a turntable (~15 rpm) in an enclosed booth and spraying 0.8 ml inoculum onto primary leaves. The booth was closed and rinsed with a water spray for 1 min between applications of different isolates of *P. graminis f. sp. tritici.*

After inoculation, the seedlings were allowed to dry under fluorescent lights for at least 1 h before they were placed in a dew chamber and incubated overnight at ± 20°C. Seedlings were removed from the dew chamber and allowed to dry for 3 h before they were placed in a greenhouse cubicle at 18-22°C. Twelve to 14 days after inoculation, stem rust infection types were assessed on the primary leaves using a 0 to 4 scale (Figure 3.1) according to Stakman *et al*. (1962). Infection types 0 to 2 were considered to show avirulence to a particular Ug99 race while 3 to 4 was interpreted as pathogen virulence. Two replications were used for all seedling greenhouse trials.

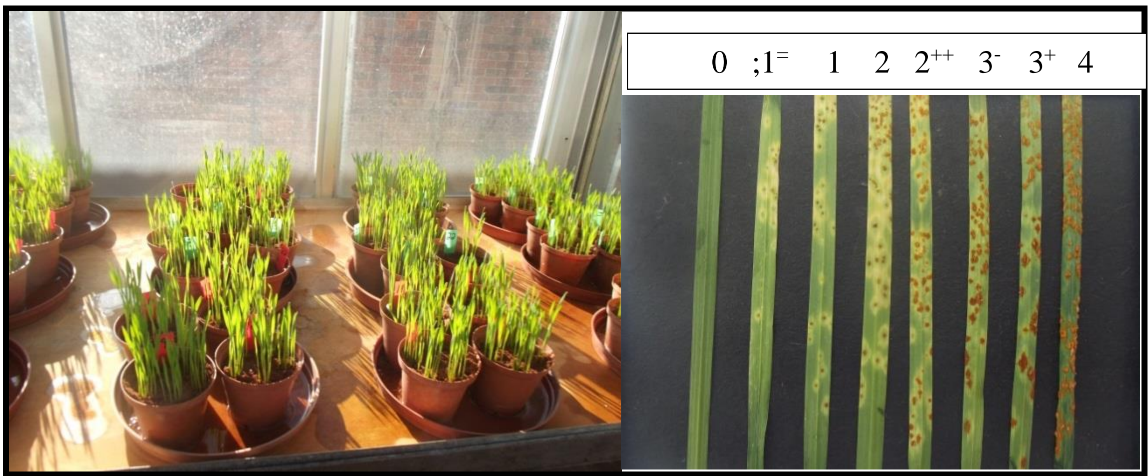

Figure 3.1: Seedling infection studies. Left: Pot layout in the greenhouse cubicle.

Right: Major scores used for recording seedling infection types (after Roelfs *et al*., 1992) with 0 to 2 (avirulent) and 3 to 4 (virulent).

3.2. Greenhouse adult plant infection studies

Steam-sterilized soil was placed in plastic pots 10 cm diameter by 15 cm in depth. Three seeds of each wheat entry were planted in each pot. Two replications were planted per entry. Pots were placed in a greenhouse cubicle free from rust. After emergence, plants were fertilized twice with Multifeed® water soluble fertilizer (19:8:16 NPK plus micronutrients), followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals for the duration of the trial as recommended by Bender *et al*. (2016). Plants were inoculated with a specific Ug99 race at flowering (Zadoks stage 58). Urediniospores were suspended in distilled water (0.7 mg/ml) and Tween 20 (0.03% v/v). Using a low pressure spray gun and air compressor at 300 kPa, the suspension was applied to stems at a rate of 100 ml inoculum per six pots, similar to the procedures for inoculating adult wheat plants with stripe rust (Pretorius *et al*., 2007b). An improvised plastic chamber measuring 100 × 85 × 60 cm, consisting of an aluminium frame covered with 200 µm greenhouse sheeting (Evadek [green tint], Gundle API Plastics Springs, South Africa), was used for incubation. The plastic-covered frame was placed in a tray filled with water to a depth of approximately 5 cm. Inside the frame, plants were placed on a metal grid raised above the water line. The temperature in the greenhouse cubicle housing the plastic chamber ranged from 16 to 22°C while the condensation dew chamber had temperatures between 20 to 28°C. After incubating inoculated plants in the

dew chamber for 20 h, plants were dried at 20°C before placing them in a greenhouse set at a 16 to 26°C night and day schedule. At 14 days after inoculation, stem rust infection response types (resistant R, moderately resistant MR, moderately susceptible MS, and susceptible S) (Roelfs *et al.*, 1992) were recorded on the two replications of each entry. Initial infection responses were recorded on flag leaf sheaths seven days after inoculation and subsequent records were done after every 24 to 48 h. Seven days after the first infection response recording, records were taken on the sheath, peduncle and on the flag leaf blade using infection type scores. Figure 3.2 shows greenhouse adult plants infected by stem rust.

Figure 3.2: Adult plant responses in the greenhouse.

Greenhouse adult plant scores (left to right): 0 = immune, R = resistance MR = moderate resistance, MS = moderate susceptible and S = susceptible.

3.3. Adult plant field response scores

Adult plant scores in the field (Figure 3.3) were based on Roelfs *et al*. (1992) namely:

(i) Modified Cobb scale that is used to determine the percentage of rusted tissue (disease severity); and

(ii) Host response to infection in the field where R = Resistance, MR = Moderate Resistance, MS = Moderate Susceptible, S = Susceptible and T = Trace.

In some cases, the stem rust field scores were converted into stem rust coefficient of infection (SRCI) for the purposes of statistical data analysis. SRCI was calculated by multiplying the disease severity score with a constant for host response: where immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8 and S = 1.0 (Roelfs *et al.*, 1992).

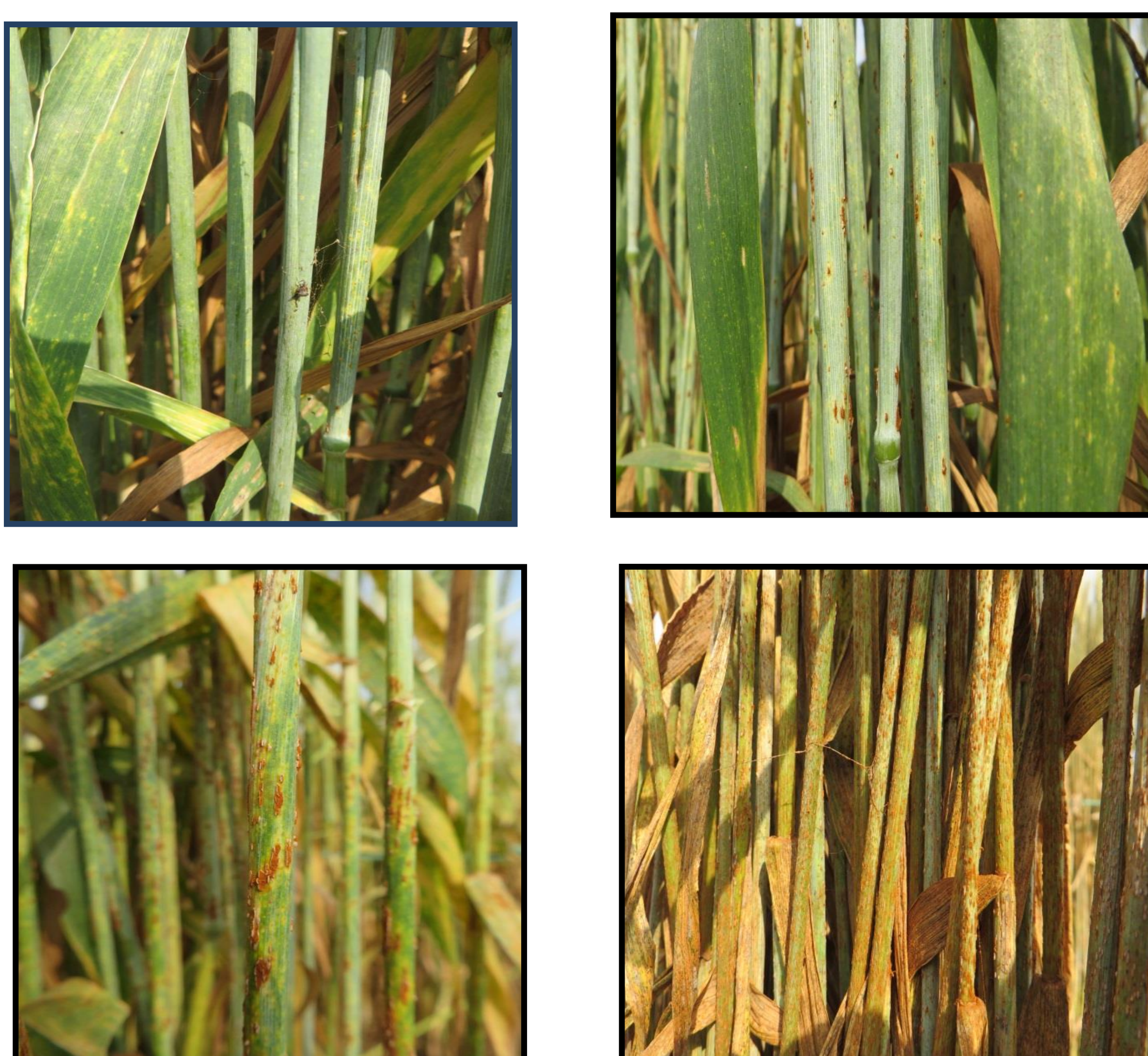

Figure 3.3: Adult plant responses in the fields.

Field adult plant responses (top left to right): Resistance response (SC8); moderate resistance (Kingbird); Bottom left to right: moderate susceptible (W6979) and susceptible (Line 37-07)

3.4. Wheat crosses to generate study populations

3.4.1. Selection of parents and type of breeding populations

The selection of lines for crossing and designation as female or male parent was based on the objective of the study project. Parents must be diverse in order to increase the frequency of polymorphism in segregating populations for marker analysis. A susceptible line, SC20 was used in the SC1 genetic studies (Chapter 5). Selected parents had *Sr* genes that have molecular markers already available to allow genotyping for tracking purposes. All selected parents were genetically pure (true to type) spring bread wheats that could be crossed easily and produce

fertile progenies. Genotyping was done for all parental material for confirmation and at various stages of the study. A recombinant inbred line (RIL) population was created for the SC1 genetic study (Chapter 5) while backcross inbred lines (BIL) were generated for the marker assisted stem rust introgression study (Chapter 6).

3.4.2. Planting method and management

Three planting dates, with fortnightly plantings were done to eliminate synchronization challenges between female and male parents. This ensures that all planned crosses were implemented. Two replications were planted per planting date. Each entry (female or male) was planted in an independent 10 cm diameter pot with a 15 cm height, with three seeds per pot. Steam sterilized soil was used to fill all the pots. Reverse osmosis water was used to water the pots throughout the study. After plant emergence till physiological maturity, plants were fertilized twice with Multi-feed® water soluble fertilizer (19:8:16 NPK plus micronutrients), followed by a water suspension of Wonder® 3:2:1, at 7-day intervals using the Bender *et al.* (2016) protocol of managing greenhouse adult plants. Weeds were manually controlled in all pots.

3.4.3. Female parent emasculation

To pollinate with desired male pollen, controlled hybridization was done. Wheat is predominantly self-pollinating, with both stamens and pistils located on the same flower and pollen is shed before or just when the flower opens. At flowering, before pollen shed (when 50% of the ear was still covered by the flag leaf), all female plants in designated pots were emasculated. Two spikes were selected from each female plant and using a scissors and forceps, anthers (three pairs) were removed from all spikelets. The emasculated spikes were immediately covered with a labelled glassine bag that was held in place by a paper clip. This prevented unwanted pollen from landing onto emasculated spikes and removal of anthers eliminated self-pollination. Information on the glassine bag included date of emasculation and female pot number. The emasculated plants were left for two days to allow further development of the stigma.

3.4.4. Pollination of emasculated female plants

Two days after emasculating female plants, the stigma became receptive and ready for pollination. Spikes that showed signs of pollinating (yellow anthers protruding from some of

the spikelets) were plucked from the designated male plants and their spikelets were cut half way using a pair of scissors. This allows anthers to quickly elongate thereby exposing mature pollen ready for shedding. The pollen was then dusted onto a specific female spike where the glassine bag is momentarily removed. Pollen was dusted along the whole length of the emasculated female spike. The pollinated female spike was immediately covered using the same bag and labelled with the date of pollination and male entry name. In between pollinations, the scissors and forceps were cleaned with paper dipped in a sterilizing agent (sodium hypochlorite). Pollination of one female plant by the designated male was done in a greenhouse cubicle free from other potted wheat plants. After pollination, plants were managed normally until harvesting.

3.4.5. Leaf sampling for genotyping

Young, healthy and tender leaf samples were taken from individual plants between four to six weeks after emergence. These samples were put in a labelled seal-ease envelope and freeze dried using an Advantage Virtis Freeze dryer before DNA extraction. Plants that were sampled were also tagged for easy tracking after result analysis.

3.4.6. Harvesting

All pollinated female spikes were harvested and threshed separately to avoid seed admixtures and grain was kept in a seal-ease envelope. Only seed from spikes where both emasculated female plant and male pollen donor were confirmed to have the gene of interest were advanced to the next stage of testing.

3.5. Genotyping of wheat populations and lines

3.5.1. Genomic DNA (gDNA) extraction

Advanced modern techniques for genetic mapping, finger printing, marker-assisted selection, and variety genetic purity require easy, faster and cost-effective extraction methods that result in pure and adequate quantities of DNA for PCR analysis. DNA extraction is complicated in plant material because of the presence of polysaccharides, proteins and DNA polymerase inhibitors such as tannins, alkaloids and phenols that requires long incubation periods or multiple precipitations during the extraction process (Abdel-Latif and Osman, 2017). Different companies have developed extraction kits such as Cetyltrimethylammonium bromide (CTAB), Zymo Research ZR-96 Plant/Seed DNA Kit™ and Sodium dodecyl sulphate (SDS) extraction

protocol (Agenbag, 2012) that are ready to use while some laboratories have customized their own extraction protocols but most of these are a modified version of CTAB that differ in time and cost (Abdel-Latif and Osman, 2017). A customized SDS gDNA extraction protocol of Pallotta *et al*. (2003) as reported by Agenbag (2012) was used in this study.

3.5.1.1. SDS gDNA extraction

Dry leaf segments measuring 20 mm x 5 mm together with 2 x 3 mm stainless steel balls were placed in a 2 ml Eppendorf tube. Tubes were arranged in 96-well holding plates and samples were ground for 2 to 5 min at a frequency of 25 revolutions per sec using a Qiagen TissueLyser (Retsch GmbH, Germany). The tubes were centrifuged in a microcentrifuge for 1 min at a frequency of 12 000 RCF. In the meantime, SDS buffer [(0.1 M Tris-HCl (pH 7.5); 0.05 M EDTA (pH 8.0); 1.25% (w/v) SDS] was preheated in a water bath. Preheated 600 µl of SDS extraction buffer was added to each sample tube and incubated in a water bath for 30 min at 65°C. Samples were centrifuged for 1 min at 12 000 RCF. Samples were placed in a freezer at -20°C for 15 min in order to cool them. Two-hundred and forty µl of 7.5 M of ammonium acetate that was kept at 4°C was added to each sample after which tubes were shaken vigorously for 30 sec. Samples were placed in a fridge at 4°C for 15 min. Plates were centrifuged at 2 250 RCF (microcentrifuge with a plate rotor fitted) for 15 min to collect the precipitated proteins and plant tissue. Single tubes were centrifuged for 5 min at a frequency of 12 000 RCF. Seven hundred µl of supernatant was withdrawn from each sample into a new set of Eppendorf tubes that contained 360 µl of iso-propanol each. The new tubes were well mixed by inversion and left to stand for 5 min at room temperature. This allowed DNA to precipitate. The plates were then centrifuged for 15 min at 2 250 RCF and single tubes were centrifuged for 5 min at 12 000 RCF using a microcentrifuge, in order to collect DNA.

The supernatant was carefully discarded and the remaining fluid was allowed to drain off the DNA pellet by inverting the tubes onto a piece of paper towel. Four hundred µl of ice-cold 70% ethanol was added to wash the DNA pellet. Sample plates were left at room temperature for 5 min then centrifuged again for 15 min at 2 250 RCF and the sample tubes for 5 min at 12 000 RCF. The supernatant was carefully discarded and the pellets were allowed to dry at room temperature. Eighty µl of pH 8 TE buffer [10 mM Tris-Cl (pH 8.0); 1 mM EDTA (pH8.0)] was added. The DNA was left to dissolve overnight in the fridge at 4°C. On the following day, the pellet was dislodged (resuspended) in the solution before centrifuging the plates for 15 min

at 2 250 RCF and the Eppendorf tubes for 5 min at 12 000 RCF. The supernatant was transferred to a new tube (1.1 ml tubes), leaving any debris behind.

3.5.1.2. DNA quantification and dilution

DNA was quantified on the Nanodrop Spectrophotometer (Nanodrop®ND-1000). It uses a 1µl sample to measure concentration within a spectrum of 220-750 nm with high accuracy and reproducibility (www.albany.edu/genomics/microarray/manuals/nd-1000-users-anual.pdf 27/6/17). It does not need dilution to take measurements on highly concentrated samples. A sample (1 µl) is pipetted onto the end of a receiving fibre and a second source fibre is brought into contact with the liquid sample. The liquid sample acts as a bridge between the two fibres. A xenon flash lamp provides the light source and the nanodrop machine utilizes a linear CCD array to analyse the light after passing through the sample. Data is logged in an archive file on the personal computer (PC) that utilizes Nanodrop software, running only on IBM compatible PC meeting set criteria (www.nanodrop.com 27/6/17). It is recommended to wipe both the receiving and source fibres after every sample to avoid contamination.

3.6. Molecular marker analysis

Selected marker analysis protocols used in this study were based on the *Sr* genes that were being tracked in Chapters 5 and 6. DNA markers that were used included single nucleotide polymorphism (SNP), Cleaved amplified polymorphic sequences (CAPS) and detection techniques included Real Time PCR (RT-PCR), Kompetitive allele-specific PCR genotyping system (KASP) and Gel electrophoresis. The marker assisted wheat (MASWheat) website (maswheat.ucdavis.edu/protocols/StemRust/index.htm) was referred to for some protocols used in this study.

3.6.1. Polymerase chain reaction (PCR)

PCR offers the means of reducing the complexity of a genome and increasing the copy number of the DNA templates to the levels required for the specific and sensitive detection of single-base changes. PCR has three stages, namely exponential phase (PCR products increase exponentially because reagents are not limiting), linear phase (linear increase in products as reagents become limiting) and plateau phase (amount of product will not change because reagents are now limiting). Conventional PCR detects products (amplicons) at the end point of the analysis.

3.6.2. Real time polymerase chain reaction (RT-PCR)

This approach follows PCR principles but amplified DNA (amplicon) is quantified as it accumulates in the reaction in real time after each amplification process (Xu, 2010; www.bio-rad.com 15/6/17). Detection is enabled by the inclusion of a fluorescent molecule (fluorophores) in each reaction well that yields increased inflourescence with an increasing amount of PCR product. The fluorescent chemistry included in real time PCR includes DNA binding dyes (intercalating dye based assays) and fluorescently labelled sequence specific primers or probes (Xu, 2010; www.bio-rad.com; www.pcrbio.com 15/6/17). Results of RT-PCR can be qualitative (presence or absence of a sequence) or quantitative (number of copies). Qualitative data can be evaluated without gel electrophoresis, resulting in reduced bench time thereby increasing throughput. Specialized thermal cyclers equipped with fluorescence detection modules are used to monitor the fluorescence signal as amplification occurs. RT-PCR can be used for different purposes including gene expression, analysis of complementary DNA (cDNA), population genotyping studies and multiplex detection of several disease targets.

3.6.3. Gel electrophoresis

Agarose is a linear polymer extracted from seaweed. An agarose gel allows negatively charged DNA, at neutral pH (7.5 to 7.8), to migrate towards the anode when an electric field is applied. Agarose gel electrophoresis is the standard method for separation, identification and purification of DNA and RNA fragments. Purified DNA fragments can be used for sequencing, PCR, cloning, *in vitro* transcription, restriction mapping and labelling. The rate of DNA migration depends on molecular size of DNA (large molecules migrate slower than smaller ones), agarose concentrations that determine porosity and gel size (4-10% with higher concentrations reducing overall mobility), DNA configuration and composition of the electrophoresis buffer (affects electrophoretic movement of DNA). Different buffer types include TAE (Tris-acetate), TBE (Tris-borate) and TPE (Tris-phosphate). Other types of gel used to separate DNA of different sizes include acrylamide, polyacrylamide and pulsed field agarose gels. Electrophoresis is done at room temperature. Ethidium bromide (EDB), mutagen toxic dye is used to stain agarose gel and as little as 0.05µg DNA can be detected. Gel imaging cameras are used to show DNA movement in form of bands.

3.6.4. Single nucleotide polymorphism (SNPs)

SNPs are individual nucleotide base differences in any part of the genome (Xu, 2010; Shavrukov, 2016). SNPs show high levels of polymorphism within organisms and have been used as molecular markers for the detection of associations between allelic forms of a gene and phenotypes especially for common diseases that have multifactorial genetics. SNPs can be used in accumulating APR genes for resistance in MAB. Haplotype-based (a haplotype is a group of genes within an organism that was inherited together from a single parent) analysis is more informative than analysis based on individual SNPs and has more power in analysing association with phenotypes. Various systems can be used to detect SNPs and these include gel electrophoresis, fluorescence resonance energy transfer (FRET), fluorescence polarization, arrays or chips, luminescence, mass spectrophotometry and chromatography. These detection systems differ in their chemistry, detection platform, multiplex rate and application. High throughput platforms for SNP detection include Illumina BeadArray™, Luminex 100 Flow Cytometry, ABI SNPlex™, ABI TaqMan™ 5`-Nuclease, ABI SNaPshot™ and KASP™ (www.cerealsdb.uk.net; www.lgcgroup.com; www.affymetrix.com and www.illumina.com, 7/8/17).

3.6.5. Cleaved amplified polymorphic sequence (CAPS)

Normally researchers will sequence a fragment closer to the gene and later amplify the fragment manually leading to CAPS. CAPS are used when SNPs occur within the recognition site of a restriction enzyme.

3.6.6. Kompetitive allele-specific PCR genotyping system (KASP)

This is a homogenous, fluorescent, endpoint genotyping technology that offers the simplest, cost effective and flexible way to determine both SNPs and insertion/deletion genotypes (https://www.lgcgroup.com/kasp/#.WVN6xuuGPI 28/6/17). KASP analysis can be done in 96, 384 and 1536 well plate format and enables bi-allelic scoring of SNPs and indels (insertions and deletions) at specific loci. SNP-specific KASP assay mix and KASP master mix are added to DNA samples, a thermal cycling reaction is then performed and followed by an end-point fluorescent read. Bi-allelic discrimination is due to the competitive binding of the two allele specific forward primers (FAM™ dye and HEX™ dye) that are in the KASP assay mix together with one common reverse primer. If the genotype at a given SNP is homozygous, only one of the two possible fluorescent signals will be generated while heterozygotes will

result in mixed fluorescent signals. It is always recommended to include positive controls for each genotype to facilitate data analysis. A minimum of 22 data points (including 2 non-treated checks (NTCs)) in each analysis will facilitate cluster analysis.

3.6.7. Applied Biosystems SOLiD™ (ABI)

A next generation DNA sequencing technology by Life Technologies was used in 2012 by CenGen laboratories to check on the presence of *Sr31* within Zimbabwe wheat lines. The platform is a high throughput DNA sequencing technology based on immobilization of DNA samples onto a solid support, cyclic sequencing reactions with the aid of automated fluidics devices and detection of molecular events by imaging (Myllykangas *et al*. 2012). ABI data are not collected directly as DNA sequences but is recorded in “colour space” in which individual values (colours) within a read provide information about two adjacent bases and colour is then decoded to sequence data (Ondov *et al*. 2008). It is based on the ability of DNA ligase to detect and incorporate bases in a very specific manner and generates sequence data of comparatively higher accuracy (Gupta *et al*. 2014). The main disadvantage of ABI is the long duration taken (6-7 days) for a whole run to be completed.

3.7. Stem rust genes used in molecular studies

KapaBiosystems (http://www.kapabiosystems.com), recently acquired by Roche with Sigma Aldrich coming as the distribution agent (2017), supplied all assay kits except for the *Sr31*gene (KASP assay) which was supplied by LGC (https://www.lgcgroup.com/products/kasp-genotyping-chemistry/#.WVVdhYSGPIU).

3.7.1. *Sr2*

Sr2 is a recessive adult plant resistant gene that has been the backbone of stem rust resistance for over 60 years. *Sr2* had a 38.7% frequency in Zimbabwean wheat germplasm (CenGen marker work in 2012) and will continue to be the backbone gene for Zimbabwean wheat breeding programmes. *csSr2* is a CAPS marker that detects three bands during molecular analysis i.e. null (absent), Marquis type band (non-*Sr2* wheat with bands at 225 and 112bp) and Hope type band (present - 172 bp) depending on the gene source.

Kapa 2G ReadyMix was used in a normal cyclic PCR reaction that followed the denaturation cycle (for 3 min at 95°C), 40 cycles of product amplification (with alternating cycles of 15 sec at 95°C, 15 sec at 60°C and 1 sec at 72°C) and a product extension cycle for 10 min at 72°C.

The PCR product was incubated at 15°C till the next stage analysis. The *csSr2* product was run in a 1% agarose gel for 50 min at 80 volts. The DNA product recovered after electrophoresis was photographed and digested by the *Pag1* enzyme. The product of digestion was run under a 2% agarose gel for 80 min at a voltage of 70 before another image of the gel was taken. Tables 3.1 summarize the protocols for *csSr2* marker used to genotype *Sr2* while Figure 3.4 shows the expected allelic bands for *Sr2*.

Table 3.1: PCR mix and program for *csSr2* marker used for *Sr2* gene

***csSr2* - Kapa 2G ReadyMix**		**PCR program**			
Reagents	**µl**	**Step**	**T°C**	**Time**	**Cycles**
dH_2O	2.50	Denaturation	95	3 min	
2X Kapa ReadyMix [1x]	10.00	Amplification	95	15 sec	
2mM dUTP [0.15 mM]	1.50		60	15 sec	X 40
csSr2 F [0.5 uM]	1.00		72	1 sec	
csSr2 R [0.5 uM]	1.00	Extension	72	10 min	
25ng/ul DNA [100ng]	4.00	Incubation	15	∞	
Total	**20.00**				

Steps	**Loading dye**	**Ladder (100bp Generuler)**	**DNA (PCR product**	**Runtime**	**dH_2O**	**10x Buffer**	***Pag1***
1% agarose Imaging	in mix	4µl	4µl	50min@ 80V	-	-	-
Digestion	-	-	-	-	1.60	2.00	0.40
2.5% agarose Imaging	in mix	4µl	12µl	80min@70V	-	-	-

Expected *Sr2* product using *csSr2* marker

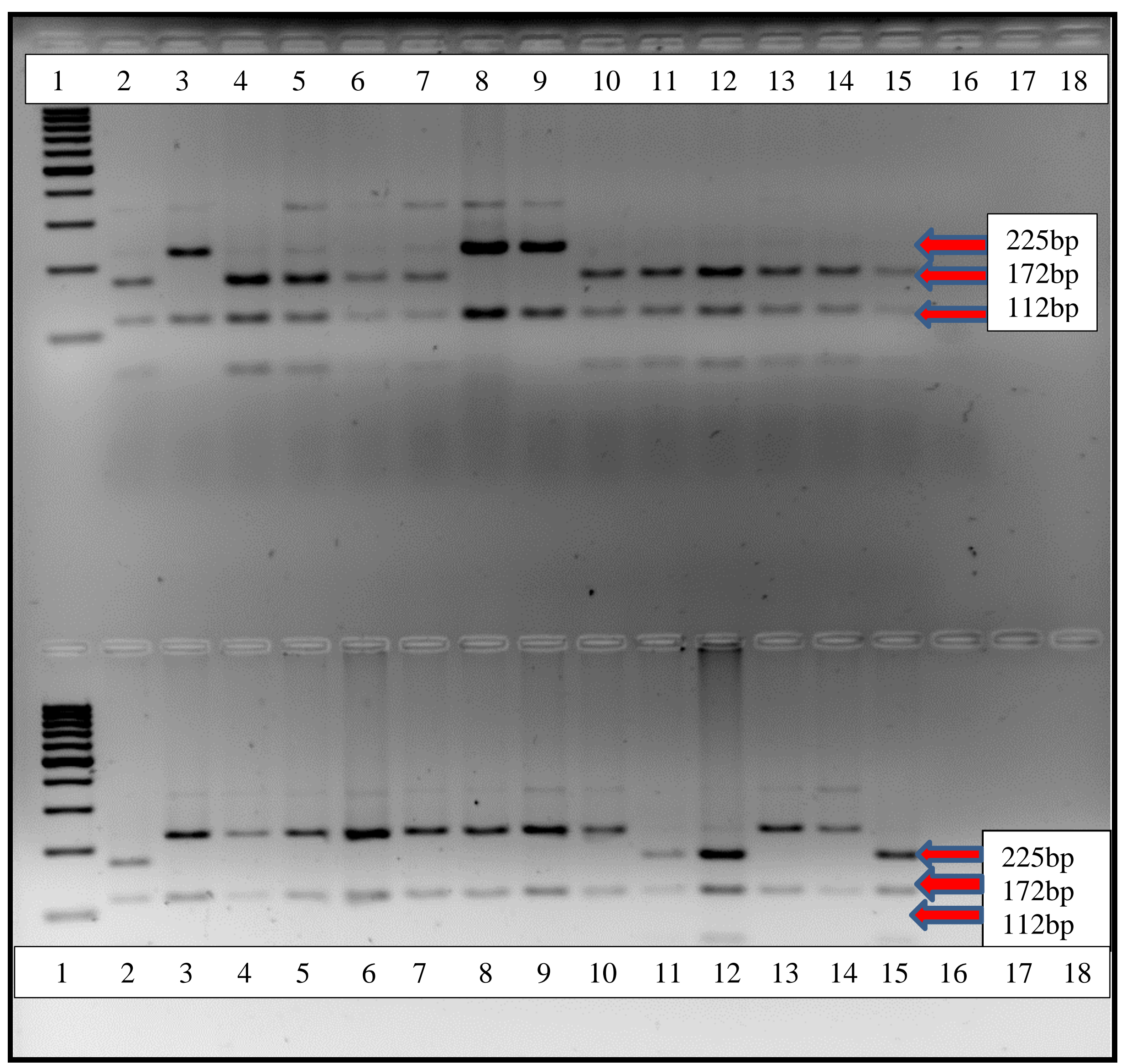

Figure 3. 4: *Sr2* Agarose gel photo.

csSr2 marker alleles showing the presence or absence of *Sr2* in 25 samples and two controls. Top (left to right): 100bp Generuler (DNA ladder, lane 1), Yr16DH70 (positive control (2) – band at 172bp), W4969 (negative control (3) – band at 225bp). *Sr2* present (Hope type allele) in lanes 4 to 7 and 10 to 15; *Sr2* absent (Marquis type allele) in lanes 8 and 9. Lanes 16 to 18 were empty. Below (Left to right): 100bp Generuler (DNA ladder (1)), Yr16DH70 (positive control (2) – band at 172bp), W4969 (negative control (3) – band at 225bp). *Sr2* present in lanes 11, 12 and 15. *Sr2* absent in lanes 4 to 10, 13 and 14. Lanes 16 to 18 were empty.

3.7.2. *Sr25*

Sr25 gene is closely linked to *Lr19* and a sequence-tagged-site (STS) marker, *Lr19STS*$_{130}$ converted from an AFLP band (Prins *et al*., 2001) was used during the molecular tracking of *Sr25*. A Kapa HRM Fast PCR Kit was used in real time PCR that followed a cycle of denaturation (3 min at 95°C), 40 cycles of amplification (5 min at 95°C and 30 min at 60°C) and an extension cycle (alternating cycles of 1 min at 95°C, 10 min at 40°C, gradual temperature increase from 70 to 90°C at a rate of 0.2°C per sec, followed by 10 min at 40°C). The product was incubated at 10°C.Bio Rad `s CFX96^{TM} Real Time System C1000 Touch Thermo Cycler was used. The marker presents a single peak when present and the presence of *Sr25* is then inferred. Table 3.2 give an *Lr19/Sr25* protocol summary.

Table 3.2: PCR mix and program for *Lr19STS*$_{130}$marker used for *Lr19* (*Sr25*) gene

Kapa HRM Fast PCR Kit 60°C		**PCR program**			
Reagents	**µl**	**Step**	**T°C**	**Time (min)**	**Cycles**
ddH_2O	1.00	Denaturation	95	3	
KAPA PCR Kit (2x)	5.00	Amplification	95	5	
$MgCl_2$ (25mM) [2.5mM]	1.00		60	30	x40
Lr19STS130-F [5pmol]	0.50	Extension	95	1	
Lr19STS130-R [5pmol]	0.50		40	10	
DNA (25ng)	2.00		70-90	0.2°C/sec	2sec/step
			40	10	
Total	**10.00**	Incubation	15	∞	

PCR=polymerase chain reaction, *Lr19STS130*-F= *Lr19STS130 forward primer, Lr19STS130*-R= *Lr19STS130* Reverse primer and HRM=high resolution melting, T°C=temperature

Expected output for *Lr19* using marker *Lr19STS*$_{130}$

(a) Presence of *Lr19 (+):* melt temperature will be between 81.0 – 82.4°C.

(b) Absence of *Lr19* (-): no peak will be observed.

3.7.3. *Sr26*

Sr26 is a gene with potential in breeding programmes because of its low frequency of deployment and resistance to current races of Ug99. Two dominant markers, operating in repulsion with one marker indicating *Sr26* presence (*Sr26#43*) and the other detecting absence (*BE518379*) resulting in a co-dominant diagnostic marker, are available (maswheat.ucdavis.edu 7/8/17). Kapa HRM Fast master mix was used in real time PCR reaction using Bio Rad `s CFX96™ Real Time System C1000 Touch Thermo Cycler. The PCR reactions were denatured for 5 min at 95°C, 45 amplification cycles (alternating cycles of 5 min at 95°C, 30 min at 61°C and 20 min at 72°C), and lastly an extension phase with four alternative cycles (1 min at 95°C, 10 min at 40°C, rapid temperature changes between 70 to 90°C at a rate of 0.2°C / sec and 10 min at 40°C). Incubation follows at 15°C. Table 3.3 summarizes the PCR protocol for detecting *Sr26* marker.

Expected outputs for *Sr26* gene using markers *Sr26#43* and *BE518379*

(a) If *Sr26* is present (+): A peak will be detected at melt temperature of 80.0°C.
(b) If *Sr26* is absent (-): A peak will be detected at melt temperature of 84.4°C.

Table 3.3: PCR mix and program for *Sr26#43* and *BE518379* markers used for *Sr26* gene

Kapa HRM Fast master mix - 61°C		**PCR program**			
Reagents	**µl**	**Step**	**T°C**	**Time (min)**	**Cycles**
ddH_2O	0.65	Denaturation	95	5	
2x Master Mix [1x]	5.00	Amplification	95	5	
25mM MgCl2 [2.5mM]	1.00		61	30	x45
Sr26#43 F [3.75 pmol]	0.375		72	20	
Sr26#43 R [3.75pmol]	0.375	Extension	95	1	
BE518379 F [3 pmol]	0.30		40	10	
BE518379 R [3 pmol]	0.30		70-90	0.2°C/sec	2sec/step
25 ng/ul DNA [50 ng]	2.00		40	10	
Total	**10.00**	Incubation	15	∞	

HRM=high resolution melting, PCR=polymerase chain reaction, F=forward primer, R=reverse primer, ng=nanogram, pmol=picomole,

3.7.4. *Sr31*

A KASP assay (Table 3.4) was used to track the dominant *Sr31* gene with a frequency of 53.1% in Zimbabwean wheat varieties (Chapter 2). The assay's objective is to detect the presence or absence of the 1RS:1BL translocation that carries the gene.

Table 3.4: KASP mix used in tracking the *1RS*:*1BL* translocation associated with *Sr31* gene

KASP assay		**KASP mix**		
Name	**µl**	**Step**	**# sample**	**T/location**
PCR grade H_2O (Bioline)	1.5	Forward primer A1 (100pmol)	12	Absent
2X KASP Reaction Mix [x1]	1.5	Forward primer A2 (100pmol)	12	present
Assay Mix *1RS:1BL_6110*	0.041	Reverse primer (100pmol)	30	
30ng DNA		PCR grade H_2O (Bioline)	46	
Total	**3.041**		100	

Expected outputs for *Sr31*

Shown in form of a KASP picture (Figure 3.5) with different positions for homozygotes (with/without the translocation i.e. red or blue), while heterozygotes will be in-between (green) in a segregating population.

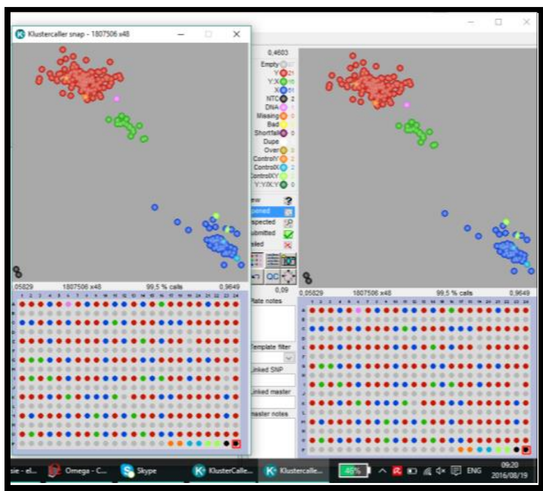

Figure 3.5: *Sr31* KASP output photo.

Sr31 (+): Homozygotes with *1RS:1BL* translocation (Red); *Sr31* (-): Homozygotes without *1RS:1BL* translocation (Blue) and *Sr31* (+ -): Heterozygotes (Green). Non-treated checks (NTCs) will be close to the zero intercept.

3.7.5. *Sr39*

Sr39 was one of the genes targeted for introgression into SC germplasm. Kapa master mix (kept at -60°C) was used in a normal PCR reaction (Table 3.5). The PCR had the following conditions for denaturation (5 min at 95°C), 40 cycles of amplification (5 min at 95°C, 1 min at 60°C, 1 min at 72°C) and lastly extension (1 min at 95°C, 10 min at 40°C, alternating cycles to increase temperature from 70 to 90°C at a rate of 0.2°C / sec and 10 min at 40°C). Incubation for at least 10 min is the last step before scoring the bands.

Table 3.5: PCR mix and program for *Sr39#22r* marker used for *Sr39* gene

Kapa HRM Fast master mix -60°C		**PCR program**			
Name	**µl**	**Step**	**T°C**	**Time (min)**	**Cycles**
ddH2O	1.40	Denaturation	95	5	
2X Master Mix [X1]	5.00	Amplification	95	5	
25mM MgCl2 [2.5mM]	1.00		60	1	x40
Sr39#22r-F [3pmol]	0.30		72	1	
Sr39#22r-R [3pmol]	0.30	Extension	95	1	
DNA (25ng)	2.00		40	10'	
			70-90*	0.2°C/sec	2sec/step
			40	10	
Total	**10.00**	Incubation	15	∞	

Table 3.6 summaries the different markers and their sequences that were used for tracking the stem rust resistant genes *Sr2*, *Sr25*, *Sr26*, *Sr31* and *Sr39.*

Table 3.6: Markers, primers and their sequences for the *Sr* genes used as part of the *Sr* introgression and SC1 genetic studies

Gene	**Marker (Technique)**	**Primer name**	**Sequence** (5`…….3`)	**Reference(s)**
Sr2	CAPS	*csSr2*-F	CAAGGTTGCTAGGATTGGAAAAC	Mago *et al*., 2011
		csSr2-R	AGATAACTCTTATGATCTTACATTTTTCTG	maswheat.ucdavis.edu 28/6/17
Lr19	STS	*BF145935*-F	CTTCACCTCCAAGGAGTTCCAC	Liu *et al.,* (2010)
(*Sr25*)	(Real Time)	*BF145935*-R	GCGTACCTGATCACCACCTTGAAGG	maswheat.ucdavis.edu 28/6/17
Sr26	PCR	*Sr26#43*-F	AATCGTCCACATTGGCTTCT	Mago *et al*., (2005) Bariana *et al*.,
	(Real Time)	*Sr26#43*-R	CGCAACAAAATCATGCACTA	(2007)., Liu *et al.,* (2010)
	PCR	*BE518379*-F	AGCCGCGAAATCTACTTTGA	maswheat.ucdavis.edu 28/6/17
	(Real Time)	*BE518379*-R	TTAAACGGACAGAGCACACG	
Sr31	SNPs	*wMAS000011* F1	GGAGCAGGTCCAGATCGCG	www.cerealsdb.uk.net 31/7/17
	(KASP)	*wMAS000011* F2	CGGAGCAGGTCCAGATCGCA	
		wMAS000011 R	GAAGCTCCGGTAGATGGAGGCTA	
Sr39	PCR	*Sr39#22r*-F	AGAGAAGATAAGCAGTAAACATG	Mago *et al*., (2009)
	(Real Time)	*Sr39#22r*-R	TGCTGTCATGAGAGGAACTCTG	maswheat.ucdavis.edu 28/6/17

PCR; Polymerase chain reaction. F: Forward primer. R: Reverse primer. *Sr*: stem rust gene designation. *Lr*: Leaf rust designation gene. CAPS: Cleaved amplified polymorphic sequence. STS: Sequence-tagged-site. SNPs: Single nucleotide polymorphism. 5`- CAA…. – 3`: DNA strand sequence from 5` to 3` end.

4. Characterization of Zimbabwean bread wheat (*Triticum aestivum* L.) lines for seedling infection type and adult plant response to Southern African races of Ug99 stem rust

4.1. Introduction

Four Ug99 stem rust races, i.e. PTKST, TTKSF, TTKSF+*Sr9h* and TTKSP, are prevalent in Southern Africa (Visser *et al*., 2009; 2011; Pretorius *et al*., 2010 and Mukoyi *et al*., 2011). With the exception of TTKSP, all of these have been detected in Zimbabwe since 2009. Annual rust surveys and sample collection were conducted across Zimbabwe by the Ministry of Agriculture's Department of Specialist Services, Seed-Co and CIMMYT. Rust race analysis was done by the University of the Free State and the Agricultural Research Council of South Africa. Despite occurrence of these broadly virulent races of *Puccinia graminis f. sp. tritici*, no work had been done to establish the level of genetic vulnerability of Zimbabwean wheat germplasm. This could be ascribed to absence of infrastructure to conduct appropriate seedling and adult plant greenhouse trials, a lack of expertize to carry out such work and un-availability of genetically pure Ug99 isolates.

The objective of this study was to determine all stage resistance and adult plant host responses of Zimbabwean germplasm using a sample of 49 lines that included old varieties, current commercial varieties and experimental lines. The study was based on the hypothesis that the stem rust resistance base in Zimbabwean wheat germplasm is narrow and vulnerable to the effects of prevalent Ug99 races.

4.2. Study objectives

The main objective was to characterize Zimbabwean wheat lines to create a data base for making breeding and production decisions. Specific objectives were:

(a) To characterize 49 Zimbabwean wheat lines by establishing their all stage resistance infection types when inoculated with PTKST, TTKSF, TTKSF+*Sr9h* and TTKSP Ug99 stem rust races;

(b) To characterize adult plant field responses of the 49 Zimbabwean wheat lines to race PTKST when grown for two seasons at Greytown in KwaZulu-Natal, South Africa;

(c) To characterize adult plant responses of the forty-nine Zimbabwean wheat lines when inoculated with PTKST stem rust race in the greenhouse; and

(d) To corroborate phenotypic data from both seedling and adult plant studies with genotypic data done in 2012 by CenGen (Pty) and by Limagrain in 2015.

4.3. Materials and methods

4.3.1. Wheat germplasm used in the study

Zimbabwean germplasm used in these studies consisted of six old commercial varieties, 18 current commercial varieties (13 from Seed-Co and five from other companies) and 25 Seed-Co experimental lines (Chapter 2).

4.3.2. Seedling infection type studies

Forty-nine Zimbabwean wheat lines were planted in pots following protocols described in Chapter 3. Four Ug99 races detected and confirmed using the North American five letter nomenclature code were used to inoculate entries (Table 4.1). PTKST is coded as UVPgt60 by the University of the Free State (UFS - UV) while Agriculture Research Council (ARC) uses 2SA107 for the same race. TTKSF is UVPgt55 or 2SA088, TTKSF+*Sr9h* is UVPgt61 or 2SA088+*Sr9h* and TTKSP is coded as UVPgt59 or 2SA106. Table 4.1 shows the four Ug99 races, their various codes and their avirulence/virulence genes using South African differential set as described by Pretorius *et al*. (2010) and Terefe *et al*. (2016). Federation*4/Kavkaz (Fed/Kvz - *Sr31*), Satu (*SrSatu*), Avocet S (*Sr26*) and Pavon 76 (*Sr2*) were used as positive and negative control lines when PTKST was used for inoculations while LCSr24Ag (*Sr24*), Sr31/6*LMPG (*Sr31*), Matlabas (*Sr9h*) and McNair (*SrMcN*) were used with the other three inoculations. Primary leaf infection types were recorded 14 days after inoculation according to Stakman *et al*. (1962).

4.3.3. Greenhouse adult plant infections

Zimbabwean wheat lines were grown in the greenhouse in pots and the adult plant inoculation protocol described in Chapter 3 was followed. Stem rust race PTKST (UVPgt60 or 2SA107) was used to inoculate plants. Inoculation was done at flowering just before pollen shedding. First pustules were noticed six days after inoculation and first records on adult plant responses were taken seven days after inoculations. A total of five record sets were taken, the last four on a weekly basis after the first recording. Disease severity (%) according to the modified Cobb scale (Peterson *et al*., 1948) and response type where 0 = immune, R = resistant, MR =

moderately resistant, MRMS = overlap between moderately resistant to moderately susceptible, MS = moderately susceptible and S = susceptible were used to record adult plant host responses.

Host responses on peduncles were recorded as described above. The 0 to 4 scale (Stakman *et al.*, 1962) was used for the infection type on the upper surface of the flag leaf blade. Scores from 0 to 2 represented low infection types (resistant) while 3 to 4 represented high infection types (susceptible). Three sets of records were taken on both the peduncles and flag leaves. Chlorotic flecks, usually recorded as seedling chlorosis (*sc*), a phenotypic marker linked to *Sr2,* was recorded on the two replications of all adult plants by recording presence (+) or absence (-) of chlorosis on the upper surface of the flag leaf blade.

Line 37-07 was used as a susceptible check for all four Ug99 stem rust races. Three lines known to be resistant to Ug99 stem rust were used as resistant checks, i.e. *Sr25* (7th SRTN-12#30 Agatha/9#LMPG6 (*Lr19/Sr25*)), *Sr26* (WBC08 (Australia)) and *Sr39* (25#2/163). These were the same lines that were used as donor parents in Chapter 6.

4.3.4. Field responses

The study was done for two seasons (2012 and 2014) at the Pannar Redgates research station near Greytown located in Kwazulu-Natal, South Africa. The station is located 29°03`South and 30°35`East at 1050 meters above sea level (masl). In 2012, mean minimum and maximum temperatures recorded were 10.1°C and 24.9°C respectively, with 1102 mm of rainfall being received. In 2014, the average minimum and maximum temperatures were 9.6°C and 25.2°C, respectively with 757.2 mm of rainfall. The 42 year average annual rainfall for the station (as of 2016) was 835.17 mm according to Hildesheim Weather summary records at Pannar seed research station in Greytown.

Each entry was planted to a single 1m row with inter row spacing of 0.76 m. Rows of Line 37-07 (susceptible check) were planted after every ten rows and the same variety was used as border rows that were planted surrounding the trial and in pathways. Weeds were controlled both manually and by herbicides. Prior to planting, 250 kg 2:3:4 N-P-K plus 0.5% Zn fertilizer was applied per hectare. Two replications were planted in 2012 and two sets of records were taken for all replications while in 2014 the trial was not replicated with only one set of records

taken. For each entry disease severity was recorded as the mean percentage stem tissue infected, accompanied by a response type, as described above. The trials were inoculated with PTKST known to be the dominant race at Greytown (Pretorius et al., 2010a). Inoculum for field trials was increased on seedlings of an *Sr31* carrier to ensure race purity. The race that infected the trial over two years was not analyzed in the laboratory but reactions on checks confirmed PTKST.

Table 4.1: Avirulence/virulence profiles of four Ug99 stem rust races used to inoculate 49 Zimbabwean wheat lines in the greenhouse for seedling infection types

Ug99 race[a]	**UFS**	**SA code**	**avirulent *Sr* genes**[b*]	**Virulent *Sr* genes**[b*]
PTKST	UVPgt60	2SA107	*Sr9h, 21, 27, 36, Kiewiet, Satu, Tmp*	*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 24, 30, 31, 38, McN*
TTKSF	UVPgt55	2SA88	*Sr9h, 24, 27, 31, 36, Kiewiet, Satu, Tmp*	*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 38, McN*
TTKSF+*Sr9h*	UVPgt61	2SA88+*Sr9h*	*Sr24, 27, 31, 36, Kiewiet, Satu, Tmp*	*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 9h, 10, 11, 17, 21, 30, 38, McN*
TTKSP	UVPgt59	2SA106	*Sr9h, 27, 31, 36, Kiewiet, Satu, Tmp*	*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 24, 30, 38, McN*

[a]TTKSF, TTKSP, PTKST and TTKSF+*Sr9h* belong to the Ug99 lineage. TTKSF+*Sr9h* differ from TTKSF by its virulence on *Sr9h* (formerly *SrWeb*).

[b] Barleta Benvenuto (*Sr8b*), Coorong (*Sr27*) Kiewiet (triticale gene, *SrKiewiet*), Satu (*SrSatu*) McNair 701 (*SrMcN*), Matlabas (*Sr9h*), Triumph (*SrTmp*)

* Avirulence/virulence according to Terefe *et al*., 2016

4.4. Results

4.4.1. Seedling infection studies for detecting all stage resistance

Inoculation with PTKST was repeated on all lines due to some "escapes" that were observed after the initial inoculation and replication 2 scores were used in this report. PTKST was the most virulent Ug99 stem rust race resulting in 40.8% of the Zimbabwean wheat lines showing high infection types (3 to 4) indicating their susceptibility to this race at seedling stage. TTKSF+*Sr9h* ranked second with 12.2% while TTKSF and TTKSP were third, respectively, with 10.2% of susceptible lines (Figure 4.1). Among the susceptible lines, 8.2% (SC7, SC13, CBI8 and SC20) were susceptible to all four Ug99 stem rust races, 4.1% (CBI2 and CBI6) susceptible to three races and 28.6% of the lines were susceptible to one stem rust race. Resistant lines against all four Ug99 races constituted 59.2% of Zimbabwean wheat lines. CBI1, an old commercial variety was the only variety that showed immunity (0 score) against all four Ug99 stem rust races.

Among the positive check varieties (Table 4.2), Federation*4/Kavkaz (*Sr31*) was susceptible to PTKST and McNair 701 (*SrMcN*) was susceptible to races TTKSF, TTKSF+*Sr9h* and TTKSP. Matlabas (*Sr9h*) was susceptible to race TTKSF+*Sr9h*. As negative checks, Matlabas (*Sr9h*) was resistant against TTKSF and TTKSP while *Sr31*/6*LMPG was resistant against TTKSF, TTKSF+*Sr9h* and TTKSP. LC*Sr24*Ag was resistant against TTKSF, TTKSF+*Sr9h* and TTKSP though it was expected to be susceptible to TTKSP because of the virulence of the race on *Sr24*. It had a score of 2++ in the two replications. Pretorius *et al*. (2012), reported ITs of 2 (TTKSF), 3 (TTKSP), 3 (PTKST) for LC*Sr24*Ag; 1 (TTKSF), 1 (TTKSP), 4 (PTKST) for *Sr31*/6*LMPG and IT of 4 (TTKSF, TTKSP and PTKST) for McNair 701 in a study to determine seedling resistance and marker analysis for *Sr2*, *Sr24* and *Sr31* using South African varieties.

Table 4.2: Seedling infection type score for 49 Zimbabwean wheat lines after inoculation with four Ug99 stem rust races.

PTKST Rep 2*: scored after re-inoculation of second leaf with PTKST (seedlings were 14 days old). LIT (resistant): ≤ 2; HIT (susceptible): ≥ 3

Mixed infection type scores: e.g. 4p;1, 1p2 where 4 plants had a score of ;1 while a single plant had a score of 2. *Sr*: Stem rust gene.

		PTKST		**TTKSF**		**TTKSF+*Sr9h***		**TTKSP**	
Entry	***Sr* genes****	**Rep 1**	**Rep 2***	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
SC1	*Sr31+*	3	4	;1=	;1=	;1= 2p3-	;1 1p3-	;1= 1p3-	;1=
SC2	*Sr2+Sr31+*	;1	1	;1=	;1=	;1=	;1 1p3-	;1=	;1=
SC3	*Sr2+Sr31*	4	4	;1= 1p3++	;1= 1p3++	;1= 1p2+	;1= 1p3	;1=	;1=
SC4	*Sr2+*	;1	;1	;1=	;1=	;1=	;1=	1- 1p2-	;1= 1p2+
SC5	Unknown	3++	4	2++	2++	2++	;1=	2+	2+
SC6	*Sr2+Sr31*	4	4	;1=	;1=	;1= 1p3+	2++	;1=	;1 1p3+
SC7	*Sr2+,*	3	3+	3	3	3++	;1= 1p3+	3+	3+
SC8	*Sr2+Sr31+*	;1	;1	;1	;1=	;1=	3+	;1=	;1=
SC9	*Sr31+*	3	3+	;1=	;1=	;1=	;1=	;1=	;1=
SC10	*Sr31*	3+	4	;1=	;1=	;1=	;1=	;1=	;1=
SC11	*Sr31*	3+	3-	;1=	;1=	;1=	;1=	;1=	;1=
SC12	*Sr31*	escape	3	;1=	;1=	;1=	;1=	;1=	;1=
SC13	*Sr2*	3	4	3+	3+	33++	3+	3+	3+
CBI1	*Sr36*	0;	0;	0	0	0	0	0	0
CBI2	*Sr31*	3	3	2++	2++	3-	3-	3-	3-
CBI3	*Sr2+Sr31*	4	4	;1=	;1=	;1=	;1=	;1=	;1=

Entry	Sr genes	PTKST		TTKSF		TTKSF+Sr9h		TTKSP	
		Rep 1	Rep 2*	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
CBI4	*Sr2+Sr31*	4	3++	;1=	;1=	;1= 1p3+	;1=	;1=	;1=
SC14	Unknown	;1	;1	1	1	1	1	1	1
SC15	*Sr31+*	1	2-	;1=	;1=	;1=	;1=	;1=	;1=
SC16	*Sr2+*	1	1	1	1	1-	1-	1-	1-
SC17	*Sr31+*	;1	;1	;1	;1 1p2+	;1=	;1=	1	1
SC18	*Sr31+*	1	1	;1=	;1=	;1=	;1=	;1=	;1=
SC19	Unknown	1	1	;1=	;1=	1-	1	1	1
CBI5	Unknown	2-	2+3	1+	1+	1	1+	1+	1+
CBI6	Unknown	3	3++	3-	3-	3-	3-	2++	2++
CBI7	*Sr31+* (mixed)	;1	23	1	1 2p3	1 1p3	1 1p2	1	1 1p2
CBI8	Unknown	3	4	3+	3+	3	3	3++	3++
PAN1	*Sr24*	1,2	1	:1=1p3-	;1=	;1=	;1= 1p3-	;1=	;1= 2p3
SC20	Unknown	escape	4	3=	3=	3	3	3	3
SC21	Unknown	escape	2	1	1	1	1	1	1
SC22	*Sr24*	2	2	pgn	;1=	;1=	;1=	2++	2++
SC23	*Sr2+Sr31+*	2	2	;1=	;1=	;1=	;1=	;1=	;1=
SC24	*Sr31+*	escape	1-	;1=	;1=	;1=	;1=	;1=	;1=
SC25	*Sr24+*	;1	;1	pgn	;1=	;1=	;1=	;1=	;1=
SC26	Unknown	1	2++	2	2	1	1	1	1

		PTKST		TTKSF		TTKSF+*Sr9h*		TTKSP	
Entry	***Sr* genes**	**Rep 1**	**Rep 2***	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
SC27	*Sr31*	3	3++	;1=	;1=	;1=	;1=	;1=	;1=
SC28	*Sr31*	4	3++	;1=	;1=	;1=	;1=	;1=	;1=
SC29	Unknown	2	2-	1	1	1	1	1	1
SC30	*Sr2+Sr31+*	;1	1	;1	1	1	1	1	1
SC31	*Sr2+*	;1	;1	1+	1+	1	1+	1	1
G1	*Sr2+Sr31*, mixed	4	4	;1=	;1=	;1=	;1=	;1=	;1=
SC32	*Sr24+*	;1	;1	;1=	;1=	;1=	;1=	1++	1++
SC33	*Sr24+,*	;1	;1, 3	;1=	;1=	;1=	;1=	1+	1+
SC34	*Sr2+*	;1	;1	1 1p3-	1	1	;1=	;1=	;1= 1p3
SC35	*Sr2+Sr31+*	;1	;1, 4	;1=	;1=	;1=	;1=	;1=	;1=
SC36	*Sr2+Sr31+*	1	;1	;1=	;1=	;1=	;1=	;1=	;1=
SC37	*Sr2+Sr31*	;1	;	;1=	;1=	;1=	;1=	;1=	;1=
SC38	*Sr31*	2+3	2, 3	;1=	;1=	;1=	;1=	;1=	;1=
SC39	*Sr2+*	1	;1	;1=	;1=	;1=	;1=	;1=	;1=
Fed/Kvz[1]	*Sr31*	4	4	Unused	Unused	Unused	Unused	Unused	Unused
Sr24 LCSr24Ag[1]	*Sr24*	Unused	Unused	1	1	1	1	2++	2++
Sr31/LMPG eps[1]	*Sr31*	Unused	Unused	;1=	;1=	;1=	;1=	;1=	;1=
Matlabas[1]	*Sr9h*	Unused	Unused	1	1	4	4	1	1
McNair 701[1]	*SrMcN*	Unused	Unused	4	4	4	4	3+	4

		PTKST		TTKSF		TTKSF+*Sr9h*		TTKSP	
Entry	**Sr genes**	**Rep 1**	**Rep 2***	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
Pavon 76[1]	*Sr2*	3+	3++	Unused	Unused	Unused	Unused	Unused	Not used
Satu[1]	*SrSatu*	0;	0;	Unused	Unused	Unused	Unused	Unused	Unused
Avocet[1]	*Sr26*	;1	;1	Unused	Unused	Unused	Unused	Unused	Unused

[1]Checks : Federation*4/Kavkaz (*Sr31*), LCSr24 Ag (*Sr24*), Sr31/LMPG (*Sr31*), Matlabas (*Sr9h*), McNair (Sr*McN),* Pavon 76 *(Sr2),* Satu *(SrSatu)* and Avocet *(Sr26)*. pgn: poor germination. ** Postulated *Sr* gene information is according to CenGen results in 2012.

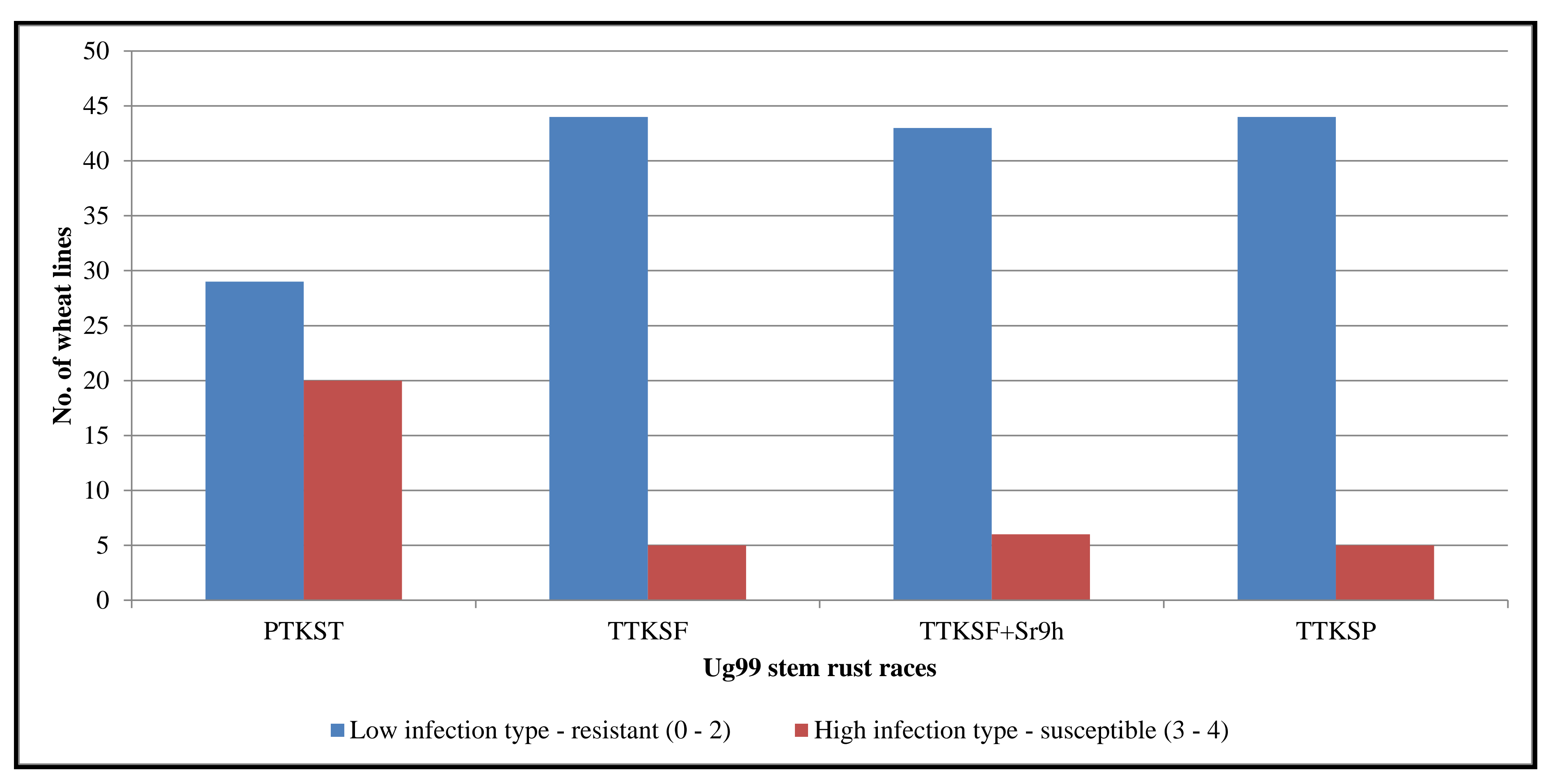

Figure 4.1: Infection types of Zimbabwean wheat lines to four Ug99 races.

Proportion of Zimbabwean wheat lines that were resistant (low infection type) or susceptible (high infection type) to four Ug99 stem rust races at seedling stage in the greenhouse

4.4.2. Greenhouse adult plant infections

4.4.2.1. Adult plant response scores

Zimbabwean wheat lines showed the whole range of responses from immunity (0) to susceptibility (S) and all the intermediate reactions (MR, MS and MRMS) as shown in Table 4.3. The host response could be grouped into six categories (Table 4.4):

(a) ***Immune***: CBI1 (2%) was the only variety that had an immune score to PTKST.

(b) ***Resistant:*** Scores ranged from TR to 40MRR; 24.5% of the germplasm including five commercial varieties, SC2 (30MRR being the highest score), SC4 (20MRR), SC8 (30R), SC14 (20MR) and SC15 (40R) and seven experimental lines whose scores ranged from 20MRR (SC16 and SC31) to 40MRR (SC26, SC30, SC18 and SC32).

(c) ***Moderately resistant:*** This category was made up of lines with scores from 50MRR to 40MRMS. This group constituted 18.4% of the germplasm, with one commercial variety (CBI7 – 60MR) and eight experimental lines ranging from 50MRR (SC36 and SC39) to 80MR (SC21).

(d) ***Moderately susceptible:*** Scores ranged from 50MRMS to 50MS. This group was made up of 8.2% of the germplasm with two commercial varieties namely SC3 and CBI5 (80MRMS) and two experimental lines SC19 (80MSMR) and SC33 (60MRMS).

(e) ***Susceptible:*** Scores ranged from 60MS to 100MSS. This group constituted 16.3% of the germplasm; two current commercial varieties, SC9 (60MS), PAN1 (60MSS), one old variety CBI3 (80MSS), and five experimental lines with scores ranging from 60MSS (SC23) to 100MS (SC27 and SC28).

(f) ***Highly susceptible***: Lines had a susceptible reaction. These constituted 30.6% of the germplasm. Ten commercial varieties with scores ranging from 60S (SC1 and SC12) to 100S (CBI6, G1, SC6, SC10 and SC11). Four are old commercial varieties with scores ranging from 60S (CBI2) to 100S (SC13 and CBI4). SC38 (40S) is the only experimental line in this category.

Table 4.3: Greenhouse adult plant scores from five successive recording dates for 49 Zimbabwean wheat lines following inoculation with Ug99 stem rust race PTKST

DAI	**7**		**14**		**21**		**28**		**35**	
Entry (gene*)	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
SC1 (*Sr31+*)	10S	10S	20S	20S	30S	30S	40S	40S	60S	60S
SC2 (*Sr2+Sr31+*)	0	0	10R	10R	10MRR	10MRR	20MRR	10MRR	30MRR	20MRR
SC3 (*Sr2+Sr31*)	10MSS	5MSS	10MSS	10MSS	20MRMS	20MRMS	40MRMS	20MRMS	80MRMS	30MRMS
SC4 (*Sr2+*)	0	0	0	0	5R	5R	10MRR	20MRR	20MRR	20MRR
SC5 (unknown)	20S	20S	40S	40S	50S	40MS	60S	40MSS	80S	60MSS
SC6 (*Sr2+Sr31*)	30S	30S	40S	40S	60S	40S	70S	40S	100S	60S
SC7 (*Sr2+*)	5S	10S	10S	10S	30S	30S	40S	40S	60S	80S
SC8 (*Sr2+Sr31+*)	0	0	0	0	0	0	10RMR	10R	10RMR	30R
SC9 (*Sr31+*)	0	0	0	0	0	0	30MRMS	30MRMS	60MS	60MS
SC10 (*Sr31*)	0	0	0	0	0	0	60MSS	40MSS	100S	60S
SC11 (*Sr31*)	10MSS	10MSS	50S	40MSMR	80S	30S	100S	60S	100S	60S
SC12 (*Sr31*)	40S	40S	60S	60S	80MS	80MS	80MSS	80MSS	100MSS	100MSS
SC13 (*Sr2*)	10S	20S	10S	20S	20S	40S	80S	100S	80S	100S
CBI1 (*Sr36*)	0	0	0	0	0	0	0	0	0	0
CBI2 (*Sr31*)	20S	20S	30S	30S	60S	60S	70MS	70MS	80MSS	70MSS
CBI3 (*Sr2+Sr31*)	30MS	30MS	60MRMS	60MRMS	60MSS	60MSS	80MSS	80MSS	80MSS	80MSS
CBI4 (*Sr2+Sr31*)	20S	20S	40S	40S	60S	40S	80S	40S	100S	80S

DAI	7		14		21		28		35	
Entry (Gene*)	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
SC14 (unknown)	0	0	0	0	20R	20RMR	20RMR	20MRR	20MRR	20MRR
SC15 (*Sr31*+)	0	0	0	0	20R	30R	30R	30R	30R	40R
SC16 (*Sr2*+)	0	0	0	0	0	0	5R	5R	20MRR	10R
SC17 (*Sr31*+)	0	0	20MR	10R	20MRR	20MRR	40MRR	30MRR	60MRR	60MRR
SC18 (*Sr31*+)	0	0	0	0	5R	5R	30MRR	30MRR	40MRR	40MRR
SC19 (unknown)	0	0	20MR	10MR	20MR	20MRR	40MRMS	30MRMS	80MRMS	80MRMS
CBI5 (unknown)	20S	20S	30S	30S	40MRMS	30MRMS	80MRMS	50MRMS	80MRMS	50MRMS
CBI6 (unknown)	30S	30S	60S	60S	70S	60S	80S	60S	100S	80S
CBI7 (*Sr31*+)	10MR	10MR	30MR	30MR	30MR	30MR	40MR	30MR	60MR	40MR
CBI8 (unknown)	10S	10S	40MS	40MS	40S	40MS	80S	40S	80S	50S
PAN1 (*Sr24*)	10MS	10MS	20MSS	20MSS	30MSS	40MSS	40MSS	60MSS	40MSS	60MSS
SC20 (unknown)	20S	20S	60S	60S	60S	60S	80S	80S	80S	80S
SC21 (unknown)	0	0	0	0	30R	5R	60MR	10MRR	80MR	50MR
SC22 (*Sr24*)	20MS	10MS	60MSMR	50MSMR	60MSS	60MS	80MSS	80MSS	80MSS	80MSS
SC23 (*Sr2*+*Sr31*+)	5MS	5MS	20MRMS	20MRMS	20MSS	30MSS	40MSS	40MSS	60MSS	60MSS
SC24 (*Sr31*+)	40R	20MR	60MRR	20MRR	60MRR	30MRR	60MRR	50MRR	60MRR	60MRR
SC25 (*Sr24*+)	5MR	5MR	50R	50R	50MR	50MR	60MRR	60MRR	60MR	60MR
SC26 (unknown)	0	0	10MR	10MR	30MR	20MR	40MR	40MR	40MR	40MR
SC27 (*Sr31*)	0	0	40MR	40MR	60MS	80MS	80MS	100MS	80MS	100MS

DAI	**7**		**14**		**21**		**28**		**35**	
Entry (Gene*)	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
SC28 (*Sr31*)	40MS	40MS	60MRMS	40MRMS	100MS	60MS	100MS	80MSS	100MS	80MS
SC29 (unknown)	0	0	0	0	5R	5R	10MR	10MR	20MR	30MR
SC30 (*Sr2*+*Sr31*+)	0	0	0	0	0	5R	20MRR	10R	40MRR	30MRR
SC31 (*Sr2*+)	MR	MR	5RMR	5R	5MR	20RMR	5MR	20MRR	5MR	20MRR
G1 (*Sr2*+*Sr31*)	20S	20S	70S	40MS	80S	40MS	100S	60MSS	100S	80MS
SC32 (*Sr24*+)	0	0	10R	TR	20MR	10MR	20MR	10MR	40MR	10MR
SC33 (*Sr24*+)	10MRMS	10MRMS	40MRMS	40MRMS	40MRMS	40MRMS	50MRMS	50MRMS	50MRMS	60MRMS
SC34 (*Sr2*+)	20R	20R	20RMR	20RMR	40MRR	40MRR	50MR	60MR	50MR	60MR
SC35 (*Sr2*+*Sr31*+)	5MR	5MR	20RMR	20R	40MRR	30MRR	60MR	40MRR	60MR	40MRR
SC36 (*Sr2*+*Sr31*+)	20R	20R	20RMR	20R	40MRR	40MRR	40MRR	50MRR	40MRR	50MRR
SC37 (*Sr2*+*Sr31*+)	10MSS	10MS	40MSS	40MSS	70MSMR	40MSMR	80MSMR	50MS	80MSS	50MS
SC38 (*Sr31*)	20S	20S	40S	40S	50MS	50MS	60MSS	60MSS	80MSS	80MSS
SC39 (*Sr2*+)	0	0	0	0	5RMR	TR	10MRR	50MRR	20MRR	50MRR
Line 37-07	10S	10S	60S	80S	70S	80S	90S	100S	90S	100S
25#2/163 BV2011[1]	10R	None	10RMR	None	20MR	none	30MR	none	30MR	none
WBC082SrBV2011[1]	10R	None	20RMR	None	40MRR	none	60MR	none	60MR	none
Agatha/9#LMPg9[1]	0	None	20RMR	None	30MR	none	40MR	none	40MR	none

[1]Effective *Sr* lines: 25#2/163 BV2011 (*Sr39*), WBC08 2SrBV2011 (*Sr26*) and Agatha/9#LMPg9 (*Sr25*). DAI=days after first inoculation.Disease severity (%, modified Cobb scale) and response type where 0=immune, T=trace, R=resistance, MR=moderate resistance,

MRMS=moderate resistance-moderate susceptible, MS=moderate susceptible S=susceptible. Postulated *Sr* gene information is according to Cengen results in 2012.

Table 4.4: Six distinct categories of greenhouse adult plant infection responses

1. Immune	**2. Resistance**	**3. Moderate resistance**
Score: 0	Score: TR – 40MRR	> 40MRR to 40MRMS
CBI1	SC2, SC4, SC8, SC14, SC15, SC16, SC26, SC29, SC30, SC31, SC32 and SC18	SC17, CBI7, SC21, SC24, SC25, SC34, SC35, SC36, SC39

Low field adult plant scores **Intermediate**

High field adult plant scores **Intermediate**

6. Very Susceptible	**5. Susceptible**	**4. Moderate susceptible**
40S to 100S	> 50MS to 30S	> 40MRMS to 50MS
SC1, SC5, SC6, SC7, SC10, SC11, SC13, CBI4, CBI6, CBI8, SC20, G1, CBI2, SC12 and SC38	SC9, CBI3, SC22, SC23, SC27, SC28, SC37 and PAN1.	SC3, SC19, CBI5 and SC33

Disease severity score (%, modified Cobb scale) and response type where 0=immune, T=trace, R=resistance. MR=moderate resistance, MRMS=moderate resistance-moderate susceptible, MS=moderate susceptible, S=susceptible.

4.4.2.2. Greenhouse adult plant peduncle scores

Host response was first scored on the peduncle of all entries seven days after inoculation and subsequently after every seven days. No severity score was included. CBI1 was the only line with an immune score (2.0%) of zero, 19 lines (38.8%) had MR score, two (4.1%) with moderate susceptible score (MS), five (10.2%) with susceptible score (S) and 22 lines (44.9%) with very susceptible score (VS). Generally, 59.2% (29 lines) of the Zimbabwean germplasm had MS to VS scores for the peduncle response. Among these susceptible lines, 51.7% (15 out of 29) were current commercial varieties, 17.2% (five) being old commercial varieties and 31.0% (9) experimental lines.

4.4.2.3. Flag leaf infection type and chlorosis (*Sr2* flecking) scores

Host infection types on flag leaves were recorded on a scale of 0 to 4 with 0 to 2 being low infection type (resistant) while 3 to 4 represents high infection type (susceptible). Scores were recorded seven days after inoculation and subsequently after every seven days. A total of 31 lines (63.3%) had scores between 0 and 2 indicating their resistance to PTKST stem rust race while the rest had high infection type scores (Table 4.5). Thirteen out of the 18 that had high infection type scores were current commercial (10) and old varieties (three).

One of the phenotypic markers of *Sr2* besides the PBC trait is the seedling chlorosis/flecking response that is temperature and light sensitive but is observed on unaffected leaves of rusted host plants (Brown 1997). Sheen *et al.* (1968) as cited by Brown (1997) listed chlorosis in adult leaves as one of the symptoms of PBC. The Zimbabwean lines were scored for the presence or absence of flecking as an indication of the presence of *Sr2*, though at adult stage. A frequency of 38.8% (19) of the germplasm showed flecking signs, 53.1% did not show any flecking while 8.2% had unconfirmed flecking signs. In total 89.5% of these results were corroborated by genotyping information by CenGen (Chapter 2) whereby 17 of the 19 lines that were recorded with flecks were also postulated to have *Sr2* by molecular analysis. Flecks were recorded on SC5 and SC9 while genotypic data postulated absence of *Sr2* on the two varieties. CenGen genotypic information also postulated presence of *Sr* in SC2 and SC6 but these did not show any flecks in the greenhouse. Comparing with genotypic data by Limagrain on (Chapter 2), genotypic data indicates *Sr2* presence in 34 lines while flecking records in the greenhouse had 12 of these with flecks, two were unconfirmed and 20 had no flecks. Limagrain's real time PCR protocol also indicated six heterozygotes with four of these

recorded as having flecks, one unconfirmed and one without flecks. Limagrain data had four lines without *Sr2* while one of these was unconfirmed and three did not have flecks in the greenhouse.

Table 4.5: Adult plant peduncle, flag leaf and *Sr2* flecking scores on three occasions for 49 Zimbabwean wheat lines inoculated with PTKST race.

* Host response where 0=immune, T=trace, R=resistance, MR=moderate resistance, MRMS=moderate resistance-moderate susceptible, MS=moderate susceptible S=susceptible. +/- presence or absence of flecks; +? unclear

** Stakman *et al*. (1962) infection types where 0 to 2 = low infection type (resistant) and 3 to 4 = high infection type (susceptible)

	First				**Second**				**Third**					
	Peduncle*		**Flag leaf****		**Peduncle***		**Flag leaf****		**Peduncle***		**Flag leaf****		***Sr2* Flecking**	
Entry (Gene*)	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**
SC1 (*Sr31*+)	MS	MS	3	3	S	S	3+	3+	S	S	3+	3+	-	-
SC2 (*Sr2*+*Sr31*+)	0	0	;	;	0	MR	;	;	MR	MR	;1	;1	-	-
SC3 (*Sr2*+*Sr31*)	S	S	3-	2	VS	VS	3	2+	VS	VS	3	2+	+	+
SC4 (*Sr2*+)	R	R	;	;	R	MR	;	;	MR	MR	;	;	+	+
SC5 (unknown)	VS	S	3+	3	VS	S	3+	3	VS	S	3+	3	+	+
SC6 (*Sr2*+*Sr31*)	S	S	2+	2+	S	S	3	2+	S	S	3	2+	-	-
SC7 (*Sr2*+)	S	S	2+	2	S	S	2+	2+	S	S	2+	2+	+	+
SC8 (*Sr2*+*Sr31*+)	0	R	;	;	0	MR	;	;	0	MR	;1	;	+	+
SC9 (*Sr31*+)	S	S	1+	2	VS	S	2	2	VS	S	2+	2+	+	+
SC10 (*Sr31*)	S	MS	4	2	VS	VS	4	2	VS	VS	4	2	-	-
SC11 (*Sr31*)	VS	S	2+	2	VS	S	2+	2+	VS	VS	3	2+	-	-
SC12 (*Sr31*)	VS	VS	2+	2+	VS	VS	3	3	VS	VS	3	3	-	-
SC13 (*Sr2*)	VS	VS	2	2	VS	VS	3	2+	VS	VS	3	3	+	+

	First				**Second**				**Third**					
	Peduncle*		**Flag leaf****		**Peduncle***		**Flag leaf****		**Peduncle***		**Flag leaf****		***Sr2* Flecking**	
Entry (Gene*)	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**
CBI1 (*Sr36*)	0	0	0	0	0	0	0	0	0	0	0	0	-	-
CBI2 (*Sr31*)	VS	VS	2+	2+	VS	VS	2+	2+	VS	VS	2+	2+	-	-
CBI3 (*Sr2*+*Sr31*)	S	S	2	2	S	S	3	2+	S	S	3	2+	+	+
CBI4 (*Sr2*+*Sr31*)	S	S	2-	2	VS	S	2-	2+	VS	VS	2_	2+	+	+
SC14 (unknown)	MR	R	;	;1	MR	R	;1	;1	MR	R	;1	;1	+?	+?
SC15 (*Sr31*+)	MR	R	;	;	MR	R	;	;	MR	R	;	;	-	-
SC16 (*Sr2*+)	R	R	;	;	MR	R	;	;	MR	MR	;	;	+	+
SC17 (*Sr31*+)	MR	MR	;	;	MR	MR	2-	;1	MR	MR	2	2	-	-
SC18 (*Sr31*+)	MR	MR	;	;	MR	MR	;	;	MR	MR	;1	;1	-	-
SC19 (unknown)	MR	MR	;	;1+	MR	MR	;	2	MR	MR	;1	2	-	-
CBI5 (unknown)	VS	VS	3	3	VS	VS	3	3	VS	VS	3	3	-	-
CBI6 (unknown)	VS	VS	4	3	VS	VS	4	4	VS	VS	4	4	+?	-
CBI7 (*Sr31*+)	MS	MS	2	2	S	MS	2	2	S	MS	2+	2	-	-
CBI8 (unknown)	S	S	2+	3	VS	VS	2+	3	VS	VS	2+	3	+?	-
PAN1 (*Sr24*)	S	S	2	2	S	S	2	2	S	VS	2	2	+	+?
SC20 (unknown)	VS	VS	3+	3+	VS	VS	3+	3+	VS	VS	3+	3+	-	-
SC21 (unknown)	MR	MR	;	;1	MR	MR	;	;1	MR	MR	;1	;1	-	-
SC22 (*Sr24*)	S	S	2+	2	S	VS	3	2	VS	VS	3	2	-	-

	First				Second				Third					
	Peduncle*		**Flag leaf****		**Peduncle***		**Flag leaf****		**Peduncle***		**Flag leaf****		***Sr2* Flecking**	
Entry (Gene*)	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**
SC23 (*Sr2*+*Sr31*+)	VS	VS	;1	2	VS	VS	2	2	VS	VS	2	2	+	+
SC24 (*Sr31*+)	MR	MR	;	;	MR	MR	;1	;1	MR	MR	;1	;1	-	-
SC25 (*Sr24*+)	MS	MS	;1-	;1-	MS	MS	;1	;1	MS	MS	;1	;1	-	-
SC26 (unknown)	S	S	2	2	S	S	2	2	S	S	2	2	-	-
SC27 (*Sr31*)	MS	MS	2	2	S	S	2+	3	VS	VS	3	3	-	-
SC28 (*Sr31*)	VS	MS	3	1,2	VS	S	3	3	VS	VS	3	3	-	-
SC29 (unknown)	MR	MR	;1	;	MR	MR	;1	;	MR	MR	;1	;1	-	-
SC30 (*Sr2*+*Sr31*+)	TR	MR	;1-	;	MR	MR	;1-	;	MR	MR	;1	;1	+	+
SC31 (*Sr2*+)	0	MR	;1	;	0	MR	;1	;	0	MR	;1	;1	+	+
G1 (*Sr2*+*Sr31*)	S	S	2+	2+	S	S	2+	2+	S	VS	2+	2+	+	+
SC32 (*Sr24*+)	MR	MR	2	2	MR	MR	2+	2+	MR	MR	2+	2+	-	-
SC33 (*Sr24*+)	VS	VS	2	2	VS	VS	2+	2+	VS	VS	2+	2+	-	-
SC34 (*Sr2*+)	0	MR	;1	;1	0	MR	;1	;1	MR	MR	;1	;1	+	+
SC35 (*Sr2*+*Sr31*+)	MR	MR	;	;	MR	MR	;1	;	MR	MR	;1	;1	+	+
SC36 (*Sr2*+*Sr31*+)	MR	R	;	;	MR	MR	;	;	MR	MR	;	;	+	+
SC37 (*Sr2*+*Sr31*+)	VS	S	3	3+	VS	S	3+	3+	VS	S	3+	3+	+	+
SC38 (*Sr31*)	VS	VS	3+	3+	VS	VS	3+	3+	VS	VS	3+	3+	-	-
SC39 (*Sr2*+)	MR	R	;	;1	MR	MR	;1	;	MR	MR	;1	;1	+	+

	First				Second				Third					
	Peduncle*		**Flag leaf***		**Peduncle***		**Flag leaf***		**Peduncle***		**Flag leaf***		***Sr2* Flecking**	
Entry (Gene*)	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**
Line 37	VS	VS	3	3	VS	VS	3	3	VS	VS	3	3	na	na
25#2/163 BV2011[1]	MR	none	;1	none	MR	none	;1	none	MR	None	;1	none	na	na
WBC082SrBV2011[1]	MR	none	;1	none	MR	none	;1+	none	MR	None	;1+	none	na	na
Agatha/9#LMPg9[1]	0	none	;1-	none	0	none	;1-	none	0	None	;1-	none	na	na

[1]Effective *Sr* lines: 25#2/163 BV2011 (*Sr39*), WBC08 2SrBV2011 (*Sr26*) and Agatha/9#LMPg9 (*Sr25*). na: not tested for *Sr2*. * Postulated *Sr* gene information is according to CenGen results in 2012.

4.4.3. Field adult plant host responses

These scores were based on two sets of scores done on two replications in 2012 while only one score was done on one replication in 2014. Seven lines (14.3%) had a susceptible reaction in 2012 with scores ranging from 5S (SC37) to 80S (SC20 and SC38). A moderate resistance reaction was shown by 32.7% of the lines, 20.4% were resistant whereas the moderate susceptible to susceptible categories had the lowest proportion of 6.1%. SC11 (40S), CBI5 (70S) and SC20 (80S) were some of the commercial varieties that were susceptible. In 2014, 38.8% of the lines showed a resistant reaction, 16.3% with moderate resistance, 12.2% moderate resistance to moderate susceptible, 10.2% resistant to moderate resistance 8.2% were susceptible and moderate susceptible, respectively while 6.1% were moderate susceptible to susceptible. Only four varieties showed a susceptible reaction with scores ranging from 30S (SC3) to 80S (SC38). Three of these lines (SC27, SC28 and SC38) had a susceptible reaction again in 2012.

Over the two seasons, considering only the highest scores (most susceptible) for the wheat lines, 10 lines (20.4%) showed a resistant reaction ranging from TR (SC8, SC4, SC2, SC16, SC30, SC39 and SC24 (mixed score of TR, 30MS)) to 5R (SC9, SC32 and SC36)). A total of 34.7% (17 lines) of the lines had a moderate resistance (MR) score ranging from TMR (SC35, SC29, SC21) to 30MR (SC12). Moderate resistance-moderate susceptible (MRMS) response was shown by 18.4% (nine) of the lines, ranging from 10MRMS (CBI4 and PAN1) to 40MRMS (SC13). Moderate susceptible (MS) reactions were shown by 12.2% (six lines) of the germplasm with a score range of 5MS, N (SC23, with some necrosis) to 60MSS (CBI8). Susceptible scores ranging from 5S (SC37) to 80S (SC38 and SC20) constituted 14.3% (seven lines). SC3 (30S), SC20 (80S) and CBI5 (70S), three of current commercial varieties were susceptible. Some lines would show an overlapping response such as SC34 (20RMR) with both a resistant and moderate resistant reactions while other lines would show mixed reactions such as SC11 (20MR, 40S) and SC22 (TR, 30MS) in one of the replications in 2012. Table 4.6 shows a summary of all the adult plant field scores for the different lines.

Table 4.6: Adult plant field stem rust scores for 49 Zimbabwean wheat lines as evaluated over two seasons

	2012				2014
	18-Oct		5 & 6 Nov		3-Nov
Entry	**Rep1**	**Rep2**	**Rep 1**	**Rep 2**	**Rep 1**
SC1 (*Sr31+*)	0	0	20MRMS	10MR	20MRMS
SC2 (*Sr2+Sr31+*)	0	0	TR	TR	TR
SC3 (*Sr2+Sr31*)	0	0	15MSS	20MR,N	30S
SC4 (*Sr2+*)	0	0	TR	TR	TR
SC5 (unknown)	0	0	10MRMS	20MRMS	20MRMS
SC6 (*Sr2+Sr31*)	0	0	20MRMS	5MR	20MR
SC7 (*Sr2+*)	0	0	10MR	10MR	10MR
SC8 (*Sr2+Sr31+*)	0	0	TR	TR	0R
SC9 (*Sr31+*)	TS	0	TMR	TR	5R
SC10 (*Sr31*)	0	TS	40MSS	20MR	20MSS
SC11 (*Sr31*)	0	0	50MSS	20MR,40S	40MS
SC12 (*Sr31*)	5S	0	5MR	5MR	30MR
SC13 (*Sr2*)	0	0	40MRMS	10MR	15RMR
CBI1 (*Sr36*)	0	0	TR	5MR	TR
CBI2 (*Sr31*)	0	TS	10MR	20MR	20MR
CBI3 (*Sr2+Sr31*)	0	0	10MRMS	20MR	10R
CBI4 (*Sr2+Sr31*)	0	0	10MRMS	5MR	10R
SC14 (unknown)	0	0	TR	5MR	0R
SC15 (*Sr31+*)	0	0	5RMR	5MR	5R
SC16 (*Sr2+*)	0	0	TR	TR	TR
SC17 (*Sr31+*)	0	0	TMR	10MR	5R
SC18 (*Sr31+*)	0	0	TR	20MR	10R
SC19 (unknown)	0	0	TR,1p40MSS	20MR	10R
CBI5 (unknown)	0	0	TR	10MR	10RMR
CBI6 (unknown)	TS	0	70S	50S	60MS
CBI7 (*Sr31+*)	TS	0	10MR	20MR	10MR
CBI8 (unknown)	TS	TS	60MSS	30MSS	20MS
PAN1 (*Sr24*)	0	0	TMR	TMR	10MRMS

	2012				2014
	18-Oct		5 & 6 Nov		3-Nov
Entry	**Rep1**	**Rep2**	**Rep 1**	**Rep 2**	**Rep 1**
SC20 (unknown)	5S	TS	70S	80S	30MSS
SC21 (unknown)	0	0	TR	TMR	5RMR
SC22 (*Sr24*)	0	0	TR,30MS	30MRMS	10MR
SC23 (*Sr2*+*Sr31*+)	0	0	TMR	5MS,N	TMS
SC24 (*Sr31*+)	0	0	TR	TR,30MS	0R
SC25 (*Sr24*+)	0	0	TR	5MR	5RMR
SC26 (unknown)	0	0	30MRMS	20MR	70MR
SC27 (*Sr31*)	0	0	30MS	50S	70S
SC28 (*Sr31*)	0	TS	40S	50S	40S
SC29 (unknown)	0	0	TR	TMR	5R
SC30 (*Sr2*+*Sr31*+)	0	0	TR	TR	0R
SC31 (*Sr2*+)	0	0	TR	TR	20MRMS
G1 (*Sr2*+*Sr31*)	0	0	TMR	30MRMS	30MSS
SC32 (*Sr24*+)	0	0	TR	TR	5R
SC33 (*Sr24*+)	0	0	30MS	10MR	50MRMS
SC34 (*Sr2*+)	0	0	5MR	TR	20RMR
SC35 (*Sr2*+*Sr31*+)	0	0	TR	TR	TMR
SC36 (*Sr2*+*Sr31*+)	0	0	TR	TR	5R
SC37 (*Sr2*+*Sr31*+)	0	0	5S	TMS,N	15MRMS
SC38 (*Sr31*)	10S	5S	70S	80S	80S
SC39 (*Sr2*+)	0	0	TR	TR	TR
Line 37-07	TS	TS	80S	70S	70S

Disease severity (%) and host response where 0=immune, T=trace, R=resistance. MR=moderate resistance, MRMS=moderate resistance-moderate susceptible, MS=moderate susceptible, S=susceptible. Line 37-07 was a susceptible check variety.

4.5. Discussion

PTKST has been detected both in Zimbabwe (2010) and South Africa (2009) and is virulent to *Sr31* and *Sr24* (Singh *et al*., 2011a), which are some of the major genes in Southern Africa germplasm. PTKST is avirulent to (ITs 0; to 2++) *Sr9h, 13, 14, 21, 22, 25, 26, 27, 29, 32, 33, 35, 36, 37, 39, 42, 43, 44, Em, Kiewiet, Satu, Tmp* and virulent (ITs 3 to 4) to *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 16, 17, 24, 30, 31, 34, 38, 41 and McN* (Pretorius *et al*., 2010; Terefe *et al*., 2016). The proportion of Zimbabwean wheat varieties that had *Sr31* alone or in combination with other *Sr* genes was 53.1% while 38.7% had *Sr2* alone and/or in combination with *Sr31* and other unknown genes. It would be expected for PTKST to be more virulent against the Zimbabwean germplasm given the dominance of *Sr31* in the genepool. Greenhouse infection type studies confirmed this given that 40.8% of Zimbabwean germplasm was susceptible to PTKST, which was the highest among the four Ug99 races.

The Zimbabwean wheat germplasm has been divided into ten different groups depending on the postulated genetic composition (refer to Figure 2.4), for the ease of this discussion.

Group 1 (*Sr2*): SC13 was postulated to have *Sr2* only and it had high infection types against all the Ug99 races in the greenhouse. Adult plants were susceptible to PTKST in the greenhouse with 100S accompanied by high rust scores on both peduncles and flag leaves. Plant chlorosis/flecking indicated the presence of *Sr2* which was in line with genotypic data. *Sr2* is an APR gene that shows partial resistance but cannot offer adequate levels of resistance on its own (Ayliffe *et al*., 2008; Njau *et al*., 2010; Singh *et al*., 2011a; 2011b), especially under high disease pressure induced in greenhouse studies. The highest field score for SC13 over two seasons was 40MRMS. This gene cannot be deployed on its own in wheat germplasm.

Group 2 (*Sr2*+): Six Zimbabwean lines, SC4, SC7 (commercial varieties), SC16, SC31, SC34 and SC39 were postulated to have *Sr2* and some unknown minor genes (Figure 2.4). Five of these lines had low infection type scores ranging from ITs 1 to ;1= against all four races with the exception of SC7 which was susceptible to all Ug99 races. These five (SC4, SC16, SC31, SC34 and SC39) had a moderate resistant greenhouse adult plant reaction ranging from 20MRR (SC16) to 60MRR (SC34), field scores that were ranging from TR (SC4 and SC16) to 20MRMS (SC31), peduncles showed moderate resistance and ; to ;1 scores for flag leaf

infection type (Table 4.5). SC7 was susceptible to PTKST in the adult plant stage in the greenhouse (80S) while the peduncle was susceptible and the flag leaf infection type score was 2+. Flecking scores confirmed all six varieties to have the *Sr2* gene corroborating genotyping results. The major gene *Sr25* was postulated by Limagrain laboratory (Table 2.5) to be present in SC4, SC16, SC34 and SC39 and in combination with *Sr2* could have provided adequate resistance at both seedling and adult plant stages. SC7 does not have *Sr25* and *Sr2* alone could not provide adequate protection against all four Ug99 races at seedling stage and against PTKST at adult stage.

Group 3 (*Sr2+Sr31*): Except for two (SC23 and SC37), these lines were susceptible to PTKST but resistant against TTKSF, TTKSF+*Sr9h* and TTKSP. The two sister lines, SC23 and SC37 were the only lines that showed resistance against all four Ug99 races at seedling stage. All varieties had high adult greenhouse scores ranging from 80MRMS (SC3) to 100S (G1 and SC6) while 30S (SC3) was the highest under field conditions. All varieties had susceptible to very susceptible peduncles and IT scores ranging from 2+ to 3+ for the flag leaf. Five lines showed the presence of chlorosis/flecks which was in agreement with the genotyping results. SC6 was the only line that did not show chlorosis. *Sr31* is susceptible to PTKST therefore its additive effect in the *Sr2+Sr31* complex did not give much protection against PTKST both at seedling (except for the SC37 and SC23 sister lines) and adult plant stages.

Group 4 (*Sr2+Sr31+*): The group had the best level of protection in both seedling and adult stages among Zimbabwean germplasm. SC8, SC2, SC30, SC35 and SC36 were resistant against all four Ug99 races at the seedling stage (Low infection type scores). Adult plants showed a resistant score range of 10RMR (SC8) to 60MSS (SC23) in the greenhouse and scores of TR (SC2, SC8 and SC30) to 5MS, N (SC23) in the field. All peduncles were moderately resistant except for SC23 which was very susceptible. Flag leaf scores were of low infection type (;1 to 2) while SC2 is the only variety that did not comply with genotyping data given that it did not show any leaf chlorosis. The minor genes involved in the *Sr2*, *Sr31* and other unknown genes complex were very effective in complementing to the resistance shown by these lines. This proves the point that has been advocated in breeding, that by pyramiding several minor genes alone or a combination of race specific (major genes) and several (4-5)

minor genes tend to give the best protection against rust disease (Knott, 1982; Singh *et al.*, 2011b and Njau *et al.*, 2010).

Group 5 (*Sr31*): All lines with *Sr31* (SC10, SC11, SC12, CBI2, SC27, SC28 and SC38) were susceptible to PTKST with IT scores ranging from 3 to 4. All seven lines were resistant against TTKSF (ITs ;1= to 2++) while only six were resistant against TTKSF+*Sr9h* and TTKSP with an IT score of ;1=. CBI2 was susceptible to PTKST, TTKSF+*Sr9h* and TTKSP with IT scores ranging from 3- to 3. CBI2 was expected to be resistant to TTKSF+*Sr9h* and TTKSP but off-type plants (seed impurity caused by admixtures) could have been phenotype. The lowest greenhouse adult plant score for this group was 100MS (SC27 and SC28 - two lines introduced from CIMMYT) with a high of 100S (SC10 and SC11). Field scores ranged from TS (CBI2) to 80S (SC38) while all peduncles were very susceptible and the flag leaf scores of 3 to 4 (except for CBI2 with a 2) showed the high level of susceptibility of this group. All the lines did not show any chlorosis indicating that these lines did not have *Sr2* according to genotyping results by CenGen. The group also confirms the fact that *Sr31* is ineffective to PTKST both at seedling and adult stages. SC10, SC12, SC11 are commercial varieties in Zimbabwe, CBI2 is an old variety and the other three lines in this group were experimental lines. Pretorius *et al.* (2010) reported the virulence of PTKST (UVPgt60) to all *Sr31* testers that included Gamtoos, *Sr31*/6*LMPG, Federation4*/Kavkaz, Kavkaz and Clement with ITs of 3+ to 4. The original Ug99 outbreak in 1998 was due to virulence of TTKSK stem rust race for *Sr31*, a gene that was widely deployed and has been effective for many years (Singh *et al.*, 2006a). There is need to replace this gene with other effective stem rust resistance genes.

Group 6 (*Sr31*+): Presence of other unknown minor genes in SC15, SC17, SC18 and SC24 provided adequate protection against all four Ug99 races at seedling stage (Table 4.2) while SC9, CBI7 and SC1 were susceptible to PTKST. Greenhouse adult scores (Table 4.3) showed moderate resistant response for five lines ranging from 40R (SC15) to 60MR (CBI7). SC9 (60MS) and SC1 (60S) were two lines that were susceptible. In the field (Table 4.6) lines showed responses ranging from TS (SC9) to 20MRMS (SC1) but peduncles were susceptible for SC1, CBI7 and SC9 (Table 4.5). Other lines had MR score for peduncles while flag leaf scores were low except for SC1 with 3+. SC9 was the only line with flecks indicating its potential to have *Sr2* which was not postulated by genotyping data (Figure 2.4 and Table 2.3). However, it is quite likely that other genes may also express leaf chlorosis and that *Sr2* presence purely based on flecking could be incorrect.

Group 7 (*Sr24*)*:* is supposed to be susceptible to PTKST (given the virulent formula in Table 4.1) but PAN1 and SC22 were resistant to all Ug99 races at seedling stage and had adult host responses of 60MSS and 80MSS, respectively in the greenhouse adult stage. The unexpected responses of PAN1, SC22 and LCSr24Ag (check) could be due to failure of the spores to infect the host due to unfavourable conditions or low spore concentration in the mix causing "escapes". Scores were lower in the field at 10MRMS (PAN1) and 30MRMS (SC22). Both lines had very susceptible peduncles while a flag leaf score of 3 for the latter, indicates susceptibility to PTKST. The presence of flecks on PAN1 suggests the presence of *Sr2* which was not indicated by genotypic data. The low infection types of PAN1 were also reported by Pretorius *et al*. (2012) when the same variety had IT1 for TTKSF, and IT2- for TTKSP and PTKST, respectively. It thus appears that the variety may be carrying another gene in combination with *Sr24*. Limagrain genotyping results (Table 2.7) postulated the presence of *Sr2/Lr27*, *Lr34/Yr18* and *Lr24/Sr24,* all in heterozygous states. Njau *et al*. (2010) highlighted the fact that *Sr24* tends to show a higher level of resistance when in combination with other sources of resistance e.g. the 1A.1R chromosomal translocation from rye. TTKST, virulent to *Sr24* was detected in East Africa in 2006 (Jin *et al*., 2008; Prins *et al*., 2016) indicating that this gene alone cannot be used in breeding programmes.

Group 8 (*Sr24*+): Three lines, SC25, SC32 and SC33 were resistant against all Ug99 races used in this study. They showed MRMS reactions both in the greenhouse and in the field at adult stages. The infection type score for the flag leaf was also low and not associated with flecking. This group has *Sr24* in combination with unknown minor genes that have additive effects responsible for stem rust resistance.

Group 9 (*Sr36*): CBI1 was the only line with *Sr36.* The gene is still effective against the four Ug99 races used in this study but race TTTSK virulence to this gene has been detected in East Africa in 2007 (Jin *et al*. 2009; Njau *et al*., 2010; Prins *et al*., 2016). It was always giving a reaction close to immune in seedling and adult stages and no chlorotic signs were seen. However the gene is not recommended for further deployment due to its non-durability and susceptibility to African race TTTSK in particular.

Group 10 (Unknown genes): Ten Zimbabwean lines with unknown genes had variable responses both at seedling and adult plant stages. SC14, SC19, SC21, SC26 and SC29 were

resistant against all four Ug99 races at seedling stage. CBI6 was resistant against TTKSP while CBI8, SC7and SC20 were susceptible to all Ug99 races (Table 4.2). SC5, CBI5, CBI6, CBI8 and SC20 were susceptible to PTKST in the greenhouse at the adult stage with scores ranging from 30S to 100S (Table 4.3). In the field, CBI6 (70S) and SC20 (80S) were susceptible while SC14 (5MR) and SC29 (5R) were resistant (Table 4.6). There were variable responses on peduncles with SC5, CBI5, CBI6, CBI2 and SC20 showing very susceptible reactions. SC5 had chlorotic symptoms suggesting the presence of *Sr2* while SC14, CBI6 and CBI8 need to be verified because the flecking was not very clear. The variable responses shown by lines in this group indicate the presence of minor genes that might be involved in different combinations thereby resulting in different host responses. Again, this indicates the complexity of gene interactions in conferring resistance.

The need for verification processes throughout the different stages of experimentation cannot be over emphasized. Recording notes from all the replications in the various trials, repeating the field evaluations over two seasons to consider seasonal variation and use of the best "positive" and "negative" checks in all trials were some of the precautions that were taken during the study. Check varieties were selected based on the stem rust gene under investigation so that both negative and positive controls were included. In greenhouse seedling trials, Federation*4/Kavkaz (*Sr31*) and Pavon 76 (*Sr2*) were included as susceptible checks against PTKST while Satu (*SrSatu*) and Avocet S (*Sr26*) were the resistant checks. Against the other three Ug99 races, LC*Sr24*Ag (*Sr24*), *Sr31*/6*LMPG (*Sr31*), Matlabas (*Sr9h*) and McNair (*SrMcN*) were used as check varieties. For instance, susceptibility of Matlabas (*Sr9h)* to TTKSF+*Sr9h* was used to differentiate this race from TTKSF. TTKSF+*Sr9h* was virulent against seedlings with *Sr2, Sr2+*, *Sr9h*, *SrMcN* and unknown genes in CBI8, CBI6 and SC20 while TTKSF was avirulent on *Sr9h* and *Sr31*. Virulence of TTKSF+*Sr9h* to *Sr9h* was reported by Visser *et al*. (2011) as the difference between the two races. In the greenhouse adult plant trial, three known stem rust resistant lines were also included as checks (25#2/163 BV2011 (*Sr39*), WBC08 2SrBV2011 (*Sr26*) and Agatha/9#LMPg9 (*Sr25*)). These same lines were used as donor parents for stem rust resistant gene introgression in Chapter 6. Line 37-07 and Morocco, two lines known to be susceptible to these races were used on border rows or within the trial plots for benchmarking and to check if inoculum was present in the environment around the trial area. Genotypic data by CenGen and Limagrain were in agreement on 16 lines out of the 44 that were genotyped by both laboratories. This is probably due to differences in

protocols and markers that were used for the different genes. When the CAPS marker for *Sr2* is used, sometimes it does not detect the gene in lines predicted to carry the gene (Mago *et al*. 2011). It is very important to use the same seed source for both phenotyping and genotyping exercises in order to avoid differences in results that might be due seed admixtures. Use of different sources of seed of the same variety for CenGen and Limagrain genotyping exercises might have have resulted in differences in some lines such as SC27 postulated to have *Sr25* by Limagrain but the line was susceptible to PTKST in seedling phenotyping. The difference in lines that have *Sr2* is also a good example why a single seed source can help to resolve these differences. Data verification and independent assessments are always critical before conclusions are made.

Different approaches for characterizing wheat germplasm were used to generate as much information as possible. Genotypic data, phenotypic data for both seedling and adult plant stages and phenotypic markers associated with genes like leaf chlorosis/flecking linked to *Sr2* were all used to characterize the Zimbabwean germplasm. Results indicate that this is feasible but requires expertize and relevant infrastructure in order to carry out this type of work. One needs to understand the conditions that are suitable for storage, bulking and inoculation using Ug99 isolates. The need for purity and controls to avoid contamination especially when using isolates of different races cannot be over emphasized.

Disease pressure in the field is usually not as high as under artificial conditions in the greenhouse and it also varies from season to season depending on weather conditions. In both seasons of field observations (2012 and 2014) rust symptoms were observed and the disease pressure was almost similar when the November scores are considered. Lines could also show mixed responses both in the greenhouse or field when scoring. Probably because of differences in inoculum races for the field trials while for the greenhouse trials possibility of seed admixtures cannot be ruled out. Field trial results indicate the dominance of PTKST race at Greytown given that it is the same area, together with Cedara also in the province of KwaZulu-Natal where first isolates of PTKST were detected in 2009 (Pretorius *et al*., 2012). Host response trends were similar between field responses and greenhouse adult host responses when the same race infected the lines. There was a difference in the level of disease pressure as measured by host plant responses.

4.6. Recommendations and conclusions

It is difficult to predict plant phenotype in terms of seedling infection type or adult plant responses given the complexity of gene interactions and the environmental differences that usually influence these interactions. For instance, SC34 (*Sr2*+) had a moderate resistant score of 60MR while SC7 (*Sr2*+) had a susceptible score of 80S when adult plants were inoculated with the same PTKST race. There could be a diverse array of unknown minor genes that tend to influence the final phenotype. Availability of polymorphic markers and use of phenotypic markers such as PBC and chlorotic/flecking symptoms for *Sr2* could be utilized by breeders as proven in this study. Institutions can invest in molecular technologies or outsource from private service providers.

Breeding for durable resistance against rust diseases (such as stem rust) needs a targeted approach and modifications to traditional breeding methods. This might be time consuming, require exposure of breeding lines to the targeted pathotype (adequate disease pressure), precision selection of targeted genes (inheritance might be due to recessive genes e.g. *Sr2*, that might easily be lost if wrong approaches are used), need for increased numbers in order to increase frequency of selecting minor genes (but at the same time without stretching limited resources), and the ability to keep track of both deployed resistant genes and disease pathotypes that exist in a targeted market through a surveillance network. Breeding for APR based on several minor genes or a combination of minor and specific major genes has been proven to be the right way to go, if durable resistance is to be achieved. *Sr2* can still act as the backbone of such approaches with the advantage of the gene already existing in some elite varieties such as SC13 in the Zimbabwean germplasm. Other sources of minor or major genes need to be explored for pyramiding onto *Sr2*. Backcrossing to already adapted varieties will ensure that introduction of APR genes will not delay commercialization and other desirable traits such as high yield, good quality and good agronomic adaptation are retained within the recurrent parent. Marker assisted breeding has to be adopted in order to track retention of recurrent genes and at the same time inclusion of quantitative APR genes. Marker assisted breeding ensures that only breeding lines with the desired genes are selected, thereby saving on time, resources, allowing selection in the absence of disease exposure and having several generations per season. All these interventions result in increased genetic gains. Zimbabwean breeding programmes need to incorporate marker assisted breeding, explore for diverse sources of resistant genes, and use *Sr2* as the backbone for pyramiding resistant genes. There is an urgent

need to replace all susceptible commercial varieties such as SC20, SC3, SC1 and CBI5 to ensure that they do not act as susceptible hosts for inoculum build up leading to the evolution of more virulent pathotypes.

Characterisation data such as generated by this study must be part of the working tools for Zimbabwean wheat breeders for their decision making. This calls for investment in terms of both infrastructure and human capacity. Greenhouses, rust collection and storage facilities for artificial seedling and adult plant inoculations need to be invested in. Human resource development in areas of rust surveillance, phenotyping and information dissemination needs to be enhanced. This might call for an independent centre of excellence that will provide phenotyping services to all breeding programmes and maintenance of database on all commercial varieties under production in the country and connecting to the international community. Rust pathogenicity surveys must be a continuous process with the participation of all stakeholders given the rapid change in pathotypes due to climate change. The Zimbabwean wheat industry has to be part of the wheat global community to keep abreast with global trends on wheat and benefit from accrued knowledge, germplasm diversity (access new sources of resistance exploited through pre-breeding normally done by CGIARs and learning institutions such as Universities) and play a part in reducing the impact of global biotic stresses such as Ug99.

5. Genetics of stem rust resistance to race TTKSF in the wheat variety SC1

5.1. Introduction

Genetic studies enable breeders to know the sources and basis of resistance/susceptibility phenotypes in leading wheat varieties. This facilitates resistant varieties to be deployed into markets where pathogen virulence is also known. Genotyping work done on Zimbabwean germplasm indicated the presence of *Sr31* in SC1, the most popular commercial variety in Zimbabwe which is also grown in Zambia and Malawi. Singh *et al*. (2006a) reported that the worldwide use of stem rust resistance gene *Sr31,* derived from rye, was rendered ineffective (Pretorius *et al*. 2000) by emergence of a unique stem rust race, Ug99 (TTKSK) in 1998. Seedling infection type (IT) studies using four prevalent races of Ug99 in Southern Africa (PTKST, TTKSF, TTKSF+*Sr9h* and TTKSP) indicated that SC1 was resistant against three Ug99 races at seedling stage with IT scores ranging from ;1 to 1+ but susceptible to PTKST with IT score of 4 (Chapter 4). Since *Sr31* is ineffective to PTKST and several related races in the Ug99 group, SC1 is potentially vulnerable should an epidemic occur.

Using a Mendelian approach, the aim of this study was to determine if there are any unknown stem rust resistance genes in combination with *Sr31* that are responsible for seedling resistance in SC1. SC20, susceptible to all four races at seedling stage with IT scores ranging from 2+3 to 4, is an old variety with unknown genes according to previous genotyping work (Soko *et al*. 2015). The polymorphism that was expected between parents would be ideal for determining segregation ratios in progeny studies. Ug99 stem rust race TTKSF+*Sr9h,* which does not contain virulence for *Sr31,* was avirulent to SC1 (*Sr31*) but virulent to SC20 (Chapter 4) and this race was used to investigate the inheritance of stem rust resistance in SC1.

5.2. Study objectives

The main objective of this study was to determine the number of genes that are responsible for stem rust resistance in SC1 and establish their mode of inheritance by studying the segregation patterns of F_2 and F_3 progenies from a SC1 x SC20 simple cross.

5.3. Materials and methods

5.3.1. Genotypes

(a) SC1

This is a white grained Seed-Co spring bread wheat variety released in Zimbabwe in 1999. It was released on the basis of high yield. The variety is widely adapted hence it was also released in Zambia and Malawi. SC1 is the leading variety in terms of seed sales by Seed-Co Zimbabwe with a 67.3% market share. Genotyping results postulated the presence of *Sr31*. Inoculation of SC1 with TTKSF+*Sr9h* in the greenhouse in 2014 resulted in a low IT.

(b) SC20

A red grained Seed-Co spring bread wheat variety that is no longer being produced commercially. SC20 was known for its early maturity and premium bread baking qualities. It succumbed to leaf rust in Zimbabwe and has shown susceptibility to all four Ug99 races at seedling stage.

5.3.2. Development of study population

A simple cross was made between SC1 (resistant parent) and SC20 (susceptible parent) to generate F_2 and F_3 progeny. Leaf samples were taken from both parental lines for genotyping prior to crossing to generate F_1 plants. This was done to ensure that only genetically pure parents were used to generate the study population. A summary of the protocol is shown in Figure 5.1.

5.3.3. Seedling greenhouse phenotyping

F_1 seeds were planted using the protocol for greenhouse studies as mentioned in Chapter 3 and allowed to self-pollinate. A population of 342 F_2 seedlings was inoculated at the one and a half leaf stage using TTKSF+*Sr9h* and IT scores were recorded 12 days later. The objective was to note the infection types of both parental lines and their F_2 progeny and to test the hypothesis of segregation (ratio of 3 resistant: 1 susceptible) of a single gene (*Sr31*) for stem rust resistance. The same process was repeated for 80 F_3 families, independently advanced from the original SC1 x SC20 F_2 population, to establish if the segregating ratio will fit a 1 resistant

: 2 segregating : 1 susceptible model for single gene inheritance. A minimum of 30 plants were tested per F_3 family.

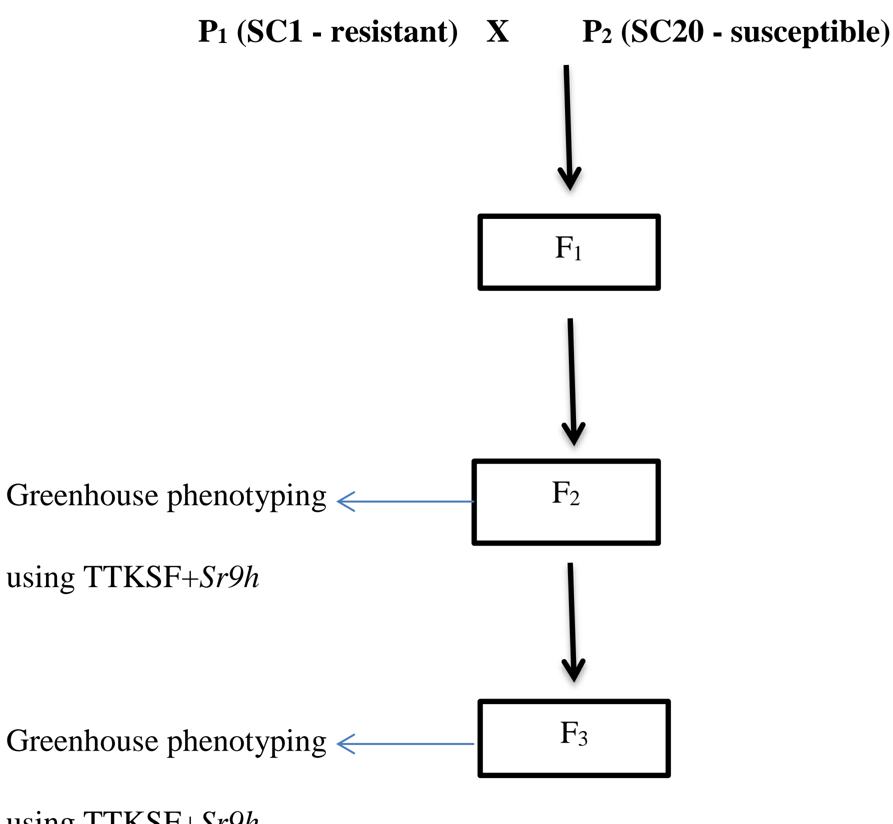

Figure 5.1: SC1 studies flow chart.

F_1, F_2 and F_3 progenies of the SC1 x SC20 cross and stages at which greenhouse seedling phenotyping was done using stem rust race TTKSF+*Sr9h*

5.4. Data analysis

The Chi-square goodness of fit test was used to determine if the observed F_2 and F_3 progeny segregation deviated significantly from the expected 3:1 and 1:2:1 ratios, respectively. The formula below was used to calculate the Chi-square statistic (χ^2) and associated p value with Microsoft Excel 2010.

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

Where χ^2: Chi-square goodness of fit test

(r-1)(c-1): Chi-square goodness of fit test degrees of freedom where r is the number of rows and c is the number of columns.

O: Observed values

E: Expected values

Decision: Reject the H_0 if $\chi^2 > \chi^2_c$critical value

Reject the H_0 if p value < 0.05%

5.5. Results

5.5.1. Phenotyping of F_2 plants using TTKSF+*Sr9h* race

A total of 342 plants were scored for infection type using the Stakman *et al.* (1962) system with 0-2 being resistant (low infection) and 3-4 being susceptible (high infection). Resistant plants (n=246) had infection types that ranged from ;$1^{=}$ to ;1 while susceptible plants (n=96) displayed 3 to 3^{++} (Figure 5.2). Parental lines had infection types of ;1^{-} for SC1 and 3^{++} for SC20. Since the Chi-square goodness of fit value ($\chi^2_{3:1}$) of 1.719 (Table 5.1) was smaller than the critical value of 3.841 at one degree of freedom, the H_0 that segregation in SC1 is due to a single, dominant stem rust resistance gene can be accepted. The p value of 0.190 was higher than 0.05 indicating that the observed ratio did not deviate significantly from a 3:1 model.

Table 5.1: Observed and expected infection types for F_2 progenies of SC1 x SC20 after inoculation with TTKSF+*Sr9h* Ug99 race, calculated chi-square value and associated p value

IT score	Observed (O)	Expected (E)	O-E	(O-E)²	((O-E)²)/E
Resistant	246	256.5	-10.5	110.25	0.429825
Susceptible	96	85.5	10.5	110.25	1.289474
$\chi^2_{3:1}$ value					1.719298
p-value					0.189783

IT: Infection type score where 0 – 2 (resistant) and 3 – 4 (susceptible). χ^2: Chi-square value at 0.05% and 1 degree of freedom.

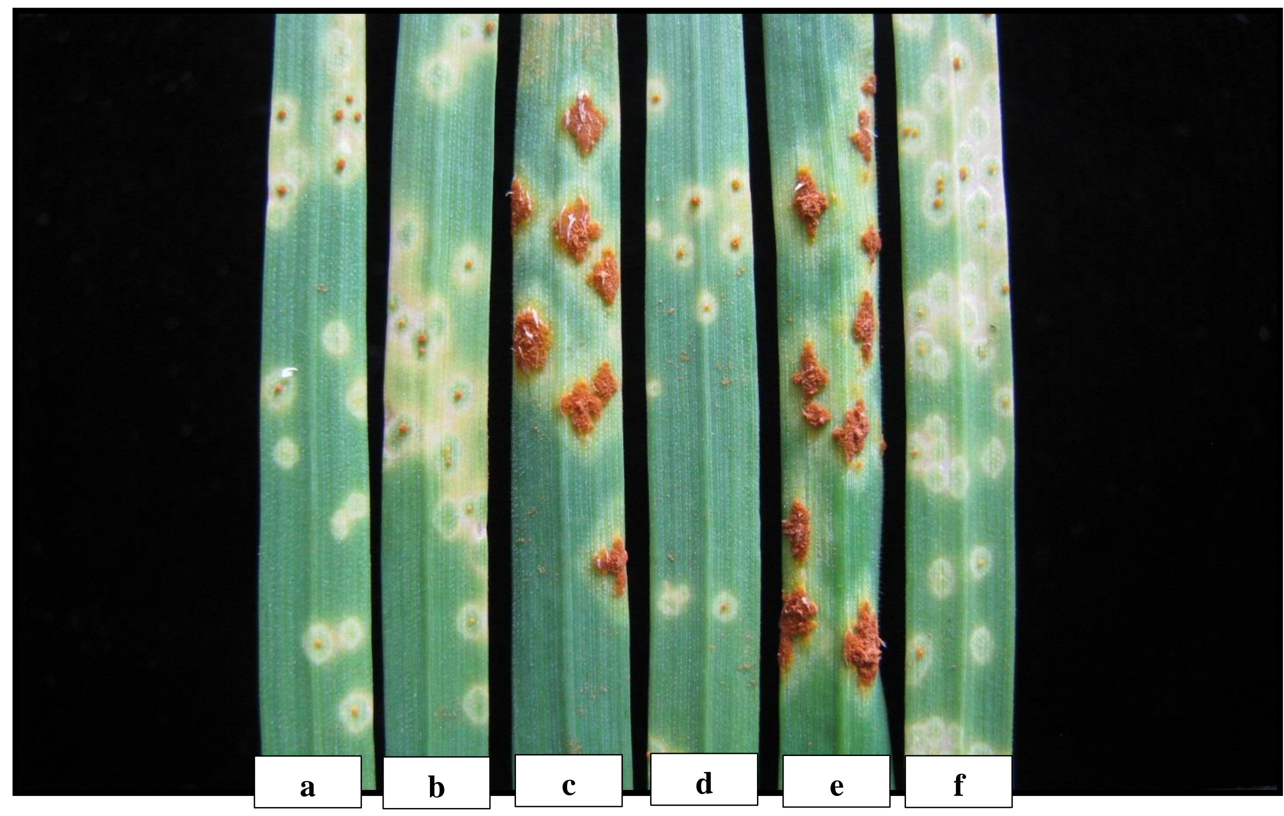

Figure 5.2: SC1 studies infection type photos.

Left to right - SC1 x SC20 resistant F_2 plants (a and b), susceptible F_2 plant (c), SC1 with IT of ;1- (resistant, d), SC20 with IT of 3++ (susceptible, e) and Federation*4/Kavkaz with IT of ;1 (resistant check with *Sr31* gene, f)

5.5.2. Phenotyping of F_3 plants using TTKSF+*Sr9h* race

The 80 F_3 families resulted in three phenotype categories after being inoculated with TTKSF+*Sr9h*. Fifteen families were grouped as homozygous resistant, 44 families were segregating and 21 families were homozygous susceptible (Table 5.2). ITs for resistant plants included scores of $;^{c}$, $;1^{=}$ and ;1 while susceptible phenotypes had IT scores ranging from 3 to 4 (Figure 3). The $\chi^2_{1:2:1}$ value of 1.7 (p=0.427) (critical value at p =0.05 for two degrees of freedom = 5.99) did not deviate significantly from the expected ratio of 1:2:1. The H_0 hypothesis of single gene resistance obtained in the F_2 was therefore confirmed and accepted in the F_3.

Table 5.2: Observed and expected infection types for F_3 progenies of SC1 x SC20 after inoculation with TTKSF+*Sr9h* Ug99 race, calculated chi-square value and associated p value

IT	Observed (O)	Expected (E)	O-E	$(O-E)^2$	$((O-E)^2)/E$
Resistant	15	20	-5	25	1.25
Segregating	44	40	4	16	0.4
Susceptible	21	20	1	1	0.05
$\chi^2_{1:2:1}$ value					1.7
p value					0.427

IT: Infection type score where 0 – 2 (resistant) and 3 – 4 (susceptible). $\chi^2_{1:2:1}$: Chi-square value at 0.05% and 2 degrees of freedom.

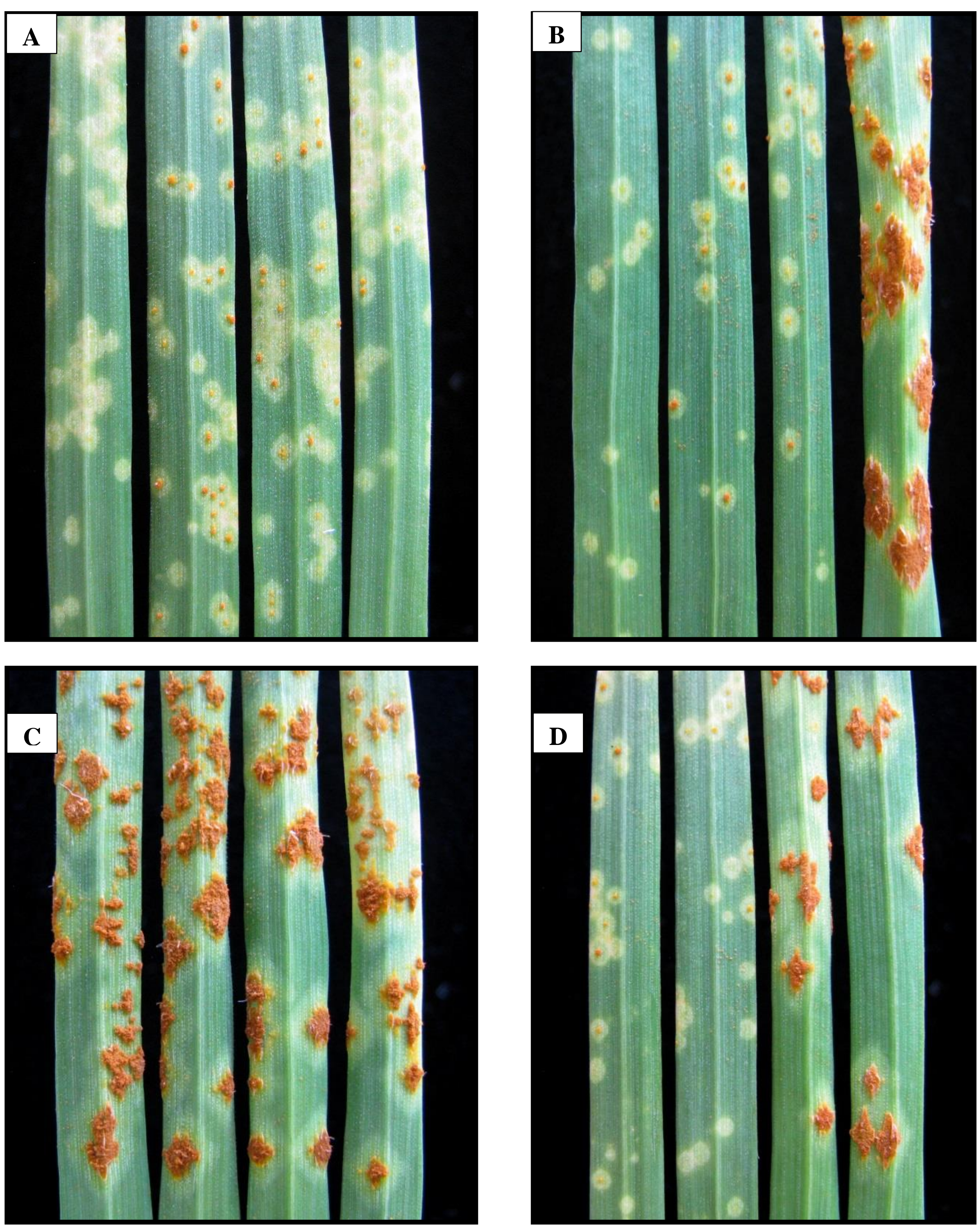

Figure 5.3: SC1 studies infection type photos.

Infection types for F_3 plants and their parents. (A) an individual SC1 x SC20 F_3 family (#41) homozygous for resistance to TTKSF+*Sr9h*, (B) an F_3 family segregating for resistance (#47), (C) an F_3 family homozygous susceptible (#42), and (D) infection types of parents SC1 (two resistant leaves) and SC20 (two susceptible leaves).

5.6. Discussion

This study used the χ^2 statistic to test the goodness of fit of SC1 x SC20 progenies into theoretical Mendelian ratios of 3:1 at F_2 and 1:2:1 at F_3 generations. Representative plants were randomly sampled from a wider range of progeny in both generations and population sizes fulfilling the expected conditions when using a χ^2test (Rana and Singhal, 2017). The seedling phenotypes showed independent segregation for one major gene among the SC1 x SC20 progenies. Based on the previous postulation of *Sr31* in SC1 using molecular markers (Soko *et al*., 2015) and the corresponding phenotype of line Federation*4/Kavkaz used as *Sr31* check here, the gene that segregated in the current population is proposed to be *Sr31*.

The presence of a single gene in SC1 is a risky scenario given previous experiences with varieties having monogenic rust resistance. In 2007, a Kenyan wheat variety "Mwamba" (*Sr24*) that occupied about 30% of the acreage was severely affected by a new Ug99 stem rust race (TTKST) virulent for *Sr24* (Singh *et al*., 2012). Historically, Eureka (*Sr6*), Fedweb (*Sr30*), Gabo (*Sr11*), Warigo (*Sr17*), Dowerin (*Sr9b*), Cook and Mengavi (both with *Sr36*) became susceptible to virulent races of stem rust soon after their releases between 1942 and 1961 in Australia (Park , 2007; Bariana *et al*., 2007; Park *et al.,* 2010; Pretorius *et al*., 2017). The detection of PTKST in South Africa in 2009, rendered *Sr31* varieties and tester lines such as Federation4*/Kavkaz, Gamtoos, Kavkaz and Clement susceptible to Ug99 (Pretorius *et al*. 2010). Matlabas, a variety with presumably *Sr9h*, is also susceptible to the TTKSF+*Sr9h* race of Ug99 (Terefe *et al*., 2016).

Breakdown of the *SrTmp* gene to TKTTF was the reason why an epidemic of this race in 2013 wiped out variety "Digalu" in Ethiopia and "Robin" in Kenya (Singh *et al*., 2015; Worku *et al*., 2016). In India, Bhardwaj (2012) reported susceptibility of the HW 2004 wheat variety released in 1995 to stem rust pathotype 62G29-1 that had virulence to *Sr24*. Likewise, the appearance of *Sr24* virulence in South Africa in 1984 led to complete susceptibility of the popular variety SST44 (Le Roux and Rijkenberg, 1987). Detection of TTTSK (virulent to both *Sr31* and *Sr36*) has also broadened the Ug99 virulence spectrum (Jin *et al*., 2009) thereby indicating the vulnerability of CBI1 (*Sr36*) the only variety in Zimbabwean germplasm with that resistance gene.

5.7. Recommendations and conclusions

In general, Zimbabwean wheat germplasm is over-reliant on race-specific stem rust resistance genes such as *Sr31* in SC1, leaving it vulnerable to Ug99. Singh *et al*. (2006a and 2008a) stated that race Ug99 is unique because it is the only known stem rust race that combines virulence to *Sr31* (1BL.1RS translocation from *Secale cereale*), *Sr38* from *Triticum ventricosum* and most of the genes of wheat origin. Therefore, dependency on race-specific genes might not be the best strategy given the broadly virulent nature of Ug99. Confirmation of *Sr31* in SC1 as the only major gene responsible for resistance to Ug99 races avirulent for this gene, is a concern given that race PTKST occurs in southern Africa.

Pretorius *et al*. (2017) recommends the practice of resistance gene stewardship which they defined as "careful and responsible management of resistance genes with the goal that they (genes) remain effective for prolonged use". Singh *et al*. (2011a) and Rouse *et al.* (2014) encouraged the use of gene combinations in order to increase resistance durability in wheat given the emergence of pathogen virulence to several genes that were effective to the original Ug99 race TTKSK, including *Sr9h.* This is part of the narrow sense approach to resistance gene stewardship that calls for specific plans targeted to reduce selection pressure on the pathogen population by increasing gene diversity. In the short term it is therefore recommended to pyramid race-specific genes that are still effective against Ug99 races onto adapted Zimbabwean wheat varieties such as SC1. Examples of such genes include *Sr22*, *Sr25*, *Sr26*, *Sr32*, *Sr35*, *Sr39*, *Sr40* and *Sr50*, and breeders are strongly recommended to stack these genes in combination with broadly effective ASR gene and/or several race non-specific APR genes (Pretorius *et al*., 2017). In the long term pyramiding onto *Sr2* backgrounds to ensure more durable resistance is recommended for the Seed-Co wheat breeding programme. The other long term option is to breed for APR based on several minor genes. The two options demand continued introgression of new sources of resistance, both race-specific genes and APR genes so as to diversify the genetic base of stem rust that is currently very narrow. Seedling bioassays and genotyping of breeding materials must be a continuous process as they can be used as aids in tracking the number of genes and their mode of inheritance in a breeding program in light of rapid changes in both the plant and pathogen environments due to climate change.

6. Introgression of *Sr25*, *Sr26* and *Sr39* stem rust resistance genes into Seed-Co bread wheat germplasm using marker assisted backcrossing

6.1. Introduction

The Zimbabwean wheat genepool is very narrow with an over-reliance on *Sr31*, a gene that is ineffective to certain members of the Ug99 race group. The second important gene in the Zimbabwean genepool is *Sr2* that is known to confer partial resistance which is not adequate under heavy disease infestation (Mago *et al*., 2011) and its effectiveness is normally enhanced in combination with other genes. *Sr24* and *Sr36* are also present in the genepool but virulent Ug99 races (TTKST and TTTSK, respectively) have been detected in Kenya in 2006 and 2007, respectively (Jin *et al*., 2008; Singh *et al*., 2011a). Therefore, it is a matter of time before these virulent races reach Southern Africa. Unless urgent action is taken to widen the genetic base, the Zimbabwean wheat industry is vulnerable to Ug99.

SC1 with stem rust resistance based on *Sr31*, commands 67% market share among Seed-Co varieties. This indicates the gravity of the issue at hand and there is a need to find other sources of resistance that can be rapidly introgressed into Seed-Co germplasm. This can lead to the necessary urgent replacement of all currently susceptible varieties. Marker assisted backcrossing (MABC) is a tool that can be used to resolve this challenge given that there is need for tracking the introgressed genes, at the same time retaining other desirable characteristics such as high yield, good industrial quality and agronomic traits. Effective stem rust resistant genes against the current 13 Ug99 races, e.g. *Sr25, Sr26* and *Sr39*, are available in elite lines and these can be used as donor sources in breeding. The study was therefore based on the hypothesis that marker assisted backcrossing is a faster and more economic way of pyramiding new sources of stem rust resistance onto adapted Zimbabwean wheat varieties.

6.2. Study Objectives

The objectives of this study were to assess the applicability of MABC to Seed-Co wheat breeding programme and at the same time introgress *Sr25*, *Sr26* and *Sr39* genes into Seed-Co wheat genepool using SC1, SC8 and SC15 as recurrent parents.

6.3. Materials and methods

6.3.1. Germplasm used

(a) Recurrent parents

i. SC1

This variety is the most popular variety in Zimbabwe commanding 67.3% of the market share for Seed-Co. Previous genotyping information (Soko *et al*., 2015) postulated that it contains *Sr31*. It also has *Lr46/Y29* for leaf rust resistance. It was highly susceptible in greenhouse adult plant studies with a high score of 60S and 20MRMS in the field to PTKST race of Ug99 (Chapter 4). SC1 is at risk in case of stem rust epidemics caused by any *Sr31*-attacking Ug99 race.

ii. SC8

SC8 is a red grained high yielding spring bread wheat variety that is medium to late maturing. It was released in Zimbabwe in 2009. The variety is very responsive to good management with a yield penalty if management is poor. Since commercialisation in 2011/12 season, 462 metric tonnes of seed of SC8 have been sold but has failed to take a market share from SC1. SC8 is postulated to have *Sr2*, *Sr31* and *Sr25* based on *Lr*19 postulation by CenGen and Limagrain results.

iii. SC15

This variety is a white grained high yielding spring wheat variety released in 2012 in Zimbabwe. Since the 2015/16 season when it was commercialised, it has sold 196 metric tonnes of seed with a potential to grow. It is postulated to have *Sr31* in combination with *Sr25* based on *Lr*19 postulation by CenGen and Limagrain results, and *Lr46/Yr29*.

(b) Donor parents

i. Agatha/9#LMPG-6

The line was used as the donor for *Sr25* which is still effective against all Ug99 races occurring in Southern Africa, and would widen the *Sr* genetic base if incorporated into Seed-Co germplasm. The line was tested in the greenhouse at the adult stage with a resistant score of 40MR against PTKST.

ii. WBC08

An Australian line used as a donor for *Sr26* in this study. *Sr26* is still effective against the Southern Africa Ug99 races and the gene has been used commercially in Australia since 1971 with the release of the cultivar Eagle (maswheat.ucdavis.edu/protocols/Sr26/index.htm 31/7/17). It showed resistance to PTKST at the adult stage in the greenhouse with a score of 60MR.

iii. 25#2/163

An Australian line used as a donor for *Sr39* in this study. *Sr39* is a gene that is still effective against Ug99 races in Southern Africa; therefore it would add value to breeding programmes in Zimbabwe. The line was resistant to PTKST at adult stage in the greenhouse with a score of 30MR.

6.3.2. Planned crosses to develop backcross inbred lines (BIL)

The study developed backcross inbred lines in a non-reciprocal crossing plan with every recurrent parent being crossed to each donor parent. A total of 9 crosses were planned based on 2012 genotypic information (Table 6.1).

Table 6.1: Original crossing plan for the targeted nine crosses to develop backcross inbred lines for the introgression of *Sr25*, *Sr26* and *Sr39* genes into Seed-Co varieties

	Agatha/9#LMPg6 (*Lr19/Sr25)*	WBC08 (*Sr26)*	25#2/163 (*Sr39)*
SC1	*Sr31+Lr19/Sr25* (1)	*Sr31+Sr26* (2)	*Sr31+Sr39* (3)
SC15	*Sr31+Lr19/Sr25* (4)	*Sr31+Sr26 (5)*	*Sr31+Sr39* (6)
SC8	*Sr2+Sr31+Lr19/Sr25* (7)	*Sr2+Sr31+Sr26* (8)	*Sr2+Sr31+Sr39* (9)

6.3.3. Establishment and management of the crossing block

Two pots were planted to each parental line with three plants per pot. Two planting dates were used to give a total of four pots per entry. Germinated plants in different pots were labelled. Leaf samples were taken from the labelled plants from each pot at three to four weeks after plant emergence. These were lyophilized before genotyping at CenGen (Pty) laboratory. The objective was to determine genetic purity of the parental lines. Seedling infection types were recorded on all parental lines in the crossing block using the Stakman *et al*. (1962) system. PTKST was used for the inoculations.

6.3.4. Genotyping of parental lines and F_1 generation

A total of 18 samples (four SC1, four SC15, four SC8, one Agatha/9#LMPG-6 (*Sr25*), two WBC08 (*Sr26*), and three 25#2/163 (*Sr39*) plants) were genotyped to confirm the presence of *Lr19/Sr25, Sr26* and *Sr39* genes in donor parents and their absence in recurrent parents. $Lr19STS_{130}$ with an expected band between 81.5-85.0 cM was used as a marker for *Lr19/Sr25* while 09US2009 was used as the positive check variety. Parental genotyping results indicated the presence of *Lr19/Sr25* in both SC15 and SC8, therefore crosses 4 (SC15/Agatha/9#LMPG-6) and 7 (SC8/ Agatha/9#LMPG6) were not included in the study. Previous genotyping work on Zimbabwean germplasm (on which parental line selection was based) did not include markers for *Lr19/Sr25* at the time.

Molecular markers *BE518379* (gene absence) and *Sr26#43* (gene presence) were used for *Sr26* with expected bands at 303 bp and 207 bp, respectively on a 3% agarose gel (maswheat.ucdavis.edu 28/7/17). Avocet S was the positive check variety used for *Sr26*. *Sr39#22r* was used as marker for the *Sr39* gene using real time PCR protocol with an expected

melt peak between 83.6–84.7°C. RL6082 was used as a positive monogenic line for *Sr39*. Other check samples used during parental screening included three 2012 Zimbabwean germplasm samples Zim 1 (SC1), Zim 8 (SC8) and Zim 30 (SC20) and Kariega. Cross 8 was not made due to synchronization problems between SC8 (late to flower) and WBC08 (very early to flower) despite having staggered plantings. Only those parental lines that were postulated to have the targeted genes were used in the crosses to generate F1 populations. After parental screening, only 6 crosses (crosses 1, 2, 3, 5, 6 and 9) were advanced in the study.

6.3.5. Backcrosses 1 and 2

Backcross 1 (BC_1) involved crossing first filial generation (F_1) plants to their respective parents. No genotyping was done at this stage. Backcross 2 (BC_2) involved crossing progenies of BC_1 (BC_1F_1) with their recurrent parents. Only BC_1F_1 plants that were genotyped and identified to have the relevant gene were used in the crosses. Genotyping results of BC_1F_1 plants failed to confirm presence of *Sr39* in plants that had been used in BC 1 for cross 9 (SC8//SC8/25#2/163). The cross was therefore dropped from further evaluations. A total of 5 crosses (Crosses 1, 2, 3 5 and 6) were advanced for further evaluation.

6.3.6. Space planted BC_2F_1

Harvested seed were space-planted in the field at Rattray Arnold Research Station (RARS) during the 2016 winter season using an intra-row and inter-row spacing of 0.3 m, respectively. Each plant was allowed to self-pollinate. Three to four weeks after emergence, 30 single plants were randomly selected from each of the five populations and these were numbered 1-30. Leaf samples were separately collected from each of the 30 plants and labelled. These were genotyped in the laboratory at CenGen (Pty). Only plants that had a gene complement from both parents were selected and included into the Seed-Co main breeding program for advancement in 2017 winter season. Figure 6.1 summarises the processes that were involved to generate the backcross inbred lines and stages at which leaf genotyping was done.

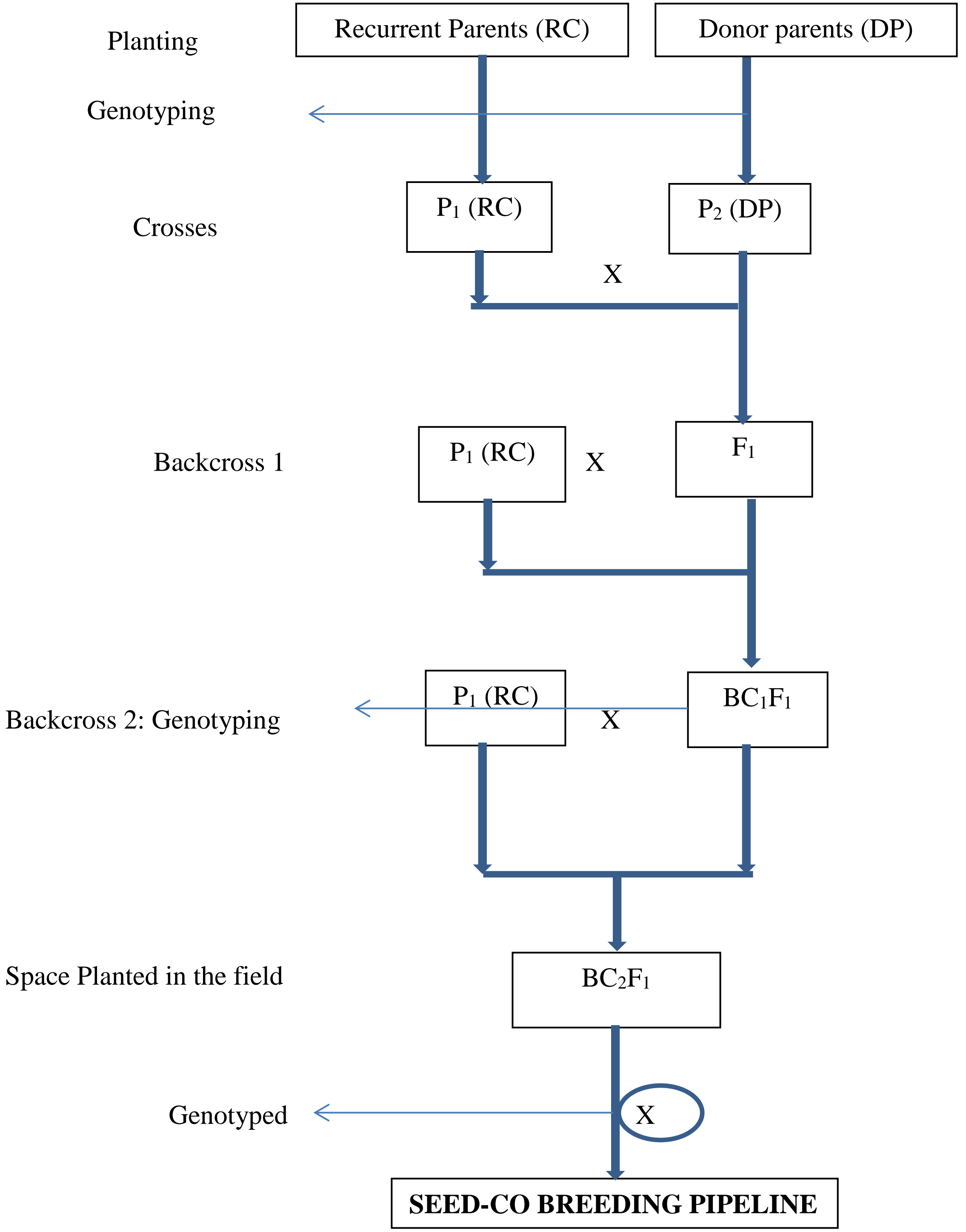

Figure 6.1: Sr gene introgression flow chart.

Introgression flow chart for *Sr25*, *Sr26* and *Sr39* into Seed-Co genepool.

X selfing of BC_2F_1 plants germplasm

6.3.7. Phenotyping of BC_2F_2

PTKST Ug99 race was used to determine the segregation pattern of BC_2F_2 seeds obtained from each of the three populations (a) SC1 (1,1,2) by WBCO8 (1,1,2) - cross 2, (b) SC1 (purified 9) by 25#2/163 (2,2,3) – cross 3 and (c) SC15 (1,2,3) by 25#2/163 (2, 1, 3) – cross 6. These three populations were positively identified to have genes from both recurrent and donor parents. A segregation ratio of one resistant to one susceptible was expected among the segregating plants that were obtained from a susceptible parent (recurrent parent) and a resistant parent (donor parent). The aim of the phenotyping stage was to validate results of genotyping done by CenGen on the BC_2F_1 space planted seeds. A total of twenty seeds were used for phenotyping from each of the positively identified single plants. Cross 2 had six plants, cross 3 with three plants and cross 6 with 17 plants.

6.4. Results

6.4.1. Parental screening

The objective of parental genotyping was to confirm that only genetically pure parents are used for easy gene tracking using relevant markers. Results of the parental screening (Table 6.2) had a significant influence on which crosses to exploit. The *Lr19/Sr25* gene was confirmed to be present in 09US2009 (check variety), Zim 8 (SC8), Agatha/9#LMPG6 (1,1,2) donor line, SC15 (1,1,3), SC15 (1,2,3) and SC15 (2,1,2). The marker failed to confirm the presence of *Lr19/Sr25* in the parental line SC8 (1,1,3) targeted for use in the crosses. WBC08 (1,1,2) and WBC08 (2,1,3) were confirmed to have *Sr26* gene as expected. This was also confirmed by the positive check variety Avocet S. Donor plants for *Sr39* gene, 25#2/163 (2,1,3) and 25#2/163 (2,2,3) confirmed the gene together with the positive check variety, RL6082. None of the three genes were confirmed in Zim 30 (SC20) and Kariega. Parental genotyping results and poor synchronization between SC8 and WBC08 (*Sr26*) resulted in crosses being reduced from nine as planned to six. PTKST was used to inoculate all seedling parental lines. Infection type (IT) scores for the parental lines indicate a low IT for all donor lines, SC8 and SC15 with a range from 1 to 2++. All SC1 parental lines had a high IT that ranged from 3 to 4 (Table 6.2).

Table 6.2: Confirmed parents that were used in stem rust gene introgression crosses

Sample	**PTKST IT***	***Sr25* *(81.5-85.0 cM)***	***Sr26* *(213)***	***Sr39* *(83.6-84.7 cM)***
SC1 (1,1,2)	3	-	-	-
SC1 (5)	3+	-	-	-
SC1 (9)	4	-	-	-
SC1 (10)	4	-	-	-
SC15 (1,1,3)	2++	+	-	-
SC15 (2,1,2)	2	+	-	-
SC8 (1,1,3)	22+	-	-	-
Agatha/9#LMPG-6 (1,1,2)	2++	+	-	-
WBC08 (1,1,2)	1	-	+	-
WBC08 (2,1,3)	1	-	+	-
25#2/163 (2,2,3)	1	-	-	+
25#2/163 (2,1,3)	1	-	-	+
25#2/163 (2,2,2)	1+	-	-	-
Zim 1 (SC1)		-	-	-
Zim 8 (SC8)		+	-	-
Zim 30 (SC20)		-	-	-
Internal CG + check		+	+	+
Kariega		-	-	-
NTC		-	-	-

* Infection type according to Stakman *et al.* (1962) with 0 to 2 = resistant and 3 to 4 = susceptible, +/- presence or absence of *Sr* gene. Numbers in brackets indicate the plant number during sampling for easy identification e.g. SC1 (5) indicates SC1 plant in pot number 5 while SC1 (1, 1, 2) indicates SC1 plant under planting date 1, pot number 1 and plant number 2. cM: centimorgan, a unit for measuring genetic linkage. Expected band for *Sr25* gene was between 81.5 – 85.0 cM, for *Sr26* the band was at 213 bp and for *Sr39* the band was between 83.6 – 84.7 cM.

6.4.2. Successful F_1 crosses and BC_1F_1

A total of six crosses were successful between recurrent and donor parents resulting in F_1 seed quantities that ranged from five for cross 5 to 15 for cross 1 (Table 6.3). These were planted and successfully crossed to their recurrent parents to generate six BC_1F_1 populations. BC_1F_1 populations were planted and genotyped prior to crossing for BC_2. Genotyping results did not confirm *Sr39* gene in BC_1F_1 plants that were used in cross 9 (SC8//SC8/25#2/163 (2, 2, 2) and therefore the cross was not advanced for further testing. This resulted in five crosses (crosses 1, 2, 3, 5 and 6) remaining.

Table 6.3: Successful F_1 crosses and the number of seeds harvested and used for establishing the crossing block for BC_1

Cross	Female	Male	Target	F_1	BC_1F_1
1	SC1 (10)	Agatha/9#LMPG (1,1,2)	*Sr31+Sr25*	15	8
2	SC1 (1,1,2)	WBC08 ((1,1,2)	*Sr31+Sr26*	8	4
3	SC1 (9)	25#2/163 (2,2,3)	*Sr31+Sr39*	13	8
5	SC15 (1,1,3)	WBC08 (2,1,3)	*Sr31+Sr26*	5	3
6	SC15 (2,1,2)	25#2/163 (2,1,3)	*Sr31+Sr39*	13	8
9*	SC8 (1,1,3)	25#2/163 (2,2,2)	*Sr2+Sr31+Sr39*	7	5

* Cross 9 was dropped before BC_2 due to unconfirmed *Sr39* gene in the parental BC_1F_1 plant (25#2/163 (2, 2, 2)). Crosses 4 and 7 were dropped after parental screening results showed the presence of *Lr19/Sr25* in the recurrent parents SC15 and SC8, respectively.

6.4.3. Successful BC_2F_1 crosses (after second genotyping)

The success rate from the genotypic results ranged from three samples for cross 2 to 11 (61%) for cross 1. Confirmed plants were used in the second backcross to generate BC_2F_1 seeds. Quantities of harvested seeds for BC_2F_1 are also indicated in Table 6.4.

Table 6.4: Successful BC_2F_1 crosses and harvested seed quantities that were space planted during 2016 winter at RARS

Cross	Female	Male	Target*	BC_1F_1 plants	(+) Samples**	BC_2F_1 seeds
1	SC1 (10)	SC1 (10)//SC1(10)/Agatha/9#LMPG6(1,1,2)	*Sr31+ Sr25*	18	11	36
2	SC1(1,1,2)	SC1 (1,1,2)//SC1(1,1,2)/WBC08(1,1,2)	*Sr31+ Sr26*	18	3	44
3	SC1 (9)	SC1 (9)//SC1 (9)/25#2/163 (2,2,3)	*Sr31+Sr39*	18	10	114
5	SC15 (1,1,3)	SC15 (1,1,3)//SC15 (1,1,3)/WBC08(2,1,3)	*Sr31+Sr26*	18	8	50
6	SC15 (2,1,2)	SC15 (2,1,2)//SC15 (2,1,2)/25#2/163 (2,1,3)	*Sr31+Sr39*	18	10	54

* *Sr*: Stem rust gene designation. ** Number of samples that were confirmed to have the targeted gene after genotyping of BC_1F_1 plants.

6.4.4. Molecular marker assays for 30 sampled space planted BC_2F_1 plants

6.4.4.1. KASP marker for *Sr31*

The most common gene, *Sr31*, was tracked in all five crosses because of SC1 and SC15 as recurrent parents. The two recurrent parents were postulated to have *Sr31* from previous genotyping work. KASP co-dominant marker wMAS000011 for 1RS:1BL_6110 (www.cerealsbd.uk.net 31/7/17) was used to track *Sr31* gene in a total of 150 samples. Federation*4/Kavkaz (*Sr31*) and Avocet S (*Sr26*) were used as positive and negative checks, respectively. The allele VIC (red) fluorophore represented gene presence (homozygous), allele FAM (blue) fluorophore indicated absence of the gene (homozygous), green samples (middle) represents heterozygotes (www.lgcgenomics.com 19/7/17). The two untreated checks (NTC) were found close to the origin and were indicated in black. The KASP images produced by the KlusterCaller™ computer software (www.lgcgenomics.com 19/7/17) are shown in Figure 6.2. The success rate for *Sr31* was 74% (both homozygotes and heterozygotes). Twenty-four percent of samples did not have the gene and the rest did not amplify. Crosses 2 and 6 had 100% homozygous gene confirmation each.

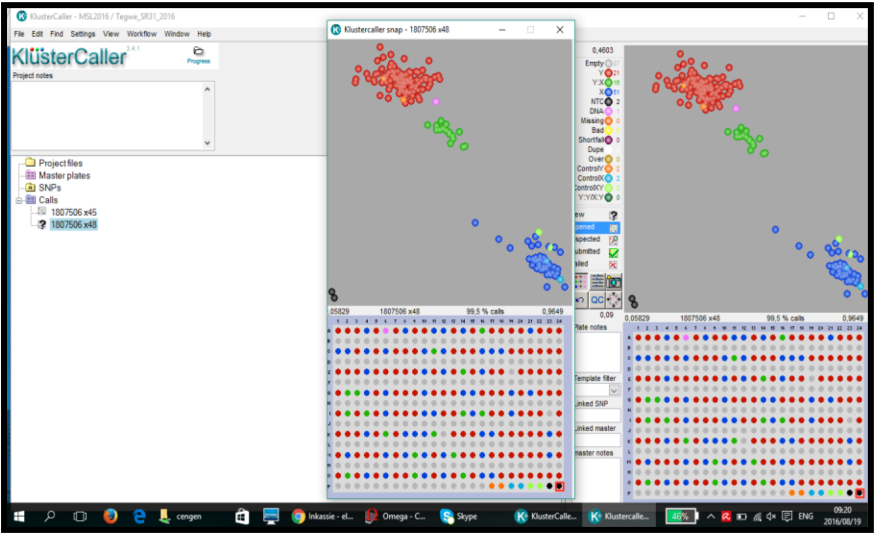

Figure 6.2: KASP image for *Sr31*.

KlusterCaller™ image for *Sr31* for two plates (five crosses). Homozygous alleles indicating gene presence are in red, homozygous alleles for gene absence are in blue, heterozygotes are in green (in the middle); black represents NTC; Fed/Kvz (*Sr31*) in orange is the positive check while Avocet S (*Sr26*) in sky-blue is the negative check.

6.4.4.2. Melt temperature outputs for *Lr19/Sr25*

Real time PCR, with molecular marker *BE145935* and expected peak between 81.0°C and 82.4°C was used to track *Lr19*, a gene closely linked to *Sr25* (Liu *et al*. 2010). W7890 was used as a positive check and had a melt temperature peak at 83.4°C (Figure 6.4) while Figure 6.3 depicts absence of the gene. *Sr25* introgression was tracked in cross 1 (SC1/2/SC1//SC1/Agatha/9#LMPG6(1,1,2)).

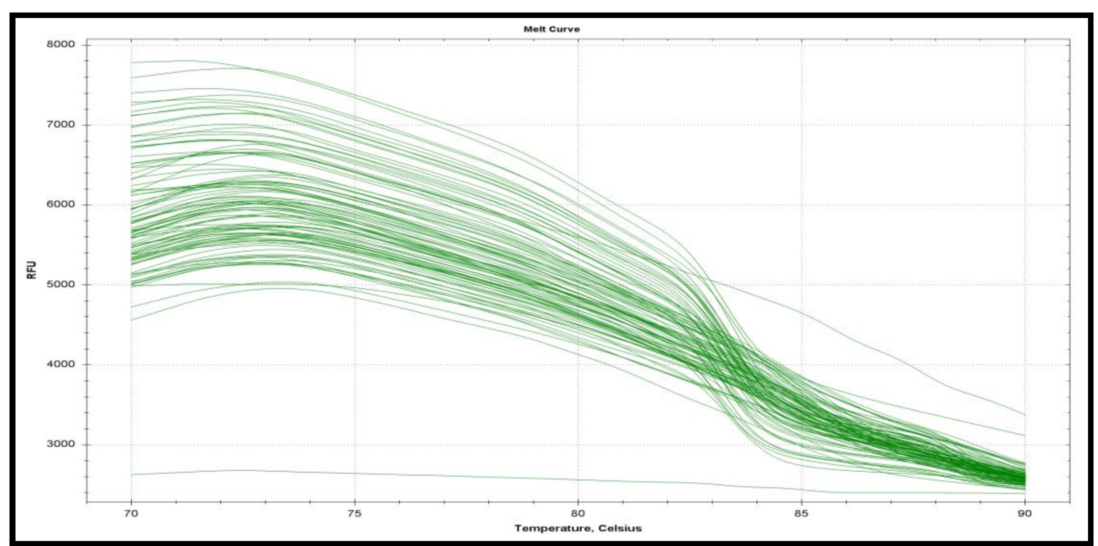

Figure 6.3: *Lr19/Sr25* melt curve showing gene absence

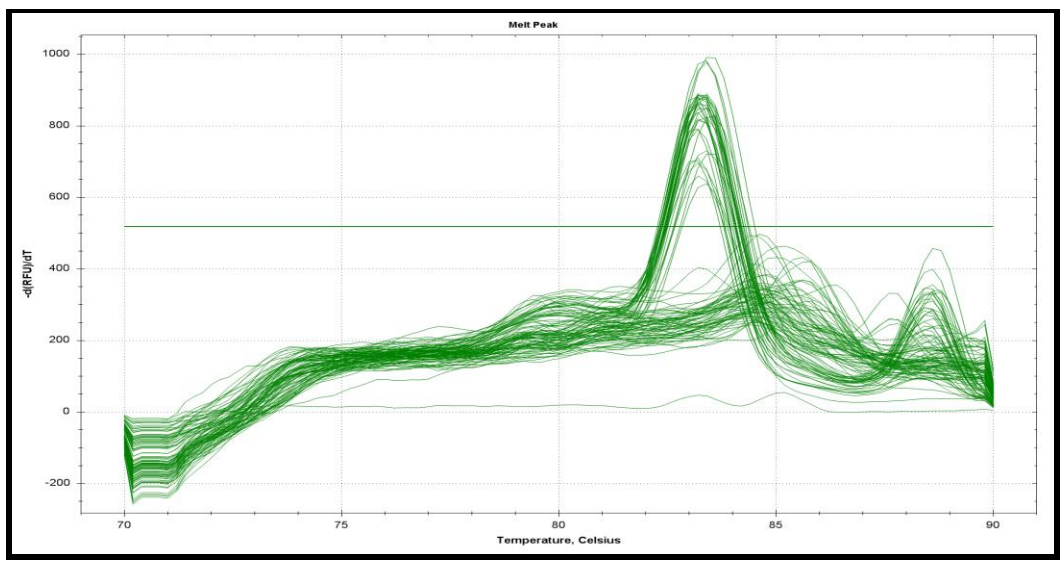

Figure 6.4: *Lr19/Sr25* melt curve showing gene presence. *Lr19/Sr25* between 83.2°C and 83.4°C.

6.4.4.3. Melt temperature image for *Sr26*

Dominant markers *Sr26#43* and *BE518379* were used to track *Sr26*. These two markers in combination (multiplexing), result in a co-dominant marker for *Sr26* (Liu *et al*., 2010; Mago *et al*., 2005). The two markers are associated with band alleles at 80.0°C (*Sr26#43*) and 84.8 - 86.0°C (*BE518379*) using a real time PCR, indicating gene presence and absence, respectively (Figure 6.6). Avocet S (*Sr26*) was used as a positive check and has a peak at 80.2°C (Cross 2) and at 80.6°C (Cross 5) while the donor parental lines amplified at 80.4°C (cross 2) and 80.2°C (cross 5), respectively. Heterozygotes would have the two melt peaks (Figure 6.6). *Sr26* was tracked in crosses 2 (SC1/2/SC1//SC1/WBC08 (1, 1, 2) and 5 (SC15/2/SC15//SC15/WB C08 (2,1,3). Melt curve images in Figures 6.5 and 6.6 shows the output of *Sr26* gene that was tracked in 60 samples.

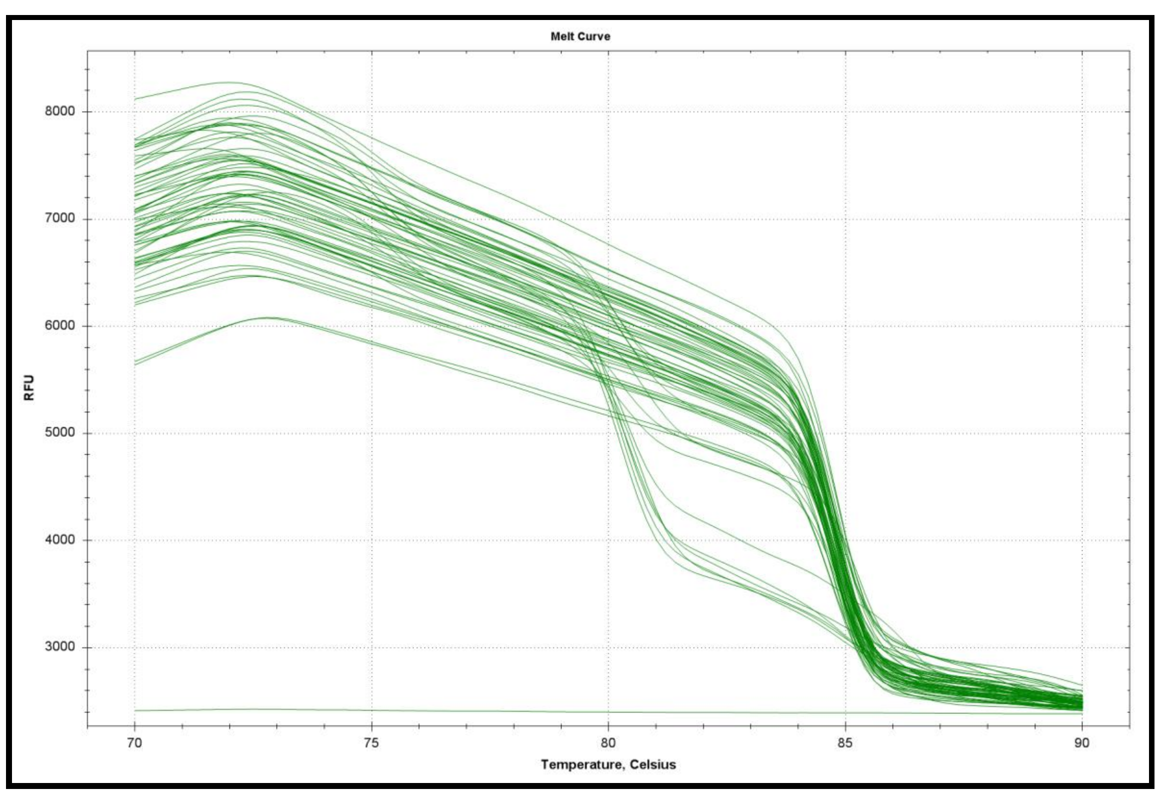

Figure 6.5: *Sr26* melt curve showing gene absence. No peak.

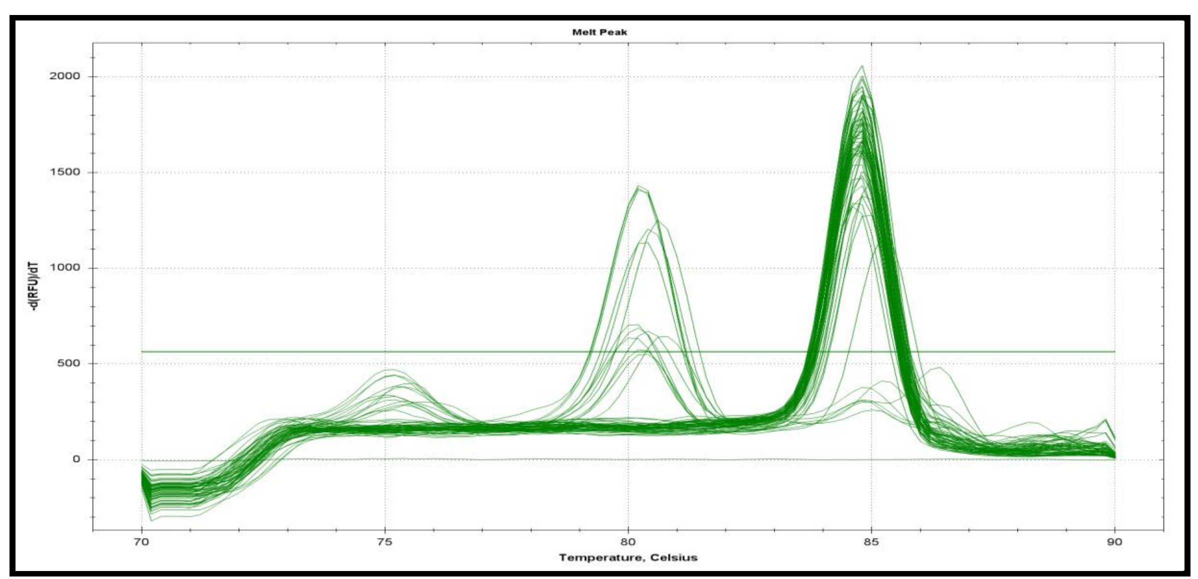

Figure 6.6: *Sr26* melt curve showing gene presence.

Sr26 gene peak at 80.0°C (Homozygous), gene absence indicated by peak between 84.5°C and 86.0°C and two peaks for heterozygotes with melt peak at both 80°C and 84.5°C - 86°C.

6.4.4.4. Melt temperature image for *Sr39*

The gene is closely linked to *Lr35 (*Mago *et al.* 2009) and was tracked in crosses 3 and 6.

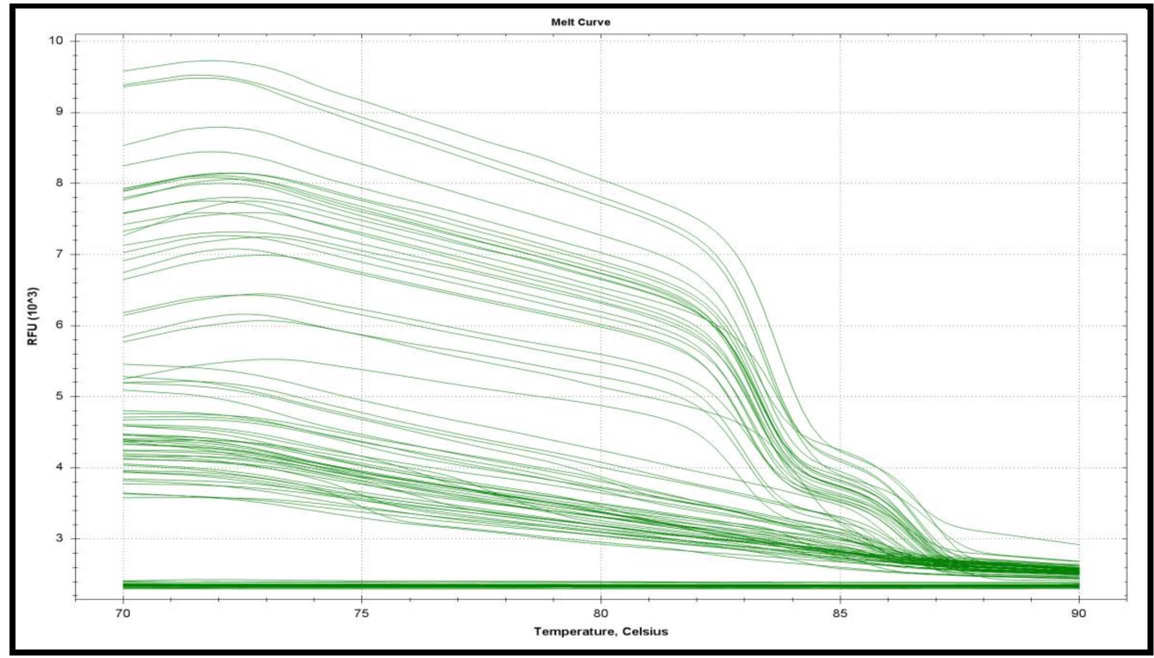

Figure 6.7: *Sr39* melt curve showing absence of gene (no peak). Samples from crosses 3 and 6.

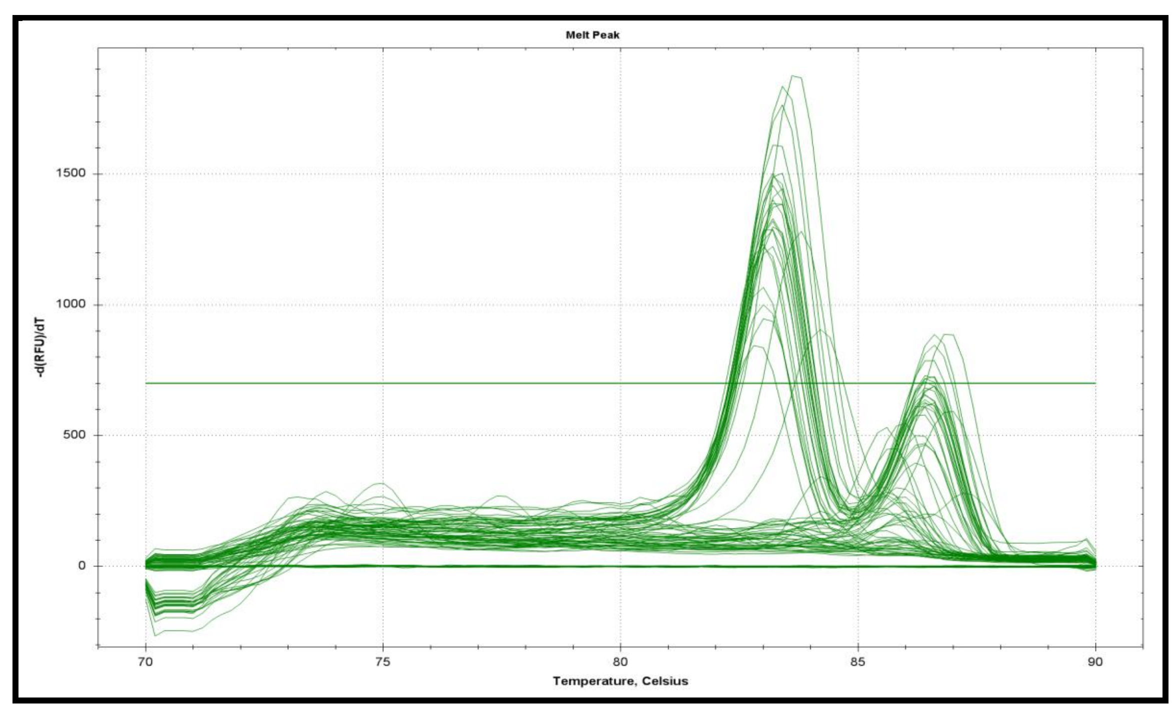

Figure 6.8: *Sr39* melt curve showing gene presence.

Presence of melt peak at 82.0°C to 84.2°C indicate *Sr39* presence (homozygous). The presence of two peaks at 82.0°C to 84.2°C and 85.0°C to 88°C indicate presence of *Sr39* in a heterozygous state. Analysed samples were from crosses 3 and 6.

6.4.4.5. Cross 1: SC1 (10)/Agatha/9#LMPG6 (1,1,2) BC_2F_1

None of the 30 randomly sampled BC_2F_1 plants were confirmed for the presence of *Sr25* by the molecular marker *BE145935* while the same marker managed to confirm *Sr25* in the donor line Agatha/9#LMPG6 (1,1,2) with a peak at 83.4°C (Table 6.5). The KASP marker for *Sr31* managed to confirm gene presence in 25 plants (20 homozygotes and 5 heterozygotes) out of the 30 samples. The marker also confirmed *Sr31* in both parents. The cross was therefore a complete failure given that not a single plant had both *Sr31* and *Lr19/Sr25* genes. This maybe due to a small sample size (n=30).

6.4.4.6. Cross 2: SC1 (1,1,2)/WBC08 (1,1,2) BC_2F_1

The two markers *Sr26#43* and *BE518379* were effective in confirming *Sr26* in a heterozygous state in four plants (Table 6.6); Z172, Z176 (peaks at 80.2 and 84.8), Z173 (80.4 and 85.0) and Z174 (80.8 and 85.2). Sample Z177 had *Sr26* in a homozygous state (80.4). All five plants

were confirmed to have *Sr31* gene by KASP marker, indicating that *Sr31* and *Sr26* were present in the five plants (16.67% success rate) which will be advanced into the Seed-Co wheat breeding programme.

6.4.4.7. Cross 3: SC1 (9)/25#2/163 (2,2,3)BC$_2$F$_1$

The marker for *Sr39*, *Sr39#22r* was able to confirm gene presence in three plants. The same plants were also confirmed for *Sr31* by KASP marker (Table 6.7) thereby indicating a 10% success rate. The plants that produced laboratory samples Z253, Z256 and Z261 will be further advanced through the Seed-Co wheat breeding programme.

6.4.4.8. Cross 5: SC15 (1, 1, 3)/WBC08 (2, 1, 3) BC$_2$F$_1$

Only one plant was confirmed to have both *Sr26* and *Sr31* indicating a 3.3% success rate for this cross (Table 6.8). The plant for sample Z318 had *Sr26* in the heterozygous state while *Sr31* was homozygous.

6.4.4.9. Cross 6: SC15 (2, 1, 2)/25#2/163 (2, 1, 3) BC$_2$F$_1$

Sr39#22r and *Sr31* KASP marker were very efficient in confirming presence of the two genes in 19 out of 30 plants giving the highest success rate over all crosses of 63.3%. Among these 12 were homozygotes for *Sr39*. The 19 selected plants were based on laboratory samples Z333 – Z345 and Z348 – Z353 (Table 6.9).

A total of 27 plants from crosses 2 (4 plants), 3 (3), 5 (1) and 6 (19) were confirmed to have genes from both the recurrent parent and donor line. These were selected and advanced for further testing within the Seed-Co breeding programme.

6.5. Seedling phenotypic results for BC$_2$F$_2$ plants (Table 6.10)

6.5.1. Cross 2: SC1 (1,1,2)/WBC08 (1,1,2)

A total of three plants out of the six phenotyped had seeds that segregated in a one resistant : one susceptible ratio while the other three showed all resistant plants.

6.5.2. Cross 3: SC1 (9)/25#2/163 (2,2,3)

All three plants originating from BC_2F_2 seed segregated in a one resistant : one susceptible ratio.

6.5.3. Cross 6: SC15 (2,1,2)/25#2/163 (2,1,3)

A total of 13 plants had seeds that segregated in a ratio of one resistant : one susceptible ratio.

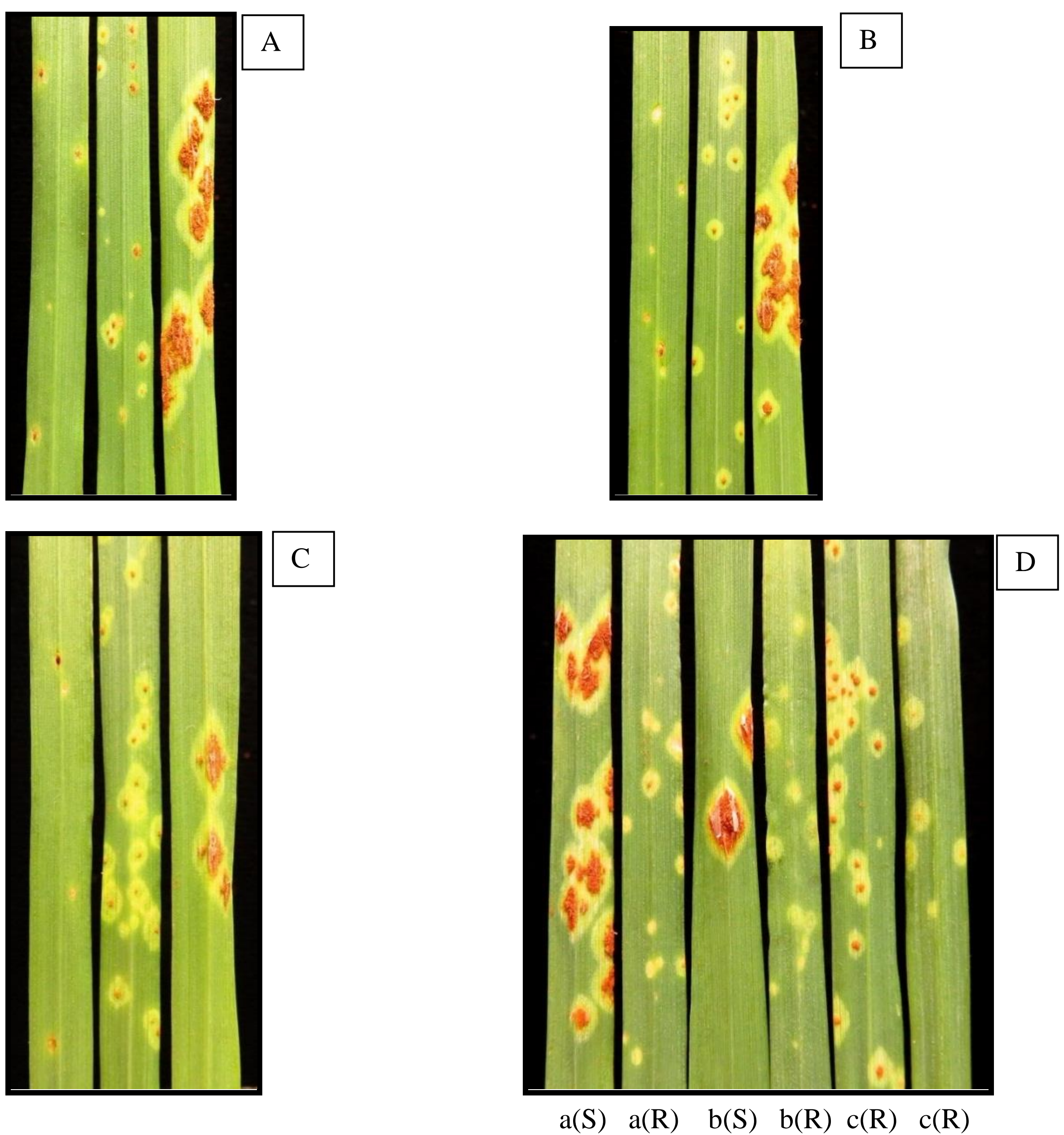

Figure 6.9: BC_2F_2 infection types for crosses 2, 3 and 6 inoculated with PTKST.

Photo A shows two resistant leaves (low infection type) on the left and one susceptible leaf (high infection type) for Cross 2 (SC1*WBC08). Photo B shows two resistant leaves (low infection type) on the left and one susceptible leaf (high infection type) for cross 3 (SC1*25#2/163). Photo C shows two resistant leaves (low infection type) on the left and one susceptible leaf (high infection type) for cross 6 (SC15*25#2/163). Photo D shows six parents of the three crosses (left - right): (a) Cross 2 - Susceptible parent SC1 (left) and resistant parent WBC08 (right). (b) Cross 3 – Susceptible parent SC1 (left) and resistant parent 25#2/163 (right). (c) Cross 6 – resistant parent SC15 (left) and resistant parent 25#2/163 (right). Second leaves of BC_2F_2 plants were inoculated.

Table 6.5: Genotyping results showing presence/absence of *Lr19*/*Sr25* and *Sr31* in 30 plant samples for Cross 1

Lab No	Plant No	Melt T°C	*Lr19*	*Sr31*	Lab No	Plant No	Melt T°C	*Lr19*	*Sr31*
#Z201	SC1 (10)	None	-	+	#Z217	15	None	-	+
#Z202	Agatha/9#LMPG6 (1,1,2)	83.4	+	+	#Z218	16	None	-	+
#Z203	1	None	-	+	#Z219	17	None	-	+
#Z204	2	None	-	+	#Z220	18	None	-	+
#Z205	3	None	-	+	#Z221	19	None	-	+
#Z206	4	None	-	-	#Z222	20	None	-	+
#Z207	5	None	-	Het	#Z223	21	None	-	Het
#Z208	6	None	-	Het	#Z224	22	None	-	+
#Z209	7	None	-	-	#Z225	23	None	-	+
#Z210	8	None	-	Het	#Z226	24	None	-	+
#Z211	9	None	-	+	#Z227	25	None	-	+
#Z212	10	None	-	+	#Z228	26	None	-	-
#Z213	11	None	-	-	#Z229	27	None	-	-
#Z214	12	None	-	Het	#Z230	28	None	-	+
#Z215	13	None	-	+	#Z231	29	None	-	+
#Z216	14	None	-	+	#Z232	30	None	-	+

Het: Heterozygotes.   +/-: presence/absence of the targeted gene

Table 6.6: Genotyping results showing presence/absence of *Sr26* and *Sr31* in 30 plant samples for Cross 2

Lab No	Plant No	Melt T°C	*Sr26*	*Sr31*	Lab No	Plant No	Melt T°C	*Sr26*	*Sr31*
#Z169	SC1 (1,1,2)	85.0	-	+	#Z185	15	84.8	-	+
#Z170	WBC08 (1,1,2)	80.4	+	+	#Z186	16	84.8	-	+
#Z171	1	85.0	-	+	#Z187	17	84.8	-	+
#Z172	2	80.2, 84.8	Het	+	#Z188	18	84.8	-	+
#Z173	3	80.4, 85.0	Het	+	#Z189	19	84.6	-	+
#Z174	4	80.8, 85.2	Het	+	#Z190	20	84.8	-	+
#Z175	5	85.0	-	+	#Z191	21	84.8	-	+
#Z176	6	80.2, 84.8	Het	+	#Z192	22	84.8	-	+
#Z177	7	80.4	+	+	#Z193	23	84.6	-	+
#Z178	8	84.8	-	+	#Z194	24	84.8	-	+
#Z179	9	84.8	-	+	#Z195	25	84.8	-	+
#Z180	10	84.8	-	+	#Z196	26	84.8	-	+
#Z181	11	84.8	-	+	#Z197	27	84.8	-	+
#Z182	12	84.8	-	+	#Z198	28	84.8	-	+
#Z183	13	84.8	-	+	#Z199	29	84.8	-	+
#Z184	14	84.8	-	+	#Z200	30	84.8	-	+

Het: Heterozygotes with 2 peaks at 80.4 and 84.8 – 86.0°C. +/-: presence/absence of the targeted gene

Table 6.7: Genotyping results showing presence/absence of *Sr39* and *Sr31* in 30 plant samples for Cross 3

Lab No	Plant No	Melt T°C	*Sr39*	*Sr31*	Lab No	Plant No	Melt T°C	*Sr39*	*Sr31*
#Z233	SC1 (9)	None	-	+	#Z249	15	None	-	-
#Z234	25#2/163 (2,2,3)	83.6, 86.8	+	-	#Z250	16	None	-	+
#Z235	1	None	-	+	#Z251	17	None	-	+
#Z236	2	None	-	+	#Z252	18	None	-	-
#Z237	3	None	-	+	#Z253	19	83.2	+	+
#Z238	4	None	-	-	#Z254	20	None	-	-
#Z239	5	None	-	+	#Z255	21	None	-	-
#Z240	6	None	-	-	#Z256	22	83.4	+	+
#Z241	7	None	-	+	#Z257	23	None	-	-
#Z242	8	None	-	+	#Z258	24	None	-	-
#Z243	9	None	-	N/A	#Z259	25	None	-	-
#Z244	10	None	-	-	#Z260	26	None	-	-
#Z245	11	None	-	-	#Z261	27	83.2	+	+
#Z246	12	None	-	+	#Z262	28	None	-	-
#Z247	13	None	-	+	#Z263	29	None	-	-
#Z248	14	None	-	+	#Z264	30	None	-	+

N/A: Not amplified +/-: presence/absence of targeted gene

Table 6.8: Genotyping results showing presence/absence of *Sr26* and *Sr31* in 30 plant samples for Cross 5

Lab No	Plant No	Melt T°C	*Sr26*	*Sr31*	Lab No	Plant No	Melt T°C	*Sr26*	*Sr31*
#Z297	SC15 (1,1,3)	84.8	-	+	#Z313	15	84.6	-	-
#Z298	WBC08 (2,1,3)	80.2	+	-	#Z314	16	84.8	-	+
#Z299	1	84.8	-	-	#Z315	17	84.8	-	Het
#Z300	2	84.6	-	-	#Z316	18	84.8	-	-
#Z301	3	84.8	-	-	#Z317	19	84.8	-	Het
#Z302	4	84.8	-	-	#Z318	20	80.0, 84.8	Het	+
#Z303	5	84.8	-	-	#Z319	21	84.6	-	+
#Z304	6	84.8	-	-	#Z320	22	84.8	-	Het
#Z305	7	84.6	-	-	#Z321	23	84.6	-	Het
#Z306	8	84.8	-	-	#Z322	24	80.2, 84.8	Het	-
#Z307	9	84.8	-	-	#Z323	25	80.2	+	-
#Z308	10	84.8	-	+	#Z324	26	84.6	-	+
#Z309	11	84.8	-	+	#Z325	27	84.8	-	Het
#Z310	12	84.8	-	N.A	#Z326	28	84.8	-	-
#Z311	13	84.8	-	-	#Z327	29	84.8	-	-
#Z312	14	84.8	-	-	#Z328	30	84.8	-	-

N/A: Nota amplified. +/-: presence/absence of targeted gene. Het: Heterozygotes for *Sr26* gene with 2 peaks at 80.2 and 84.8°C

Table 6.9: Genotyping results showing presence/absence of *Sr39* and *Sr31* in 30 plant samples for Cross 6

Lab No	Plant No	Melt T°C	*Sr39*	*Sr31*	Lab No	Plant No	Melt T°C	*Sr39*	*Sr31*
#Z329	SC15 (1,2,3)	None	-	+	#Z345	15	83.4, 86.6	+	+
#Z330	25#2/163(2,1,3)	83.0	+	-	#Z346	16	None	-	+
#Z331	1	None	-	+	#Z347	17	None	-	+
#Z332	2	None	-	+	#Z348	18	83.2, 86.6	Het	+
#Z333	3	83.2, 86.4	Het	+	#Z349	19	83.2	+	+
#Z334	4	83.2	+	+	#Z350	20	83.4, 86.6	Het	+
#Z335	5	82.8	+	+	#Z351	21	83	+	+
#Z336	6	83.2	+	+	#Z352	22	83.2	+	+
#Z337	7	83.2, 86.4	Het	+	#Z353	23	83.2	+	+
#Z338	8	83.2, 86.4	Het	+	#Z354	24	None	-	+
#Z339	9	83.2	+	+	#Z355	25	None	-	+
#Z340	10	83	+	+	#Z356	26	None	-	+
#Z341	11	83.4, 86.6	Het	+	#Z357	27	None	-	+
#Z342	12	83.4	+	+	#Z358	28	None	-	+
#Z343	13	83	+	+	#Z359	29	None	-	+
#Z344	14	83	+	+	#Z360	30	None	-	+

+/- Presence or absence of targeted gene   Het: Heterozygotes

Table 6.10: Phenotypic results of BC_2F_2 plants inoculated with PTKST Ug99 race

Cross	RP: *Sr* gene	DP: *Sr* gene	Lab (Plant)	Low IT	High IT
2	SC1 (1,1,2): *Sr*31	WBC08(1,1,2): *Sr*26	#Z172(2)*	;1=, ;1	4
			#Z173(3)*	;1	
			#Z174(4)*	;1=, 1	2+3
			#Z175(5)	;1	3+
			#Z176(6)*	:1	
			#Z177(7)	;1, ;n	
3	SC1 (9): *Sr*31	25#2/163(2,2,3): *Sr*39	#Z253(19)	;1=, 1	4
			#256(22)	;1=n	4
			#Z261(27)	;1=	4
6	SC15 (2,1,2): *Sr*25, *Sr31*	25#2/163(2,1,3): *Sr*39	#Z333(3)*	;1, 2	3+
			#Z335(5)	;1, 1, 2	
			#Z336(6)	;1n, 1	
			#Z337(7)*	;1-, 1, 2	
			#Z338(8)*	;1n, 1	
			#Z339(9)	;1=	
			#Z340(10)	;n, 1	
			#Z341(11)*	;1, 1	
			#Z342(12)	;n, ;1	
			#Z343(13)	;1=n,1	3++
			#Z344(14)	;n, :1	
			#Z345(15)	;1=n, 1	4
			#Z348(18)*	;n, 1	
			#Z350(20)*	;1, 2	
			#Z351(21)	;1, 2	
			#Z352(22)	;1-, 1	
			#Z353(23)	;1-, 1	

RP: recurrent parent, DP: donor parent, IT: infection type, * donor gene was in heterozygous state. Low IT: 0 – 2 and High IT: 3 - 4

6.6. Discussion

The genetic base of Zimbabwean wheat was very narrow and three race-specific genes in the Zimbabwe genepool, *Sr31, Sr24* and *Sr36* are already ineffective to Ug99 races. PTKST and TTKST are virulent to both *Sr24* and *Sr31*, with the former detected in both Zimbabwe and South Africa and, the latter, in Kenya; TTKSP virulent to *Sr24* is present in South Africa; TTTSK virulent to *Sr36* was detected in Kenya and it is anticipated that more complex virulence will likely occur due to mutations (Pretorius *et al.*, 2000; Mukoyi *et al.*, 2011; Singh *et al.*, 2008a, 2011a; Jin *et al.*, 2008). *Sr2* has provided durable broad-spectrum adult plant resistance since 1940 but it does not offer adequate protection on its own under high disease pressure (Hayden *et al.*, 2004; Singh *et al.*, 2011a; Njau *et al.*, 2010). This indicates the vulnerability of the Zimbabwean germplasm to Ug99. Therefore there was an urgent need to find other sources of resistance that could speedily be incorporated into the germplasm.

Sr25, *Sr26* and *Sr39* were targeted for introgression into the Zimbabwean wheat germplasm because all three are still effective against the current 13 identified Ug99 races and advanced suitable breeding lines with these genes are available for use in breeding programmes to minimize genetic drag and introgression of unwanted deleterious genes into recurrent parents. The three genes are also associated with other benefits besides improved disease resistance. These include yield improvement, e.g. CIMMYT germplasm with *Sr25* showed yield improvements of 10-15% under irrigated conditions and is part of best performing wheat varieties in Egypt (Misr 1) and Afghanistan (Muquawin 09) (Singh *et al.*, 1998; 2011b). *Sr25* is closely linked to the ASR gene *Lr19* while *Sr39* is closely linked to leaf rust gene *Lr35* (Dundas *et al.*, 2007; Bariana *et al.*, 2007). Labuschagne *et al.* (2002) reported significantly higher protein content and water absorption with a line that had *Lr35* (*Sr39*) compared to its recurrent parent without *Lr35*, traits that are important for the baking industry.

According to Singh *et al.* (2011b), the fastest way to reduce susceptibility of important wheat cultivars is to systematically incorporate combinations of diverse resistance genes through limited or repeated backcrossing. Two backcrosses to the recurrent parents were done in this study to ensure that economically important traits of the recurrent parents are retained and three genotyping processes were done to ensure parental genetic purity and tracking of the targeted genes without prior exposure of the progenies to any pathotypes. Singh *et al.* (2000; 2004) used a single backcross followed by selected bulk method and a large number of segregating populations for achieving combinations of APR genes for resistance to stripe and leaf rust in

wheat. Bariana and McIntosh (1995) used a single backcross approach to capture total variation in the durably resistant cultivar Bersee. A single backcross with a large number of BC_1F_1 was found to be useful in achieving targeted combinations of APR genes through segregation analyses of individual BC_1F_2 families by Bariana *et al.* (2004).

Prior to parental screening of the study material, selection of recurrent parents was based on the fact that SC1 is the most popular variety in Zimbabwe but stem rust resistance is based on the susceptible *Sr31* gene rendering SC1 susceptible. SC15 is an upcoming variety whose resistance unfortunately was based on *Sr31*. The all stage resistance of SC8 was based on a combination of *Sr2* and *Sr31*, but is later to mature than the other two varieties. Genotyping work done in 2012 used markers for *Sr2 (csSr2)*, *Sr31 (iag95)*, *Sr24 (Sr24#50)*, *Sr36 (wmc477 and stm773-2)*, *Lr34/Yr18 (cssfr6)* and *Lr46/Yr29 (ncw1* and *ncw7).* During that time, markers for *Sr25*, *Sr26* and *Sr39* were not included in the analysis. Results of parental screening in this study revealed that SC8 and SC15 carry the *Sr25* gene. This resulted in deviation from the original objectives, with all crosses involving the introgression of *Sr25* using SC8 and SC15 being dropped. Continuation with such crosses would not make sense given that the genes are already present in the recurrent parent.

Gene stacking or pyramiding for disease resistance is complicated as it requires a strong human resource commitment and investment in infrastructure for both phenotyping and genotyping exercises that are necessary. Phenotyping when breeding for stem rust disease resistance requires greenhouses for seedling bioassays. Bariana *et al*. (2007) mentioned that not all programs are equipped with a specialized testing facility to screen their breeding material at seedling stage effectively as is done by most cereal rust workers. Facilities at University of the Free State in South Africa were used for such bioassays during this study. Screening of progenies requires exposure to the targeted disease thus the need for “hot spot” testing sites where nurseries are planted under high disease pressure. Some of the targeted pathotypes might not be currently prevalent on breeding sites. Stem rust severity is usually high in the southern part of Zimbabwe while the Seed-Co breeding programme is located at Rattray Arnold Research Station in the northern part of the country where severity is non-existent or very low. This requires investment in greenhouses for artificial inoculations or use of MAB in which selection is based on gene presence/absence without exposing the progenies to the disease.

CenGen (Pty) laboratory at Worcester in South Africa was well equipped for MABC during this study.

In the absence of genotyping platforms, knowledge on morphological markers has been and can still be used to track introgressed genes in the progenies as part of "indirect selection". This demands experience thereby making retention of experienced staff a priority, which is a challenge in many institutions. These morphological markers also have their own limitations and have to be complemented by genotypic marker data. *Sr2* is associated with PBC and high temperature induced seedling chlorosis (HTISC) but these *Sr2* markers have limitations. Sometimes they are difficult to select in the field because the resistance phenotype which is only expressed at the adult stage, and the level of PBC expression is influenced by genetic background and environment (Mago *et al*., 2010; Hayden *et al*., 2004). Singh *et al.* (2013) and Juliana *et al.* (2015) as cited by Pretorius *et al.* (2017) reported QTL associated with PBC in the absence of *Sr2*. Therefore there is need for molecular markers to complement morphological markers as an aid to decision making.

Field verification of selected material has to be done at some point to test materials under farmer conditions, however these adult plant field bioassays for Ug99 races can only be conducted in regions where the targeted pathotypes are already present (Liu *et al*., 2010). Practising shuttle breeding is an option with progenies taken to screening sites such as Njoro in Kenya (screening global "hot spot" for Ug99) at some point. CIMMYT `s Mexico-Kenya wheat shuttle breeding programme has been a success developing widely adapted wheat varieties not only resistant to Ug99 stem rust but also to other wheat diseases (Sing *et al*., 2011b; Pretorius *et al*., 2017). This calls for programme coordination so that timely operations are accomplished in order to shuttle between testing sites. Prior knowledge of genotypic make up of parental lines is also critical to undertake gene pyramiding. This requires investment in genotyping platforms or out-sourcing the service. Crosses involving SC8 and SC15 as recurrent parents and WCB08 as the donor for *Sr25* had to be discontinued. This shows how critical it is to have adequate genotypic information to aid in decision making. Genotyping platforms that are affordable and have short turnaround time are a critical component in gene pyramiding. In this study, cross 9 involving SC8 and line 25#2/163 (*Sr39*) had to be dropped after initiation due to absence of *Sr39* in the donor line. Marker information enabled such decisions to be made.

According to Bariana *et al*. (2007), knowledge of existing pathogen virulences in the targeted market and ability to collect, store and use the rust pathogens for artificial inoculations in bioassay studies is also critical in breeding for resistance. The three genes targeted for introgression in this study (*Sr25*, *Sr26*, and *Sr39*) were selected on the basis that they are still effective against the current Ug99 pathotypes including the four pathotypes (TTKSF, TTKSF+*Sr9h*, PTKST and TTKSP) that are prevalent in Southern Africa. In most countries, Zimbabwe included, pathogenicity surveys are not systematically performed and most of the breeding is "anticipatory", with fewer tools to aid decision making. These surveys tend to avail information on the status of prevailing pathotypes in a market or region to allow breeders to plan accordingly for the most effective gene deployment. It also allows decisions on variety replacement to be done depending on changes in pathogen virulences.

The need for widening the genepool in a breeding programme cannot be over-emphasized and use of wild relatives as sources of resistance genes is one of the options. The risk involves introgression of deleterious traits into the gene pool through genetic drag. *Sr25/Lr19* gene segment on chromosome 7DL was not widely used because it was linked to a gene associated with the accumulation of undesirable levels of yellow flour pigment, until mutants with white flour and shortened chromosome segments like "Agatha-28" were available for transfer into Australian and CIMMYT wheat backgrounds (Singh *et al*., 2011a; maswheat.ucdavis.edu 28/7/17). *Sr26*, used in Australia since 1971, was found in a variety called Eagle. However there was limited use due to reported 9% yield penalty that was associated with the original long chromosome segment (maswheat.ucdavis.edu 28/7/17).

It is because of the above mentioned challenges that marker assisted breeding methods can be used to complement the phenotyping process. Marker assisted backcrossing was used in this study because robust markers are available for resistant genes *Sr25*, *Sr26* and *Sr39* while genotypic data was also available for the recurrent parents used in this study. Molecular markers ensure selection of a target gene based on the presence of the linked genotype and therefore success of selection is dependent on the close genetic association and robustness of a given marker across different genetic backgrounds (Bariana *et al*., 2007). This eliminates the need for disease nurseries (though these will be required for field verification during later stages) in order to expose progenies to high disease pressure for screening. According to Pretorius *et al*. (2017), molecular markers allow for early screening, reduces turnaround time for decision making, favour stacking and selection of several desired genes within the

constraints of population size. Ultimately, MAB enhances efficiency and increases genetic gains within a short period of time given that selection intensity is high; it is precise (only progenies with targeted genes are selected for further evaluation) and allows several generations to be quickly screened within a single breeding season. MAB addresses most the factors that enhance genetic gains as addressed by so called "breeders` equation";

$R_t = (i*r*V_g)/y$

where R_t is the genetic gain over time, i is the selection pressure, r is the selection accuracy/precision, V_g is the genetic variance and y is the number of years per plant breeding cycle.

The MABC strategy used in this study managed to eliminate two crosses in which the targeted genes were not confirmed thereby saving time and resources assuming these two would have been taken through the various testing stages under the current conventional breeding practice by the SC wheat breeding programme. Despite the small number of plants that were used in this study (a sample of 30 plants per cross), the various molecular markers were able to confirm presence of targeted genes in the four successful crosses.

Markers for *Sr26* (*Sr26#43* and *BE518379*) had a 10% success rate in confirming the gene in crosses 2 (4 plants) and 5 (2 plants). This success rate was within a range that has been recorded by other researchers. Liu *et al*. (2010) reported success rates of 0% on 170 CIMMYT lines and 11.9% on 42 wheat lines used to validate the marker. Marker for *Lr19/Sr25*, (*BE145935*) failed to confirm gene presence in 30 samples from Cross 1. Other researchers have used the same marker with Liu *et al*. (2010) recording 2.9% success rate in confirming presence of *Sr25* gene in 170 CIMMYT lines and 7.1% (3 lines) in the 42 lines used for marker validation studies. The marker can therefore be used in MABC on progenies of different backgrounds. The KASP marker for *Sr31* was also successful in confirming the *Sr31* gene in all the five populations (150 plants) with a 74% success (accuracy) rate. *Sr39#22r* was able to identify plants with the *Sr39* gene with a 36.7% success rate (3 lines in cross 3 and 19 lines in cross 6).

6.7. Recommendations and conclusions

The three targeted genes *Sr25* (though already present), *Sr26* and *Sr39* were successfully introgressed into the Seed-Co genepool using SC1 and SC15 as recurrent parents. The selected

plants will have to be genotyped after gene fixation (F_6). The genotyping process has to be done in order to identify pure lines that have the desired genes that can be included in replicated trials for potential commercialization. The identification of *Sr25* in SC15 will require genotyping for *Lr19/Sr25* gene in progenies from crosses 5 and 6 to confirm that the gene is also present in pure lines to be developed from those crosses. Bioassays should be conducted on all progenies from successful crosses to verify the presence of the genes when inoculated with known Ug99 races.

The availability of robust genetic markers that can be used on progenies with different genetic background makes MAB attractive as a complementing tool to conventional breeding methods. Pathogens are rapidly changing and breeding programmes also need to take advantage of rapid methods of characterizing germplasm. MAB can therefore play a part in anticipatory breeding by allowing gene pyramiding so that breeders are always ahead of impending epidemics. Breeding programmes need to revisit breeding strategies so as to adopt methodologies that can favourably select for desired genes that might be difficult to retain using conventional means. The use of backcrossing methods, normally avoided by many breeders, is one such method that results in speedy introgression of a desired trait into an already adapted cultivar, resulting in speedy genetic gains. Progenies selected based on marker confirmation have to be verified in the field under natural conditions or in the green house under artificial conditions. Breeding programmes, SC included, have to observe the values of resistance gene stewardship in coming up with short, medium and long term breeding goals to ensure that disease pathogens such as Ug99 are kept at bay.

7. Yield loss associated with different levels of stem rust resistance in bread wheat

7.1. Introduction

Ug99 or TTKSK, an African strain of stem (black) rust caused by *Puccinia graminis* Pers. *f. sp. tritici* Eriks. & E. Henn. (*Pgt*) virulent for the widely used *Sr31* resistance gene in wheat (*Triticum aestivum* L.), was first observed in Uganda in 1998 and characterized in 1999 (Pretorius *et al*., 2000; Singh *et al*., 2008a; Hiebert *et al*., 2010). Isolates of *Pgt* belonging to the Ug99 race group are virulent to a broad spectrum of resistance genes, rendering 90% of the wheat varieties grown worldwide susceptible to stem rust (Pretorius *et al*., 2010; Singh *et al*., 2011a; 2015). Since first detection of the original Ug99 isolate, 13 races belonging to the Ug99 lineage have been identified in 13 countries namely, Uganda, Kenya, South Africa, Ethiopia, Sudan, Yemen, Iran, Tanzania, Zimbabwe, Eritrea, Mozambique, Rwanda and most recently Egypt (Singh *et al*., 2011a; http://rusttracker.cimmyt.org 16/6/17). The continued emergence of new Ug99 races will persist to render once effective *Sr* genes ineffective. This was confirmed by Bhavani *et al*. (2010) who observed that new strains of Ug99, with added virulence to *Sr24* and *Sr36*, resulted in more than half of the TTKSK resistant lines being susceptible.

Zimbabwe and South Africa tend to have common rust pathotypes because of their geographical proximity and Ug99 races TTKSF, TTKSF+*Sr9h*, and PTKST have been identified in both countries while TTKSP has been observed only in South Africa (http://rusttracker.cimmyt.org, 16/6/17). In Zimbabwe, stem rust is regularly recorded at Lowveld sites (Mukoyi *et al.*, 2011) whereas in South Africa the disease is most prevalent in the Western Cape (Terefe *et al*., 2016). The use of resistant varieties has been an important control strategy in both countries. Moreover, resistance must be durable as utilization of single, race-specific genes has often led to breakdown of resistance due to rapid pathogen population replacement by new virulent races (Herrera-Foessel *et al*., 2008; Singh *et al*., 2015; Pretorius *et al*., 2017).

In addition to the threat posed by the Ug99 race group, other highly virulent stem rust races not related to Ug99 have recently appeared in Ethiopia, Turkey, Germany and Sicily. Olivera *et al*. (2015) reported extensive crop failures due to stem rust epidemics, primarily caused by race TKTTF, in the wheat cultivar Digalu (*SrTmp*) in Ethiopia in 2013-2014. Six races, including

TKTTF, were detected in Germany in 2013 (Olivera *et al*., 2017). The 2016 epidemic in Sicily, which occurred on bread and durum wheat affecting several thousand hectares, was caused by a highly virulent TTTTF race of *Pgt* (http://rusttracker.cimmyt.org, 16/6/17). According to present knowledge none of these races have spread to Southern Africa but following examples from pathosystems such as soybean rust, wheat leaf rust and wheat stem rust (Pretorius *et al.*, 2015), such an event is plausible.

Despite the importance of stem rust as a re-emerging disease and emphasis on breeding for more durable resistance types, often expressed as adult plant resistance (APR), little is known about the level of yield protection under epidemic conditions. In this study the effect of stem rust on yield, yield components and protein content was studied in wheat cultivars and lines with different types of resistance. The degree of yield protection in APR lines was of particular interest.

7.2. Materials and Methods

7.2.1. Experimental design

A split-plot design with three replications was used in both the 2014 and 2015 seasons. Main plots were fungicide-sprayed and non-sprayed treatments whereas subplots were seven wheat varieties or lines. Prosaro™ (active ingredients 125 g/l prothioconazole and 125 g/l tebuconazole), a registered fungicide in South Africa, was applied three times during the 2014 season and four times in 2015. The relevant plots were sprayed at a rate of 400 ml/ha using a backpack sprayer with an attached hand-held boom.

7.2.2. Trial management

Normal wheat agronomic practices were followed during the two seasons of testing. During seedbed preparation, 250 kg 2:3:4 (38) N-P-K plus 0.5% Zn fertilizer were applied per hectare. A seeding rate of 80 kg/ha was used with a gross plot size of 24.3 m^2 (eight 4 m rows with 0.76 m inter-row spacing). Each plot consisted of three rows of a respective trial entry flanked by three and two border rows of Line 37-07 (susceptible variety) on either side. Main plots were separated by eight 4 m rows of Line 37-07. Aphox™ (pirimicarb) was used to control aphids at a rate of 250 g/ha while Confidor™ (imidachloprid) was applied twice at a rate of 290 g/ha in 2015. Stem rust-susceptible border rows of Line 37-07 were inoculated on several occasions

with urediniospores of Ug99 race PTKST suspended in Soltrol 130 isoparaffinic oil (Chevron Phillips, Borger, Texas). The spore suspension was applied with an ultra-low volume sprayer (ULVA, Micron Group, Bromyard, England) in the late afternoon. As infection assurance, certain sections of inoculated borders were covered with plastic sheeting to create high humidity conditions overnight (Figure 7.1).

Figure 7.1: Field photos.

Field layout of plot rows under bird nets (left in 2015) and inoculation of Line 37-07 with plastic cover over night to ensure adequate infection (right in 2014).

7.2.3. Germplasm used

Three susceptible varieties (SC1, SC3 and Line 37-07), three APR lines (Kingbird, W1406 and W6979) and an all stage resistance (ASR) variety (SC8) were used in the study (Table 7.1).

7.2.4. Site information

The study was conducted during the 2014 and 2015 seasons at the Pannar research station near Greytown located in KwaZulu-Natal, South Africa. The station is located 29°03`S and 30°35`E at 1050 metres above sea level. Table 7.2 indicates weather records during the two seasons of testing. The annual long-term rainfall measured over 42 years is 835.17 mm according to the Pannar Hildesheim Weather summary. The study was done under nets for bird protection.

Table 7.1: Seven wheat varieties used in the study, their parentage and classification of resistance to PTKST

Variety	Resistance type	Pedigree	Remarks
SC1	Susceptible	Unavailable	White grained Seed-Co variety released in Zimbabwe in 1999 (Havazvidi and Mabandla, 1999)
SC3	Susceptible	Unavailable	A red grained Seed-Co variety released in 2004 in Zimbabwe (Havazvidi and Mabandla, 2004)
SC8	ASR	Unavailable	Red grained Seed-Co variety released in 2009 in Zimbabwe, (Soko *et al*., 2009)
Line 37-07	Susceptible	Kasyob/Genaro-81/Cham4	Selection from 2007 Stem Rust Trap Nursery, South Africa (Prins *et al*., 2016)
W1406	APR	Penjamo-62/908- Frontana-1)//Kentana-54-B	Stem rust resistant line by Genome Resource Unit (GRU), Norwich Research Park and released in Kenya as Kenya_TK_42 (Prins *et al*., 2016)
W6979	APR	Klein-Atlas/Tobari-66//Centrifen/3/Bluebird/4/Kenya-Fahari	Hard red grained spring wheat variety with stem rust resistance by Genome Resource Unit (GRU), Norwich Research Park and released in Kenya in 1982 as Kenya-Popo (Prins *et al*., 2016)
Kingbird	APR	Tam-200/Tui/6/Pavon-F-76//Carianca-422/Anahuac-F-75/5/Bobwhite/Crow//Buckbuck/Pavon-F-76/3/Yecora-F-70/4/Trap-1	A CIMMYT variety with APR (Kimani *et al*., 2015; Singh *et al*., 2015)

APR: Adult plant resistance ASR: All stage resistance

Table 7.2: Summary of site information and crop management practices

Activity/Record	2014	2015
Date of planting	29 May 2014	17 June 2015
Annual rainfall (mm)*	757.2	747.0
Monthly average minimum temperature (°C) *	9.6	10.3
Monthly average maximum temperature (°C) *	25.2	26.1
Monthly average temperature (°C) *	16.3	17.0
Annual total heat units*	2684.6	2983.9
Monthly average soil temperature (°C) *	18.7	18.2
Monthly average Dew point (°C) *	10.3	10.7
Monthly average Relative humidity (%)*	75.4	76.0
Prosaro @ 400 ml/ha application dates	30th July	10th September
	21st August	22nd September
	18th September	20th October
	7th October	-
Aphox @ 250g/ha application date	Not applied	9th September
Confidor @ 290g/ha application date	Not applied	23rd September
	Not applied	28th October
Dates for disease scoring	3rd October	2nd October
	17th October	16th October
	4th November	23rd October
	-	30th October

* Weather records according to Pannar’s Hildesheim Weather summary

7.3. Records taken

Yield per net plot (g): At harvest ripeness, three rows per treatment were cut and threshed. Grain weight was recorded in grams from each nett plot.

Hectolitre mass (HLM) (g): also known as test density or weight was recorded in grams for each plot using a Cox funnel, 0.5 l container, hardwood striker and digital electronic scale to measure test density in grams per 0.5 l (g/0.5 l).

Field stem rust scores: were recorded using the modified Cobb scale where disease severity is the percentage of tissue infected, in combination with host response as immune (0), resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) (Roelfs *et al*., 1992).

Grain protein content (GP) as a percentage: was determined at 14% moisture using AACC International Approved Method 39.25.01 of 1999 (www.aaccnet.org 16/6/17).

Thousand kernel weight (TKW) in grams: A seed counter (NUMIGRAL model) was used to count 1000 grains from each sample and an electronic digital scale (DHAUS Scout) was used to record weight of the 1000 grains.

Derived variables

(a) Yield in kg/ha at 12.5% moisture basis (mb): Net plot yield (g), moisture at harvesting (%) and nett plot area (m^2) were used in the following formula:

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

(b) HLM was converted to kilograms per hectolitre (kg/hl) using the formula:

HLM (kg/hl) = HLM (g/0.5l)/5

(c) Stem rust coefficient of infection (SRCI): was calculated by multiplying the severity score and a constant for host response where trace severity (T) = 1%, and host responses R = 0.2, MR = 0.4, MS = 0.8 and S = 1.0 according to Roelfs *et al*. (1992). For instance, SRCI with a field score of 100S would result in a SRCI value of 100, i.e. 100 x 1 (constant 1 for susceptible) and TS = 1 (1 x 1). Where necessary, interpolations were used for overlapping response classes for instance 40MRMS = 24 (40*((0.4+0.8)/2)).

(d) Area under disease progression curve (AUDPC): was calculated according to Campbell and Madden (1991) using calculated SRCI and the following formula was used:

$$AUDPC = \sum(t_{i+1}-t_i)((y_{i+1}+y_i)/2)$$

Where y_i: SRCI score of the ith observation, and t_i: time at the ith observation and $t_{i+1} - t_i$: the time interval (days) between two consecutive observations.

7.4. Data analysis

GenStat 14th Edition (www.genstat.co.uk) was used to calculate analysis of variance (ANOVA) for single site and the least significance differences (LSD) were used to compare the means for the variables wheat grain yield (kg/ha), TKW (g), HLM (kg/hl) and SRCI (%). The analysis was based on the model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + g_{ik} + e_{ijk}$$

Where Y_{ijk}: observation from ith main plot, jth block and kth subplot

μ: overall mean

α_i: is the main plot effect (fungicide treatment) at ith main plot level where i = 1 (Non-sprayed) and 2 (Sprayed).

β_j: is the subplot effect (variety) at jth subplot level where j = 1…..7.

$\alpha\beta_{ij}$: Interaction effects of main plot by subplot factors (Fungicide treatment by variety)

g_{ik}: is the main plot error distribution

e_{ijk}: is the subplot error distribution

7.5. Results

7.5.1. Grain yield

In 2014, sprayed plots had a mean yield of 6220 kg/ha compared to non-sprayed plots at 4854 kg/ha. The differences in yield were attributed to varieties ($P<0.01$) and interaction effects between treatment and varieties ($P<0.05$). Susceptible varieties recorded yield losses that

ranged from 17.1% (SC3) to 46.8% (Line 37-07) while yield losses for APR varieties were in a range of 13.9% (Kingbird) to 20.2% (W6979). The ASR variety SC8 recorded a 10.4% loss in 2014. Figure 7.2 summarizes the yield performance of the seven wheat varieties during the 2014 winter season. In 2015 (Table 7.16, Appendix 7), sprayed plots had a mean yield of 4798 kg/ha that was significantly ($P<0.05$) higher than yield for the non-sprayed plots (3813 kg/ha). Again, Line 37-07 recorded the highest loss in yield (49.2%) while SC8 recorded a 1.3% gain due to fungicide treatment. Susceptible varieties recorded the highest yield losses of between 25.2% (SC3) and 49.2% (Line 37-07) while APR varieties recorded losses between 4.9% (Kingbird) and 23.6% (W1406). These yield differences were attributed to treatment ($P<0.05$), variety, and interaction effects ($P<0.001$), respectively. Figure 7.3 summarises yield performance in 2015. In general, grain yield of sprayed plots was higher in 2014 with a mean of 6220 kg/ha compared to 4797 kg/ha in 2015. Table 7.16, (Appendix 7) summarises yield performance of the seven varieties when sprayed and non-sprayed during the 2014 and 2015 seasons. A mean yield loss of 21.4% was recorded over the two seasons (Table 7.9 and Table 7.17, Appendix 8).

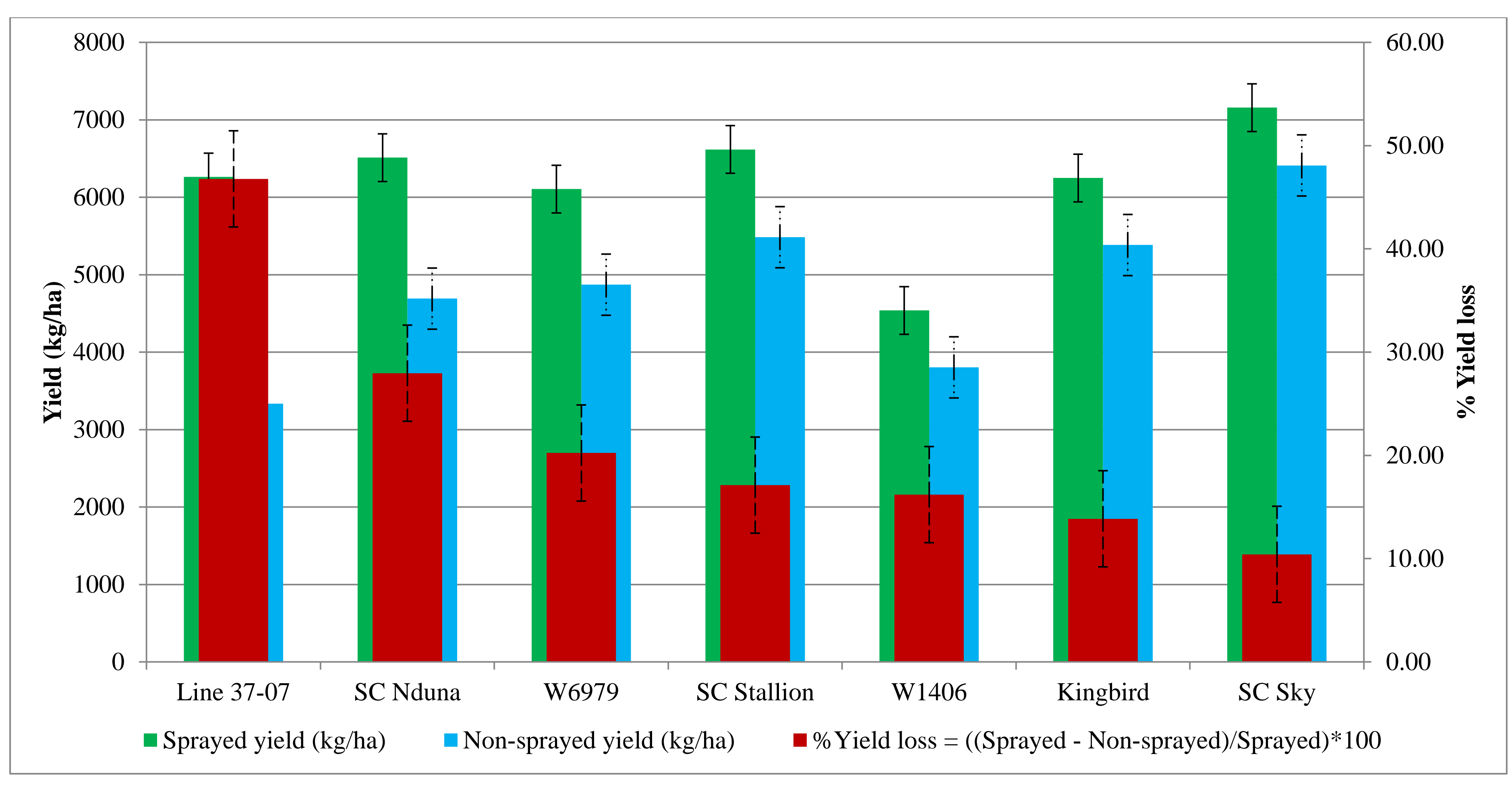

Figure 7.2: 2014 Grain yield data.

Sprayed, non-sprayed mean grain yield (kg/ha) and yield loss (%) by seven wheat varieties. Error bars represent standard error. Line 37-07, SC1 and SC3 are susceptible; W6979, W1406 and Kingbird (APR) and SC8 (ASR)

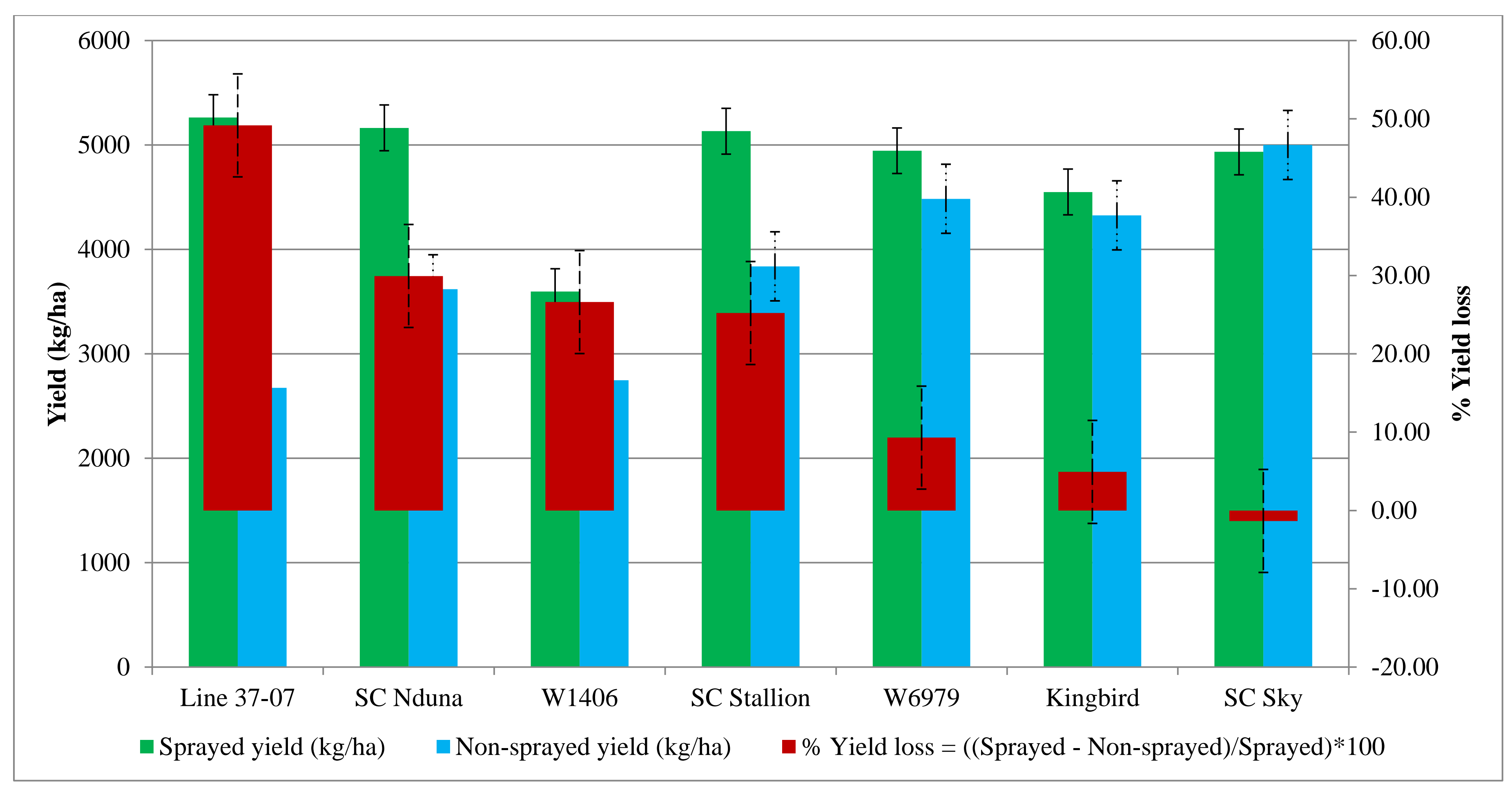

Figure 7. 3: 2015 Grain yield data.

2015 Sprayed, non-sprayed mean yield (kg/ha) and yield loss (%) by seven wheat varieties. Error bars represent standard error.

Line 37-07, SC1 and SC3 are susceptible; W6979, W1406 and Kingbird (APR) and SC8 (ASR)

7.5.2. Thousand kernel weight (TKW)

In 2014, significant differences (P<0.001) in TKW were observed between sprayed and non-sprayed varieties with susceptible varieties Line 37-07, SC1 and SC3 recording the highest losses of 28.1%, 13.3% and 17.2%, respectively. Varieties known to show APR recorded lower TKW losses that ranged between 4.1% for Kingbird and 11.6% for W1406 (Table 7.3). During the 2015 season, differences in TKW between sprayed and non-sprayed treatments were significant at P<0.01, between varieties (P<0.001) and due to interaction effects (P<0.001). Susceptible varieties recorded the largest reductions in TKW when non-sprayed with a range of 28.1% for SC3 to 48.7% for Line 37-07 (Table 7.4). Losses in entries with APR ranged from 20.4% (Kingbird) to 29.6% (W1406). Compared with fungicide sprayed plots over two seasons, stem rust reduced TKW by 20.3% (P<0.05) (Table 7.9). Significant (P<0.001) reductions in TKW were also observed between varieties with SC3 recording a 22.2% loss and Line 37-07 38%. APR varieties recorded mean losses that were between 10.6% for SC8 and 20.5% for W1406. Interaction effects between fungicide treatment and variety were significant (P<0.01) with susceptible varieties benefiting when sprayed. Seasonal differences in TKW were highly significant (P<0.001) with a TKW loss of 12.2% in 2014 compared with 29.2% for 2015. Fungicide treatment and seasonal interaction effects were highly significant (P<0.001) with mean TKW of 41.0 g and 37.2 g for sprayed varieties in 2014 and 2015, respectively. TKW for non-sprayed treatments dropped from 36.1 g in 2014 to 26.4 g in 2015 (Tables 7.3 and 7.4). Table 7.10 and Table 7.11 (Appendices 1 and 2, respectively) summarises the TKW results for the seven varieties in 2014, 2015 and their overall mean over the two seasons.

7.5.3. Grain protein content

In 2014, varieties were significantly different (P<0.001) in grain protein content ranging from 10.59% for SC8 to 12.15% for W6979 when sprayed. In non-sprayed treatments, grain protein ranged from 9.95% (Line 37-07) to 12.78% (W1406). Five (SC1, SC3, SC8, W1406 and Kingbird) of the seven wheat varieties had lower grain protein contents when sprayed than non-sprayed. Only Line 37-07 and W6979 had grain protein content increases of 11.56 and 1.98%, respectively when sprayed (Table 7.3). In 2015, differences due to fungicide treatment, varieties and interaction effects were significant at P<0.05, P<0.01 and P<0.05, respectively. Spraying resulted in higher mean grain protein content (13.53%) compared to non-sprayed (12.65%). All varieties, except SC8, recorded higher protein contents when sprayed (Table

7.4). Over two seasons, spraying resulted in higher grain protein content than non-sprayed though the trend varied from variety to variety. SC1, SC8, W1406 and Kingbird had higher protein contents when not sprayed than when sprayed (Table 7.9). Table 7.14 (Appendix 5) summarises effects of fungicide treatment on protein content of the seven varieties in 2014 and 2015 while Table 7.15 (Appendix 6) summarises the overall mean performance over the two seasons.

7.5.4. Hectolitre mass or test density (kg/hl)

During 2014 season, test density for sprayed treatments ranged from 74 kg/hl (W1406) to 81 kg/hl (Line 37-07) when sprayed and from 70 kg/hl (W1406) to 78 kg/hl (Kingbird) when non-sprayed. Line 37-07 recorded a 12.4% gain in test density when sprayed while an average benefit of 5.1% was recorded on sprayed plots as compared to non-sprayed. Significant differences in test density were due to treatment ($P<0.01$), variety ($P<0.001$) and interaction effects of treatment by variety ($P<0.05$). In the second season, sprayed plots again recorded higher test densities compared with non-sprayed plots. Sprayed plots had test densities ranging from 67 kg/hl for Kingbird and W1406 to 71 kg/hl for SC1 and W6979, respectively. Mean test density for 2015 was lower than in 2014. Susceptible varieties recorded higher losses in test density (13% to 30%) as compared to APR varieties (7-16.4%). Results are summarized in Tables 7.3 and 7.4. Over the two seasons of testing, sprayed plots (74 kg/hl) had better test density than non-sprayed plots (66 kg/hl) by 10.8% (Table 7.9). APR varieties recorded lower test density reductions (4% to 11.3%) than the susceptible varieties (9.5% to 20%). Table 7.12 (Appendix 3) summaries 2014 and 2015 effects of fungicide treatment on HLM of the seven varieties while Table 7.13 (Appendix 4) summaries the overall effect over the two seasons.

Table 7.3: 2014 Mean effects of sprayed and non-sprayed treatments on quality components (TKW, HLM and grain protein content) and percentage loss for seven wheat varieties

	TKW (g/1000 grains)			HLM (kg/hl)			Grain protein content (%)		
Variety	Sprayed	Non-sprayed	% Loss	Sprayed	Non-sprayed	% Loss	Sprayed	Non-sprayed	% Loss
SC1	37.67	32.67	13.27*	79	73[†]	7.59*	11.57	11.89	-2.77
SC3	40.67	33.67	17.21*	79	74	6.33*	11.17	11.19	-0.18
SC8	40.33	38.67	4.12	78	76	2.56	10.59	10.96	-3.49
Line 37	42.67	30.67	28.12*	81	71	12.35*	11.25	9.95	11.56*
W1406	37.33	33.00	11.60*	74	70	5.41*	11.50	12.78	11.13*
W6979	48.33	45.00	6.89	80	77	3.75*	12.15	11.91	1.98
Kingbird	40.67	39.00	4.11	78	78	0.00	11.18	12.08	-8.05*
Mean	41.10	36.10	12.17	78.00	74.00	5.13	11.35	11.54	-1.6
Source	SE	SED	LSD	SE	SED	LSD	SE	SED	LSD
Treatments	0.881	1.245	5.359	0.1	0.2	0.9	0.151	0.214	0.921
Variety	1.269	1.795	3.705	0.9	1.3	2.6	0.208	0.294	0.606
Interactions	1.881	2.66	5.544	1.2	1.7	3.4	0.311	0.44	0.921
Comparison: same level of main[a]	1.795	2.539	5.239	1.3	1.8	3.7	0.294	0.415	0.857

*: Treatment means with significant differences between sprayed and non-sprayed means at $p < 0.05$, according to Least Significant Difference (LSD) test. % Loss = ((Sprayed – Non-sprayed)/Sprayed) * 100. [a]: Used to compare variety means within the same level of treatment (Sprayed or non-sprayed). SE: standard error of means. SED: standard deviation. LSD: Least Significant Difference at 0.05%.

Table 7.4: 2015 Mean effects of sprayed and non-sprayed treatments on quality components (TKW, HLM and grain protein content) and percentage loss for seven wheat varieties

	TKW (g/1000 grains)			HLM (kg/hl)			Grain protein content (%)		
Variety	Sprayed	Non-sprayed	% Loss	Sprayed	Non-sprayed	% Loss	Sprayed	Non-sprayed	% Loss
SC1	36.00	22.33	37.97*	71	57	19.72*	12.94	12.62	2.47
SC3	34.33	24.67	28.14*	69	60	13.04*	13.07	11.73	10.25*
SC8	35.00	28.67	18.09*	70	61	12.86*	12.82	13.18	-2.81
Line 37	39.00	20.00	48.72*	70	49	30.00*	13.79	11.51	16.53*
W1406	36.00	25.33	29.64*	67	56	16.42*	13.43	13.38	0.37
W6979	44.33	35.00	21.05*	71	66	7.04*	14.63	12.86	12.10*
Kingbird	36.00	28.67	20.36*	67	60	10.45*	13.99	13.30	4.93
Mean	37.24	26.38	29.16	69.00	59.00	14.49	13.53	12.65	6.50
Source	SE	SED	LSD	SE	SED	LSD	SE	SED	LSD
Main	0.609	0.861	3.705	0.9	1.2	5.4	0.096	0.136	0.585
Variety	0.716	1.013	2.091	1.1	1.6	3.3	0.261	0.369	0.762
Interactions	1.118	1.581	3.362	1.7	2.4	5.1	0.355	0.502	1.032
Comparison: Same level of main[a]	1.013	1.433	2.957	1.6	2.3	4.7	0.369	0.522	1.077

*: Treatment means with significant differences between sprayed and non-sprayed means at $p < 0.05$, according to Least Significant Difference (LSD) test. % Loss = ((Sprayed – Non-sprayed)/Sprayed) * 100. [a]: Used to compare variety means within the same level of treatment (Sprayed or non-sprayed). SE: standard error of means. SED: standard deviation. LSD: Least Significant Difference at 0.05%.

7.5.5. Stem rust development

Stem rust was recorded three times starting at Zadoks growth stage 71 on October 3rd, October 17th and November 4th in 2014. The highest severity records on individual, non-sprayed plots were 100S (Line 37-07), 100MSS (SC1), 90MRMS (W6979), 70MRMS (W1406), 50S (SC3), 40MRMS (Kingbird) and 10MR (SC8) (Table 7.6). Stem rust infection on sprayed plots was negligible except for the highly susceptible Line 37-07 which recorded the highest score of 20S. Moreover, natural leaf rust infection occurred in the trial area with Line 37-07 recording a 100S infection score in non-sprayed plots at the final assessment, W6979 (60S), W1406 (20S), Kingbird (30S), SC1 (40S) and SC8 (0R) (Table 7.6). Non-sprayed plots had a higher SRCI (15.5) compared with sprayed plots (0.9), indicating a reduction of 94.2% due to the fungicide treatment (Table 7.5). SRCI scores for the susceptible varieties (SC1, SC3 and Line 37-07) were high on non-sprayed plots (24.4, 11.9 and 48.4%, respectively) compared to the sprayed plots. Values for AUDPC for non-sprayed susceptible varieties ranged from 343 (SC3) to 1635 (Line 37-07). Conversely, SRCI scores for both sprayed and non-sprayed plots for APR varieties were below 12. Non-sprayed AUDPC values for APR varieties ranged from 129 (Kingbird) to 325 (W6979). There was a tight linear relationship between the level of yield loss as estimated in sprayed versus non-sprayed treatments and the level of disease on each variety as estimated by AUDPC from the derived SRCI values, indicated by a significantly high regression coefficient (R^2) of 0.9945% (Figure 7.5), when yield loss (%) was plotted against mean AUDPC differences.

In 2015, stem rust was recorded on October 2nd, 16th, 23rd and 30th. The highest severities at the final assessment date for the non-sprayed plots were 100S (Line 37-07 and SC1), 80MSS (W6979), 80MRMS (W1406), 70S (SC3), 50MSS (Kingbird) and 15MRMS (SC8) (Table 7.7). Non-sprayed plots had a higher SRCI (28.6%) compared with sprayed plots (1.2%), indicating a reduction of 95.8% in field response (Table 7.5). Similar to the previous season, SRCI scores for the susceptible varieties (SC1, SC3 and Line 37-07) were high on non-sprayed plots (51.1, 28.2 and 64.8, respectively). On the contrary, non-sprayed plots for the APR varieties ranged from 15.1 (Kingbird) to 22.7 (W6979). AUDPC for non-sprayed, susceptible varieties ranged from 688 (SC3) to 1632 (Line 37-07). SRCI scores for both sprayed and non-sprayed plots for APR varieties were equal to or below 23 (Table 7.5). The values for the non-sprayed AUDPC for APR varieties ranged from 290 (Kingbird) to 473 (W6979). As in 2014, leaf rust was also noticed in 2015 with first symptoms being recorded on 2nd October and by 30th October it had

caused early maturity on two lines (Line 37-07 and W1406). Leaf rust on other lines was not severe with scores as 40MSS (SC1 and W6979), 30MSS (SC3), 10MS (Kingbird) and 0 (SC8) (Table 7.8). A similar linear relationship was also observed between level of yield loss and disease levels on the varieties with R^2 of 0.8283% (Figure 7.6).

Table 7.5: Mean sprayed and non-sprayed SRCI , reduction in SRCI (%) and AUDPC scores for the seven varieties in 2014 and 2015

		2014					2015				
		Sprayed		Non-sprayed		SRCI	Sprayed		Non-sprayed		SRCI
Resistance type	Variety	SRCI	AUDPC	SRCI	AUDPC	Reduction (%)	SRCI	AUDPC	SRCI	AUDPC	Reduction (%)
S	SC1	0.3	10.95	24.4	768	98.77	1.7	29.40	51.1	1223.05	96.67
S	SC3	2.3	63.00	11.9	342.95	80.67	0.6	8.40	28.2	687.50	97.87
ASR	SC8	0.0	0.00	0.5	14.40	100.00	0.3	4.90	2.4	35.00	87.50
S	Line 37-07	2.3	63.00	48.4	1635.4	95.25	4.1	76.15	64.8	1632.00	93.67
APR	W1406	0.0	0.90	7.6	208.65	100.00	0.3	4.20	15.8	303.90	98.10
APR	W6979	0.3	7.20	11.1	324.85	97.21	1.0	16.45	22.7	472.50	95.59
APR	Kingbird	0.6	17.10	4.6	128.90	86.96	0.3	4.55	15.1	289.75	98.01
	Mean	0.9	23.16	15.5	488.98	94.19	1.2	20.54	28.6	663.39	95.80

SRCI (%): Mean Stem Rust Coefficient of Infection as a % (3 scores in 2014 and 4 scores in 2015)

SRCI %Loss = ((Unsprayed SRCI – Sprayed SRCI)/Non-sprayed SRCI) * 100

AUDPC: Area under disease progress curve

S=susceptible ASR=all stage resistance APR=adult plant resistance

Table 7.6: Adult plant field stem and leaf rust scores (sprayed and non-sprayed plots) for seven wheat varieties that were grown at Greytown during 2014 season

Trt	Date	Rp	SC1	SC3	SC8	Line 37	W1406	W6979	Kingbird
Stem rust sprayed	3/10	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
	17/10	1	TS	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
	4/11	1	TS	10MRMS	0	20S	TR	5MR	TMR
		2	0	5S	0	0	0	0	TMR
		3	TS	10S	0	TS	TR	TMR	5MR
Stem rust non-sprayed	3/10	1	TS	0	0	TS	0	0	0
		2	TS	TS	0	5S	TS	TS	TMR
		3	TS	TMS	0	5S	TS	TS	0
	17/10	1	TS	0	0	5S	TMS	0	0
		2	10S	10S	0	60S	TMS	10MR	5MR
		3	40S	TS	0	90S	TMS	20MR	TMS
	4/11	1	40MR	20MRMS	TMR	70S	25MR	20MR	10MR
		2	60S	40MS	TMR	100S	30MR	60MR	15MR
		3	100MSS	50S	10MR	100S	70MRMS	90MRMS	40MRMS
Leaf rust sprayed	3/10	1	0	0	0	TS	0	0	0
		2	0	0	0	5S	0	0	0
		3	0	0	0	0	0	0	0
	17/10	1	0	0	0	5S	0	0	0
		2	0	0	0	20S	0	0	0
		3	0	0	0	0	0	0	0
L. rust non-sprayed	3/10	1	15S	10S	0	80S	10S	Z50S	10S
		2	10S	TS	0	80S	5S	10S	0
		3	0	0	0	5S	0	TS	0
	17/10	1	40S	Z20S	0	100S	20S	Z60S	30S
		2	20S	20S	0	100S	20S	60S	TS
		3	10S	0	0	100S	5S	20S	TS

Trt: Treatment, Rp: replication, 0: immune, T: Trace, R: Resistance MR: Moderate resistance, MRMS: Moderate resistance-moderate susceptible, S: susceptible and Z: leaf rust spores will be aggregated at the base of the leaf sheath.

Table 7.7: Adult plant field stem rust scores (sprayed and non-sprayed plots) for seven wheat varieties grown at Greytown during 2015 season

Trt	Date	Rp	SC1	SC3	SC8	Line 37	W1406	W6979	Kingbird
Stem rust sprayed	2/10	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	5S	0	0	0
	16/10	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
	23/10	1	0	0	0	TS	0	TMR	0
		2	TMR	0	0	TMR	0	0	0
		3	5MS	0	0	10S	TRMR	5MR	TMR
	30/10	1	TR	5S	TR	5S	0	0	0
		2	TS	TR	0	5R	0	TR	TR
		3	15S	10R	5MS	30SMS	5MRMS	15MRMS	5MRMS
Stem rust non-sprayed	2/10	1	TS	0	0	TS	0	0	0
		2	TS	TS	0	TS	0	0	0
		3	TS	TS	0	5S	0	0	TS
	16/10	1	40S	15S	0	50S	TMS	10MRMS	5MRMS
		2	30S	20S	0	40S	5MR	15MRMS	5MR
		3	50S	40S	0	80S	5MRMS	10MS	10MSS
	23/10	1	50S	30S	TMR	100S	10MRMS	40MRMS	20MR
		2	80S	30S	TR	100S	20MRMS	60MRMS	20MR
		3	80S	50S	5R	100S	70MRMS	50MRMS	40MRMS
	30/10	1	100S	40MSS	15MRS	100S	40MRMS	40MRMS	50MRMS
		2	80S	50MSS	15MRS	100S	50MRMS	80MSS	50MSS
		3	100S	70S	15MRMS	100S	80MRMS	70MSS	50MSS

Trt: Treatment, Rp: replication, 0: immune, T: Trace, R: Resistance MR: Moderate resistance, MRMS: Moderate resistance-moderate susceptible and S: susceptible.

Table 7.8: Adult plant field leaf rust scores (sprayed and non-sprayed plots) for seven wheat varieties grown at Greytown during 2015 season

Trt	Date	Rp	SC1	SC3	SC8	Line 37	W1406	W6979	Kingbird
Leaf rust sprayed	2/10	1	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
	16/10	1	0	0	0	TS	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0
	23/10	1	0	0	0	10S	0	0	0
		2	0	0	0	0	0	0	0
		3	0	0	0	5S	0	0	0
Leaf rust non-sprayed	2/10	1	0	0	0	20S	0	0	0
		2	0	0	0	10S	0	0	0
		3	0	0	0	0	0	0	0
	16/10	1	5S	10S	0	90S	No score	10MS	5MS
		2	10S	10S	0	80S	No score	20MSS	0
		3	20S	20MSS	0	80S	5MS	20MSS	TMS
	23/10	1	10MS	20MS	0	Dead	Dead	20MS	10MS
		2	40MSS	30MS	0	Dead	Dead	40MSS	10MS
		3	40MSS	20MSS	0	Dead	5MS	20MSS	10MS

Trt: Treatment, Rp: replication, 0: immune, T: Trace, R: Resistance MR: Moderate resistance, MRMS: Moderate resistance-moderate susceptible and S: susceptible.

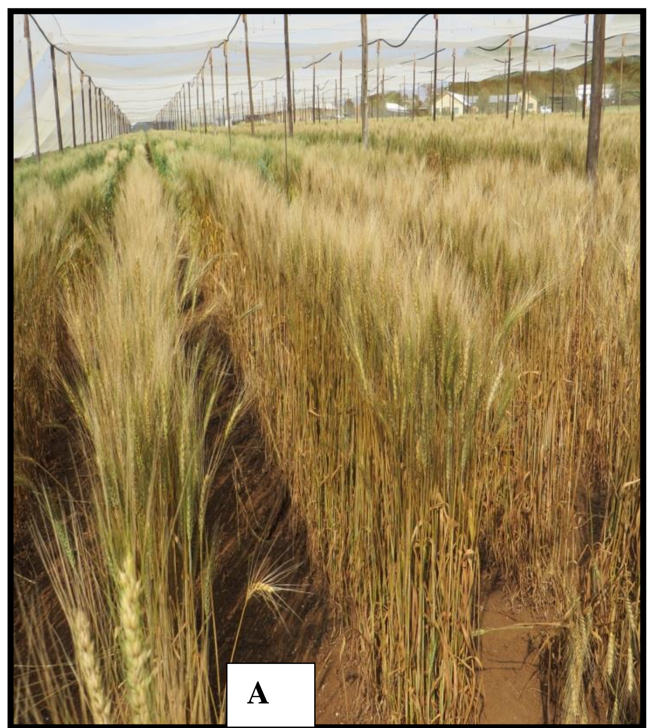

Figure 7.4: Field photos at advanced growth stages.

Line 37-07 plot (A-susceptible) and SC8 plot (B-all stage resistance) at 128 days after planting in 2015.

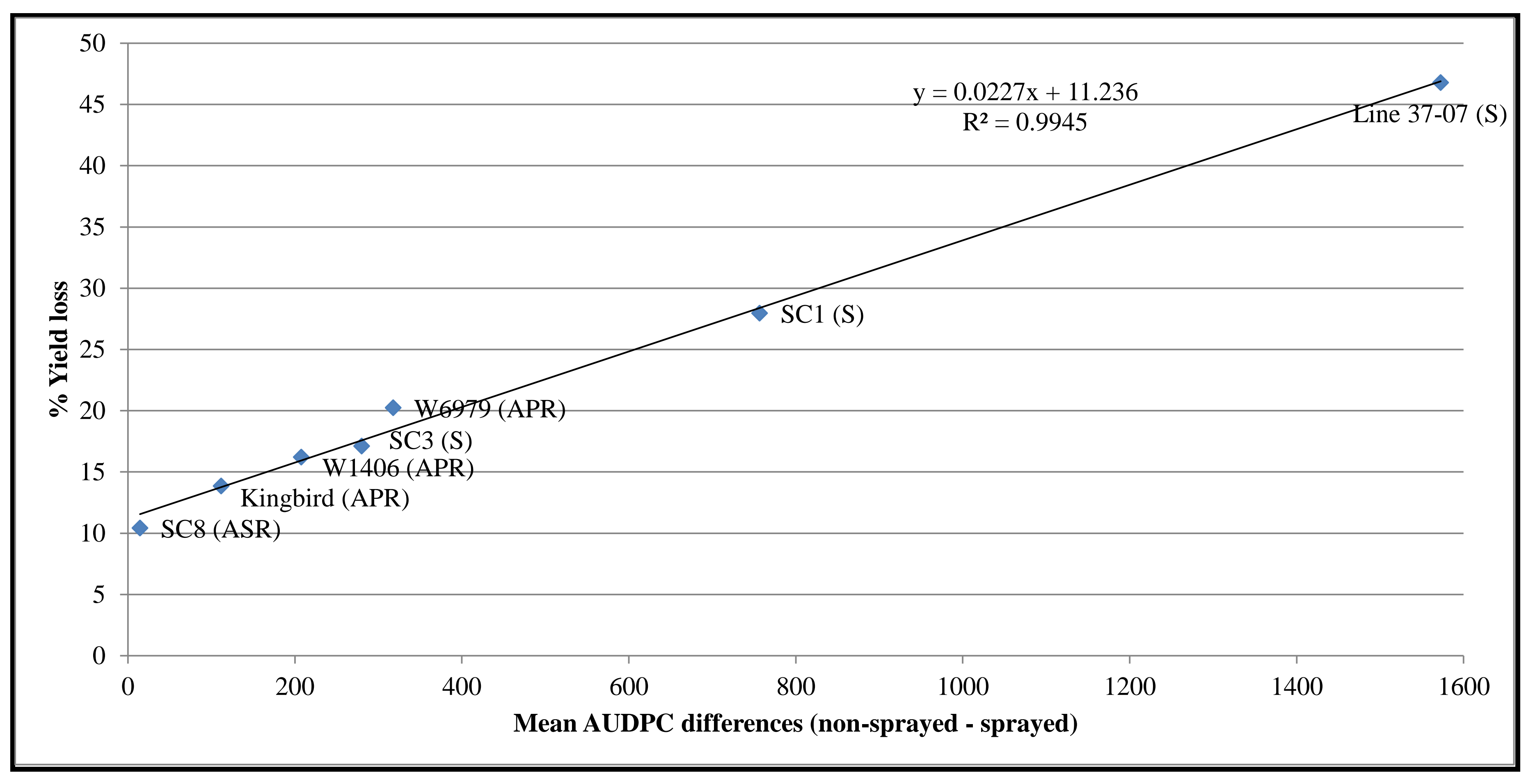

Figure 7.5: 2014 AUDPC plotted against percentage yield loss.

Mean AUDPC differences (non-sprayed - sprayed) plotted against yield loss (%) for seven wheat varieties grown at Greytown in 2014.

S: Susceptible (Line 37-07, SC1 and SC3); APR: adult plant resistance (W6979, W1406 and Kingbird); ASR: all stage resistance (SC8).

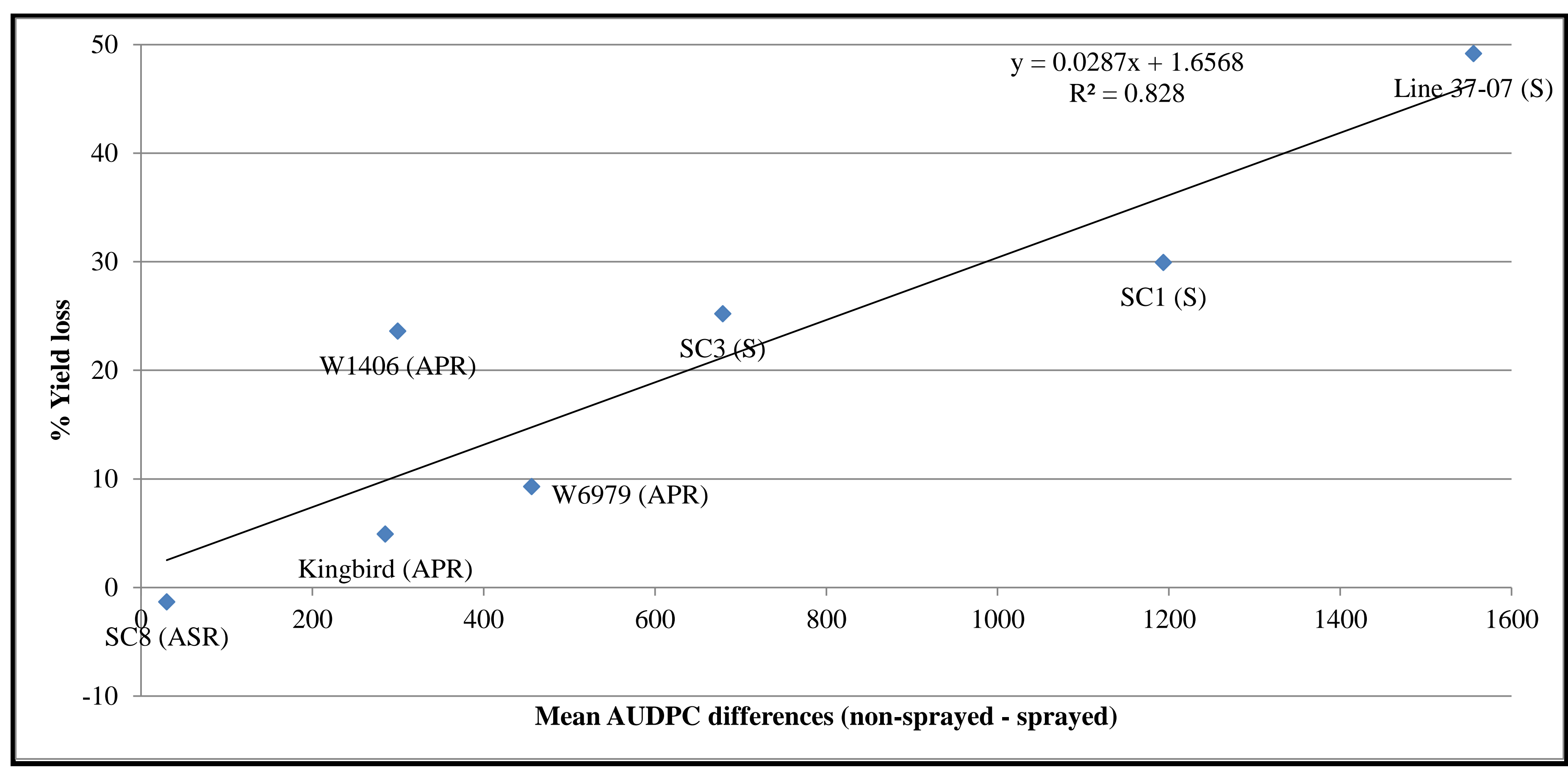

Figure 7.6: 2015 AUDPC plotted against percentage yield loss.

Mean AUDPC differences (non-sprayed - sprayed) plotted against yield loss (%) for seven wheat varieties grown at Greytown in 2015.

S: Susceptible (Line 37-07, SC1 and SC3); APR: adult plant resistance (W6979, W1406 and Kingbird); ASR: all stage resistance (SC8).

Table 7.9: Mean (2014 and 2015 combined) % loss on TKW, HLM, protein, yield and mean non-sprayed SRCI and AUDPC values for seven wheat varieties

Resistance		Mean % loss (gain)				Mean Sprayed		Mean Non-sprayed	
type	**Variety**	**TKW**	**HLM**	**Protein**	**Yield**	**SRCI**	**AUDPC**	**SRCI**	**AUDPC**
S	SC1	25.3	13.3	(0.1)	28.8	1.0	20.2	50.0	995.4
S	SC3	22.2	9.5	5.5	20.7	1.5	35.7	26.0	515.2
ASR	SC8	10.6	8.1	(3.1)	6.4	0.2	2.5	1.7	24.7
S	Line 37-07	38.0	20.0	14.3	47.9	3.2	69.6	80.8	1633.7
APR	W1406	20.5	11.3	(5.0)	19.5	0.2	2.6	15.5	256.3
APR	W6979	13.7	4.0	7.5	15.4	0.7	11.8	22.5	398.7
APR	Kingbird	11.7	4.2	(0.8)	10.1	0.5	10.8	12.2	209.3
	Mean	**20.3**	**10.8**	**2.7**	**21.4**	**0.7**	**21.9**	**29.8**	**576.2**

TKW: Thousand kernel weight SRCI: Stem rust coefficient of infection HLM: Hectolitre mass

AUDPC: Area under disease progress curve S=susceptible ASR=all stage resistance APR=adult plant resistance

7.6. Discussion

Stem rust has been a recurrent threat to wheat production, causing severe yield losses, throughout recorded history (Dean *et al.*, 2012). Furthermore, commercial losses due to stem rust have impacted on national and global policies on disease management. In North Dakota, South Dakota and Minnesota in the USA, 5.67 million hectares of wheat were seriously affected by stem rust in 1916, leading to adoption of the barberry eradication policy the following year (Roelfs, 1982). In Australia, periodic stem rust outbreaks have caused extensive damage since 1889, but the 1973 epidemic resulting in 25%-30% losses (Hodson, 2011), worth more than AU$200 million, was described as "the most severe in the Australian wheat industry" by Watson and Butler as cited by Park (2007). A follow-up conference in 1974 resulted in a highly successful, nationally coordinated rust control approach (Hodson, 2011). In Africa, a modern semi-dwarf variety 'Enkoy' covered large areas in Ethiopia when stem rust caused an average yield loss of 42% in 1993-94 (Dubin *et al.*, 2009). Olivera *et al.*, (2015) reported almost 100% yield loss on the wheat cultivar 'Digalu' during a severe stem rust epidemic caused by a pathotype that did not belong to the Ug99 race group, in Ethiopia during November 2013 to January 2014.

The quantification of losses due to stem rust has been attempted previously. Dill-Macky *et al.* (1990) recorded 59% yield loss on a susceptible wheat variety 'Oxley' compared to two susceptible barley varieties 'Corvette' (22%) and 'Galleon' (48%) when inoculated with an Australian stem rust strain known to be virulent on both crops. In fungicide efficacy experiments in Australia using different active ingredients, rates and timing of applications, Loughman *et al.* (2005) reported yield losses between 10% and 45%. In Southern Africa, accurate economic analyses of the effect of rusts are not available but previous studies have attributed a mean yield loss of 35% over a range of genotypes to stem rust (Pretorius *et al.*, 2007). Despite the re-emergence of stem rust as an important disease of wheat during recent years, few studies have quantified the effects of Ug99 on yield and associated traits in present-day varieties, in particular those that carry APR. Many studies have mentioned that APR, which is the current focus in several breeding programs, can provide resistance to one, or even multiple pathogen species, with expectations of durability (Yu *et al.*, 2011; Singh *et al.*, 2015; Pretorius *et al.*, 2017).

In the current study, mean grain yield loss in the susceptible control Line 37 was 48%. Although this figure is inflated by the fact that the line was also infected by leaf rust, it is within the

expected range of losses caused by stem rust infection (Loughman *et al.*, 2005). Working on the effects of stem rust race Ug99 on grain yield and yield components using 15 bread wheat varieties over two seasons, Macharia and Wanyera (2012) recorded mean grain yield losses ranging from 6% to 66%. Wanyera *et al.* (2016) managed to increase grain yield by 66%, grain weight by 42% and hectolitre mass by 17% in a fungicide trial using Nativo 300 SC® (trifloxystrobin 100 g/l + tebuconazole 200 g/l).

SC1 (40S in 2014 and 100S in 2015) and SC3 (10S in 2014 and 70S in 2015) recorded relatively high stem rust severities and mean yield losses of 28% and 21%, respectively. Previous genotyping work by Soko *et al.* (2015) postulated SC1 to carry *Sr31*, and SC3 to carry *Sr2+Sr31*. Both SC1 and SC3 also carry the leaf rust gene *Lr46/Yr29/Sr58*. Since *Sr31* is not effective to race PTKST, the high disease severity and associated loss in SC1 was expected. However, some protection was provided by *Sr2* and *Sr58* in SC3 as reflected in the traits measured. Over the two seasons, SC1 had higher losses in yield, TKW and HLM as compared to SC3. SC1 had high levels of stem rust disease as compared to SC3 with mean SRCI of 50 compared to 26. For grain protein content, SC1 gained 0.1% (though not significant) compared to a highly significant (P<0.001) loss of 5.5% for SC3 (Table 7.15, Appendix 8). Non-sprayed susceptible varieties had AUDPC values that ranged from 515 (SC3) to 1634 (Line 37-07). SC1 had a low infection type (1+) while SC3 was susceptible (3=) in previous work on infection types (Soko *et al.*, 2015).

Among the three APR lines, Kingbird had the best protection with a yield loss of 10.1% over the two seasons. It had also the lowest losses in TKW (11.7%) and HLM (4.2%) as compared to the APR lines W1406 and W6979. Non-sprayed plots of Kingbird had an average SRCI of 12.2 over two seasons which was also the lowest among the APR lines. Field scores ranged from 0 to TMS (2014) and 0 to 50MSS (2015). Singh *et al.* (2011a) reported that Kingbird was susceptible as seedlings in greenhouse tests with Ug99 but showed high levels of adult plant resistance in the field trials in Kenya and Ethiopia in 2005. Likewise, Njau *et al.* (2010) observed that Kingbird was susceptible at the seedling stage (infection type 3+C) but with good adult plant resistance, scores ranging from 5M to 5MSS, over four seasons of testing in Kenya. Kingbird also showed PBC, a morphological marker for *Sr2*, a trait confirmed by Mago *et al.* (2011). Although *Sr2* is an important component in gene combinations, the slow rusting phenotype conferred by the gene alone was not adequate under heavy disease pressure in Kenya (Singh *et al.*, 2006 and Njau *et al.*, 2009). Bhavani *et al.* (2011) postulated the additive effects

of three to four genes involved in stem rust resistance in Kingbird with QTL on chromosomes 1AL, 3BS (*Sr2*), 5BL (minor gene involved in *Sr2* complex), 7A and 7DS (pleiotropic gene *Lr34/Yr18/Sr57*).

The APR variety W1406, had the highest yield loss among the three APR lines, i.e. 19.5% over two seasons. The yield loss of 23.6% in 2015 was noticeably higher than the 2014 loss of 16% that was highly significant at P<0.001 (Table 7.17, Appendix 8). The lower yields were supported by losses in TKW and HLM of 20.5% and 11.3%, respectively over two seasons. This line recorded a 5% gain in grain protein content that might be due to low carbohydrate accumulation as indicated by the low HLM. The non-sprayed SRCI of 15.5overall was second highest among the three APR lines. Over the two seasons, W1406 had disease scores that ranged from immune (0) to 70MRMS in 2014 while in 2015 the field scores ranged from 0 to 80MRMS. The high disease score in 2015 is reflected in the higher yield loss during that season. Stem rust severity was higher in 2015 with mean SRCI of 28.6 for non-sprayed plots compared to SRCI of 15.5 for the same plots in 2014 (Table 7.5). In addition, these field scores were higher than Prins *et al*. (2016) who recorded 0R to 5R in 2009 at Njoro (off and main season), 10R in 2011 at Greytown and between TR to 15MR over four years of field testing in a doubled haploid (DH) field trial in South Africa. In their genetic diversity studies on African germplasm, using diversity arrays technology (DArT) , simple sequence repeats (SSR) and KASP™ SNP markers, Prins *et al*. (2016) identified four QTL that might be responsible for stem rust resistance in W1406; *Lr34/Yr18/Sr57*, *QSr-ufs-3B*, *QSr-ufs-4D* and *QSr-ufs-2B*. Major stem rust resistance effects were attributed to *Lr34/Yr18/Sr57* and *QSr-ufs-4D*. W1406 also tested negative for *Sr2* during the same studies.

The other APR line, W6979 recorded a highly significant (P<0.001) yield loss of 15.4% over two seasons (Table 7.17, Appendix 8) with annual losses of 20.2% and 9.3% in 2014 and 2015, respectively (Table 7.16, Appendix 7). In terms of yield components, W6979 lost 13.7% in TKW and 4% in HLM. These losses were lower than for W1406. The small loss in HLM might explain why the yield of W6979 was not severely affected. W6979 experienced a 7.5% reduction in grain protein content over the two seasons. The non-sprayed SRCI of 22.5 was the highest among the three APR lines but this was not reflected in the yield loss. Field records ranged from 0 to 20MR in 2014 and 0 to 80MSS in 2015. Macharia and Wanyera, (2012) recorded a yield loss of 51% in Kenya-Popo (=W6979) in a study to establish the effects of Ug99 on fifteen cultivars over two seasons at Mau-Narok and KALRO-Njoro, Kenya. The line

had a mean field severity score of 2.3% and a seedling infection type of 3+ when inoculated with TTKSK in trials conducted in the USA, Kenya and South Africa (Bajgain *et al.* 2016). Njau *et al.* (2009) had reported a seedling infection type of 3 for TTKS when inoculations were done in Minnesota, and field scores of 60S (2006) and 10MS (2007) giving a mean severity score of 35% in field trials at Njoro. Studies by Prins *et al*. (2016) indicated that W6979 had field scores ranging from 0R to 5R in 2009 at Njoro (off- and main season), 40R in 2011 at Greytown and between TR to 30MR over four years of DH field testing in South Africa. Their mapping work postulated that W6979 has five QTL (*Lr34/Yr18/Sr57*, *QSr-ufs-6A*, *QSR-ufs-2D*, QSr-ufs-*3D*, *QSr-ufs-2B*) responsible for stem rust resistance, with *QSr-ufs-6A* having a major effect over seasons and locations. The differences in level of yield loss and disease scores are most likely due to seasonal effects influencing stem rust onset and development. Despite the confirmation of APR in Kenya Popo (W6979) in several studies (Njau *et al*., 2009; Macharia and Wanyera, 2012; Bajgain *et al*., 2016; Prins *et al*., 2016), the stem rust phenotype of this line appears to vary depending on environmental conditions.

SC8 showed effective, major-gene stem rust resistance both in the seedling and adult plant stages which explains the low non-significant yield loss of 6.4% over two seasons (Table 7.17, Appendix 8). SC8 was rated clean on leaf rust infection and this also contributed to the lower yield loss when compared with the APR lines. The TKW loss of 10.6% was the lowest among all lines while HLM loss of 8.1% was higher than the two APR lines (W6979 and Kingbird). The high HLM reduction might have resulted in shrivelled grains that caused less grain carbohydrates and a 3% gain in protein content. SC8 had a low infection type in previous greenhouse work (data not shown) and it had a stem rust field record of TR over two seasons in this study. The resistance in SC8 is based on a complex combination of genes (both APR and ASR genes) that include *Sr2*, *Sr31, Lr19/Sr25* (according to genotyping work done in Chapter 6) and other unknown minor *Sr* genes along with the pleiotropic *Lr46/Yr29/Sr58* gene (Soko *et al*., 2015). The ASR gene(s) in SC8 managed to offer adequate and better protection against PTKST throughout the growing period thereby resulting in better yields over the three APR lines that were only protected at the adult plant stage.

Effective and durable resistance in wheat varieties is the preferred way of providing protection against losses due to Ug99. The level of protection against stem rust is determined by the nature and number of both race specific (major) and non-race specific (minor) genes that are combined in a variety. As demonstrated in this study, the ASR of SC8 will offer adequate and

better protection than APR, in the absence of corresponding virulence in the pathogen. However, when a resistance gene is defeated as in SC1 (*Sr31*), significant losses occur. Even the occurrence of *Sr2* and *Sr58* in SC3 added some protection against loss. In context with previous studies, the present work showed that APR can safeguard a variety against stem rust but that the level of protection may vary depending on gene complexities, environment, time of first rust occurrence, and amount of inoculum involved. Kingbird was not affected to the same degree as the other two APR lines, most likely as a result of the number of major QTL involved. It is also conceivable that certain QTL, by means of additive effects, will confer a lower rust phenotype than others.

Singh *et al*. (2011b) indicated that a single APR gene when alone, does not confer adequate resistance especially under high disease pressure but a combination of four to five such genes may result in near immunity. In the present study not all APR lines performed similarly in terms of yield and yield components measured. Although extremely high inoculum levels were experimentally induced here, the possibility of such an event in a rust-conducive, commercial production cannot be ruled out completely. Breeding programmes should thus embrace a combined APR and ASR approach to overcome the impact of Ug99 and other threatening rusts. If rust resistance is a breeding priority it is important that commercial varieties be thoroughly characterized in terms of genotype and phenotype. By confirming complex resistance based on single genes for which virulence is not known to exist, more informed predictions of durability become possible.

7.7. Recommendations and conclusions

Results showed that the level of protection against Ug99 stem rust varies in wheat varieties due to the number of effective resistance genes that are involved. Several genes increase the level of protection. The study also revealed that it is difficult to predict the level of protection that can be conferred by varieties due to other gene complexities (including pleiotropic effects) that are involved when genes work in combination. Comparing the two types of resistances, the study revealed that ASR offers better protection against Ug99 compared to APR as indicated by the better performance of SC8 as compared to the three APR lines (Kingbird, W1406 and W6979). However, since this study was only done at one site where disease pressure was very high due to repeated inoculation; it will be ideal to test the same germplasm along with additional lines under natural conditions in multi-location farmers' fields to verify and generalize these findings. It is also advisable for the Zimbabwean wheat breeding programmes

to pyramid several effective stem rust resistance genes so as to enhance the level of field protection for farmers, by either breeding for APR which has shown better protection than the single race-specific genes in susceptible varieties, or breeding for ASR as shown by SC8. A combination of both APR and ASR resistance genes within a variety is ideal because if there is breakdown of one set of genes the other will still provide significant protection.

8. Evaluation of bread wheat lines for disease, quality and yield stability using additive main effects and multiplicative interaction (AMMI) and genotype and genotype-environment (GGE) models

8.1. Introduction

Wheat in Zimbabwe is an irrigated crop grown during the winter season in the months of May through mid-November when it is cooler and dry with the potential of giving high yield and good quality (Mtisi and Mashiringwani, 1988; Mugabe; Nyakatawa, 2000). Large and small scale (A2) commercial farmers and irrigation schemes dominate the production sector. A2 is a product of the 2 000 land reform programme in Zimbabwe with the aim of allowing farmers with their own resources to venture into commercial farming (Makadho *et al*., 2006). Agricultural Rural Development Authority (ARDA), which is the country's major irrigator with 26 estates and 7 620 ha covering 6.3% of the total country's 120 410 ha of potential irrigable land (Makadho *et al*., 2006), is included within the irrigation schemes. Wheat is produced across all five natural regions of Zimbabwe. Yield potential of wheat in Zimbabwe is significantly influenced by temperature as a result of altitude (Havazvidi, 2008), therefore yields decline from Highveld (≥ 1200 meters above sea level [masl]) to Lowveld (< 800 masl) regions.

An ideal wheat variety is one that is suitable for production in all the natural regions and at the same time meeting requirements by various stakeholders in the wheat value chain. The farmer is interested in high yield and good agronomic traits; the milling industry expects varieties with good milling qualities such as high flour yield; the baker demands varieties that have good baking qualities such as high loaf volume and the consumer is interested in a good quality loaf. In order to identify such varieties there is need for multiple environment testing (MET) across all representative regions of Zimbabwe. MET data is expected (a) to allow prediction of variety yield performance based on limited experimental data, (b) to allow identification of stable varieties that maintain their superior rankings across environments, and (c) to aid in decision making on variety deployment on current and future markets (Crossa, 1990). After field evaluations, the SC winter cereals laboratory in collaboration with the milling and baking industry undertake wheat quality assessments to ensure that only varieties that combine high yield, desirable agronomic and quality traits are commercialized. Above all, the concept of

disease resistance gene stewardship has to be considered in commercializing suitable varieties. Pretorius *et al*. (2017) defined resistance gene stewardship as "careful and responsible management of resistance genes with the goal that they may remain effective for prolonged use".

The breeder is therefore interested in comparing genotypes with the aim of choosing those that show superior performance in different environments. This objective is always complicated by genotype-environment interactions (GEI). Crossa (1990) highlighted that MET data is difficult to analyse because of the systematic and intrepretable manner in which genotypes respond to environments (structural patterns), the unpredictable and uninterpretable sources of variation (non-structral noise) and interaction effects that exists among genotypes (G), environments (E) and genotypes and environments interactions (GEI). Rodrigues *et al*. (2014) referred to GEI as "the frequency of differential responses of genotypes across environments i.e. location-by-year combinations in multiple environment trials". Therefore GEI reflects the different responses of the genotypes to environmental conditions. Hongyu *et al*. (2014) referred to GEI 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 mediocre in another.

Inefficiency in GEI analysis can present problems to breeders leading to wrong varietal recommendations resulting in low variety adoption by farmers and decreased food security. Several statistical methods are available that can be used to understand GEI effects. Standard analysis of variance (ANOVA) with its additive model can identify GEI as a source of variation but it does not analyse it, principal components analysis (PCA) contains no sources for additive genotypes or environment main effects given that is a multiplicative model while linear regression (LR) can analyse GEI terms only if they fit a specific regression model and can only account for a small portion of the interaction sum of squares (Zobel *et al*., 1988). Both additive main effects and multiplicative interaction (AMMI) and genotype and genotype-environment (GGE) analysis allow for (a) analysis of mega-environments (b) evaluation of environments and (c) evaluation of genotypes (Yan *et al*., 2007). The AMMI model is very efficient, accurate, cost-effective in analysing GEI resulting in better variety recommendations, enhanced genetic gains by breeding programmes and is superior for agricultural reasons because it analyses all the three sources of variation i.e. G, E main effects and GEI effects (Gauch and Zobel, 1989; Nachit *et al*., 1992; Gauch, 2006). Superiority of the model is based

on the fact that ANOVA, PCA and LR are all included in the model and the model is flexible since it does not require a specific experimental design except for a two way data structure (Zobel *et al*., 1988; Gauch and Zobel, 1997). AMMI and GGE can be used to demarcate mega-environments and select varieties for specific environments (narrow adaptation) or several mega-environments (wide adaptation) thereby increasing accuracy for enhanced variety recommendations, repeatability, deployment and genetic gains (Falkenhagen, 1996; Yan *et al*., 2007; Hongyu *et al*., 2014).

GGE biplots are superior to AMMI in delineating mega-environments (ME) and genotype evaluation because it explains more genotype and genotype-environment interaction (G+GE) effects, GGE biplot environment analysis is more superior especially the discriminating power vs. representativeness view and this model has the inner-product property of a biplot (Yan *et al*, 2007). Inner-product property of a biplot allows it to approximate the yield and ranking of each genotype for any chosen environment (Gauch *et al*., 2008). This demarcation of environments reduces the effects of GEI with suitable varieties being deployed to suitable environments. ME has been defined by www.wheatatlas.org (26/8/17), Rajaram *et al*. (1994) and Jiankang *et al*. (2003) as "a broad, not necessarily continuous area, occurring in more than one country and frequently transcontinental, defined by similar biotic and abiotic stresses, cropping system requirements, consumer preferences, and, for convenience, by a volume of production". Yan and Rajcan (2002) defined ME as a group of locations that share the same genotypes (or group of genotypes) that are consistently superior over a number of seasons. The former definition applies to a global scale while the latter applies at regional or national level.

Breeders and seed companies are interested in varieties that are widely adapted and stable across environments and seasons. This allows for an enlarged market size leading to increased seed volumes and seed shipment across several mega-environments in times of shortages. SC1 is a good example of such a variety being grown throughout Zimbabwe, Zambia and Malawi. Varieties with narrow adaptation can also be released for a specific "niche" market when returns from such a market justifies the investments. Selection of a stable genotype overcomes the challenge of "unpredictable" year to year interactions that often cause large genotype-year and genotype-location-year interactions and cannot be solved by demarcation of mega-environments (Francis *et al*., 1978). Several definitions of stability have been given by various researchers depending on stability parameters being used. Francis *et al*. (1978) defined a stable genotype as a genotype that provides high and consistent performance over different

environments and over years. Genotype mean yield is normally used to describe genotype performance in the presence of GEI but it does not indicate consistency performance of the genotype thus the need for stability assessments.

The objectives of the study were (a) to compare performance of SC experimental lines against current commercial varieties in order to identify lines that show wide or specific adaptation to the testing environments for potential commercialization and use as parental lines in future wheat crosses, (b) to evaluate interaction effects on performance of the 16 lines using AMMI analysis, and (c) to characterize testing environments using AMMI and GGE biplots to determine the value of information generated by each site. The study was based on the hypothesis that there are no performance differences between the 16 wheat lines when grown across twelve environments in Zimbabwe.

8.2. Materials and methods

8.2.1. Germplasm used

A total of 16 wheat genotypes were tested over the 2014 and 2015 winter seasons. The genotypes comprised four current SC commercial varieties, one Pannar commercial variety, two commercial varieties by Crop Breeding Institute (CBI), one Limagrain line and eight SC experimental lines (Table 8.1).

8.2.2. Site information

A total of eight locations (Table 8.2 and Figure 8.1) with one in Harare Metropolitan province (Agricultural Research Trust Farm – ART), two in Mashonaland East province (Rattray Arnold Research Station owned by SC (RARS) and Panmure Experiment Station owned by Agronomy Research Institute (PAN)), two in Mashonaland West province (both owned by SC i.e. Stapleford Research Centre (SRC) and Kadoma Research Centre (KRC)), two in Manicaland province (Mr. Franklin's Sisal farm (SISAL) and Government's Save Valley Experiment Station (SAVE)), and one location at Government owned Chiredzi Research Station (CHZ) were used in the study. Twelve trials (which made up the environments) were planted at these locations and three locations namely RARS, ART and SRC hosted at least two trials. Trials were defined by planting date and irrigation management. All trials planted during the month of May being the optimum planting time for wheat in Zimbabwe were referred to as normal (N) environments e.g. RARSN, and those planted in the month of June being referred to as

"late" (L) environments i.e. RARSL, ART Land SRCL. Deficit irrigation was practised at ART resulting in ART deficit (ARTD). Under deficit management, the objective was to reduce the number of irrigation cycles so as to reduce costs without prejudicing yield potential. This was achieved by reducing the number of irrigation cycles (lengthening the irrigation period) during the non-critical stages of crop growth i.e. between tillering and booting.

Table 8.1: Zimbabwe wheat lines used in multi-environment trials in 2014 and 2015

Genotype	**Variety**	**Source**	**Status**
G1	SC35	Seed-Co	Experimental
G2	SC40	Seed-Co	Experimental
G3	PAN2	Pannar	Commercial
G4	SC41	Seed-Co	Experimental
G5	SC15	Seed-Co	Commercial
G6	SC42	Seed-Co	Experimental
G7	SC43	Seed-Co	Experimental
G8	SC18	Seed-Co	Experimental
G9	SC1	Seed-Co	Commercial
G10	SC44	Seed-Co	Experimental
G11	LG1	Limagrain	Experimental
G12	SC8	Seed-Co	Commercial
G13	SC36	Seed-Co	Experimental
G14	SC14	Seed-Co	Commercial
G15	CBI5	Crop Breeding Inst.	Commercial
G16	CBI6	Crop Breeding Inst.	Commercial

Table 8.2: Information on sites used during multi-environment trials in 2014 and 2015 winter seasons

No	Site	Management	NR	Altitude	Latitude (S)	Longitude (E)	Potential
1	RARSN	Normal	II	1351	17°67'35"	31°21'24"	High
2	ARTN	Normal	II	1521	17°70'88"	31°06'15"	High
3	SISALN	Normal	III	1070	18°54'95"	32°34'67"	Medium
4	PANN	Normal	III	885	17°29'97"	31°61'98"	Medium
5	SRCN	Normal	II	1466	17°42'54"	30°54'22"	High
6	CHZN	Normal	V	428	21°01'19"	31°33'97"	Low
7	ARTD	Deficit	II	1521	17°70'88"	31°06'15"	High
8	KRCN	Normal	III	1142	18°19'11"	29°51'06"	Medium
9	SAVEN	Normal	IV	452	20°23'20"	32°33'40"	Low
10	RARSL	Late	II	1351	17°67'35"	31°21'24"	High
11	ARTL	Late	II	1521	17°70'88"	31°06'15"	High
12	SRCL	Late	II	1466	17°42'54"	30°54'22"	High

NR: Natural regions I to V. Normal: planted in May. Late: planted after May. Deficit: irrigation reduced between tillering and flowering. High potential: sites ≥ 1200 masl. Medium potential: sites between 800 – 1200 masl. Low potential: sites < 800 masl. Altitude: height above sea level in metres. Latitude in degrees (°), minutes (') and seconds (") south (S) and Longitude to the east (E).

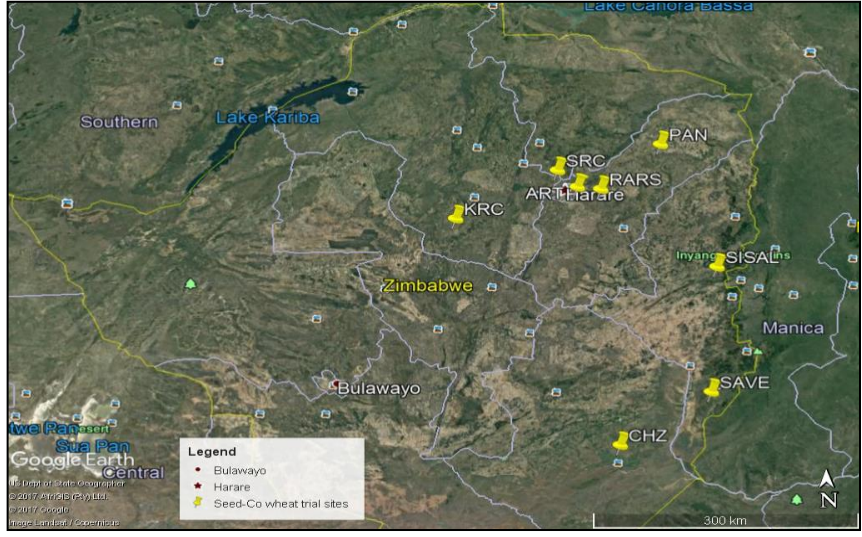

Figure 8.1: Zimbabwean location of trial sites.

Sites used in multi-environment trials during 2014 and 2015 winter seasons

8.2.3. Trial management

Recommended management practices for wheat were followed at all sites with weed management being done by a combination of herbicides and hand weeding where necessary. Aphids were controlled using Dimethoate where necessary and bird scarers were engaged at all sites from grain filling up to harvesting. Overhead sprinkler irrigation was used at all sites (Table 8.3).

8.2.3.1. Land preparation

Trial sites were ploughed, followed by discing and rolling to create a fine seedbed. The fine seedbed was necessary since trial plots were machine planted and this ensured an even sowing depth and ease of movement of the machinery during planting.

8.2.3.2. Fertilization

Basal fertilizer was broadcasted using a Vicon spreader prior to discing. The discing also assisted in incorporating basal fertilizer into the soil. Ammonium nitrate (34.5%N) was used as top dressing fertilizer in two split applications. The first application was done at four to five weeks and second at six to eight weeks after crop emergence (Table 8.3). Top dressing fertilizer was broadcasted by hand in each plot, followed immediately by light (20mm) irrigation.

8.2.3.3. Seeding rate

A seeding rate of 120kg/ha was used for all plots over two seasons. The objective was to achieve a plant stand of 250 to 300 plants per m^2.

8.2.3.4. Plot sizes and harvesting

Gross plots of $12m^2$ (10 rows by 6 m by 0.2 m) were planted using a modified small plot 10-row seed drill. At four weeks after emergence, it was reduced to $11m^2$ (10 rows by 5.5 m by 0.2 m) by removing 0.25m on either end of plot length to avoid overlaps and create 0.5m 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. Harvested grain was collected into a 5kg harvesting bag that was labelled with the plot number.

8.2.3.5. Experimental design

A lattice square design with 16 wheat genotypes, replicated four times was used over two seasons, at all testing sites.

Table 8.3: Summary of management practices at 12 environments used in Zimbabwe during 2014 and 2015 winter seasons

Yr.	Activity	RARSN	ARTN	SISALN	PANN	SRCN	CHZN	ARTD	KRCN	SAVEN	RARSL	ARTL	SRCL
2014	Pr. Crop	Soya	Soya	Soya	Fallow	Bean	Fallow	Soya	Maize	Maize	Soya	Soya	Bean
	DOP	4/5	9/5	15/5	6/5	5/5	29/4	10/5	10/5	2/5	1/6	14/6	7/6
	DOH	19/10	14/10	8/10	8/10	25/10	30/9	13/10	17/10	2/10	19/10	4/11	11/11
	N@planting	28	42	32	28	28	28	42	28	28	28	42	28
	TN(kg/ha)	166	182	170	166	166	166	182	166	166	166	182	166
	P_2O_5(kg/ha)	56	84	63	56	56	56	84	56	56	56	84	56
	K_2O(kg/ha)	28	42	32	28	28	28	42	28	28	28	42	28
	Irrig. (mm)	340	396	660	428	405	300	338	486	416	310	396	340
	Herbicides	M+B	M+B+P		M+B	M+B	M(Rt)	M+B+P	M+P	M (Rt)	M+B	M+B+P	M+B
2015	Pr. Crop	Soya	Soya	Soya	Maize	Bean	Maize	Soya	Maize	Maize	Soya	Soya	Bean
	DOP	10/5	6/5	20/5	5/5	11/5	2/5	6/5	8/5	30/4	10/6	8/6	16/6
	DOH	29/10	17/10	5/10	15/10	26/10	9/10	9/10	1/10	10/10	29/10	3/11	5/11
	N@planting	42	36	28	21	21	21	36	42	21	42	36	21
	TN(kg/ha)	180	191	166	159	159	159	191	180	159	180	191	159
	P_2O_5(kg/ha)	84	120	56	42	42	42	120	84	42	84	120	42
	K_2O(kg/ha)	42	90	28	21	21	21	90	42	21	42	90	21
	Irrig (mm)	330	396	507	549	410	373	338	300	400	300	406	390
	Herbicides	M+B	M+B		M+B	M+B	M+B(Rt)	M+B	M+B	M+B(Rt)	M+B	M+B	M+B

Yr: Year. DOP: Date of planting. DOH: Date of harvesting. N@planting: amount of nitrogen applied as basal fertilizer. TN: Total nitrogen. Pr.: Previous crop. M: MCPA 40EC applied @ 750ml/ha. B: Banvel® applied @250ml/ha. P: Puma applied at 350ml/ha and Rt: Regent®200 SC pesticide (termite control) applied @ 200g/l. Letter N after site code indicates normal planting (planting done before 1st of June, L for late planting (after 31st May) and D for deficit irrigation. Soya: Soybean. Irrig: total amount of irrigation water applied during the season.

8.3. Records taken for the study

Days to anthesis: Number of days when nett plot plants had 50% of their anthers shedding pollen.

Leaf rust scores: Disease severity (%) using the modified Cobb scale representing the percentage area of leaf tissue affected by leaf rust and host responses where immune = 0, R=resistance, MR=resistance, MS=moderate susceptible, S=susceptible was recorded at all 12 environments.

Nett plot yield: Harvested grain from each nett plot was weighed using an electronic scale to determine the weight of grain in grams.

Grain moisture (%), test density (kg/hl), starch content (%), wet gluten (%) and grain protein content (12.5% moisture basis): A near-infrared analyzing machine by Agroservices (formerly Labotec) Pvt (Ltd) model NIR9500 was used to record these variables on all samples from the 12 environments.

Derived records

Nett plot yield (g) was adjusted to yield at 12.5% moisture basis in kilogrammes per hectare using the formula:

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

Leaf rust coefficient of infection (LRCI) as a percentage: A product of field disease severity score (%) and a constant for field host response where immune=0.0, resistance=0.2, moderate resistance=0.4, moderate susceptible=0.8 and susceptible=1.

8.4. Data analysis

Days to anthesis (flowering), yield (kg/ha), LRCI , test density (kg/hl), starch content (%), wet gluten (%) and grain protein content at 12.5% moisture basis (%) were subjected to a combined MET analysis using the AMMI model in Genstat 14th Edition (Payne *et al*., 2011). Data from all 12 environments collected over two seasons was used. AMMI model applies the singular value decomposition (SVD) to the residuals of additive two-way analysis of variance

(ANOVA) so as to explain the GEI effects that are identified by the standard ANOVA but left unexplained (Gauch, 2013 and Rodrigues *et al*., 2014). AMMI first extracted the additive main effects using ANOVA and then interaction (GE) effects using PCA (Crossa *et al*., 1991; Yan *et al*., 2007; Gauch *et al*., 2011). The AMMI model used for the analysis was according to Gauch (1992) where:

$$Y_{i,j} = \mu + \alpha_i + \beta_j + \sum^{N}_{n=1} \lambda_n \Upsilon_{n,i} \delta_{n,j} + \varepsilon_{i,j}$$

Where; $Y_{i,j}$ is the yield of genotype i at environment j

μ is the general trial mean

α_i is genotype deviations from mean (μ)

β_j is the environment deviations from the mean

λ_n is the singular value for Interaction Principle Component (IPC) axes n

$\Upsilon_{n,i}$ is IPC score for genotype for axes n

$\delta_{n,j}$ is IPC score for environment for axes n

$\varepsilon_{i,j}$ is the residual error term (including multiplicative and model residuals errors)

A full AMMI model was adopted in this study though not all PCA were significant. Statistics from the ANOVA were used to determine the level of noise that was defined by Gauch (1992) as the difference between the true mean and its yield estimates. Level of noise in GEI component was determined only for yield using the formula (Gauch, 1992):

Noise = [100*(Interaction deg. of freedom * error mean squares)]/Interaction sum of squares

The signal to noise ratio approach (Gauch, 1992) was used to select the best AMMI model with models that are close to the estimated noise levels being the best. A total of six AMMI biplots were plotted for yield where genotype means, environment means and combined genotype and environments means were plotted against IPCA1 and IPCA2, respectively.

GGE biplot within Genstat 14th edition was used to create a scatter plot of PC1 scores of genotypes and environments against respective scores for PC2 from SVD of environment-

centered genotype-environment data (GED) (Yan *et al.*, 2007) in order to analyse genotypes and environments i.e. a "which-won-where" view of GGE biplot. Comparison biplots were plotted for genotype evaluation using environments for scaling and for environment analysis using genotype scaling. Both genotype and environment analysis was based on comparison with the average environment coordination (AEC) view referred to as "ideal genotype" or "ideal environment". The general GGE model by Cornelius *et al.* (1996) was used.

$\bar{Y}_{ij} = \mu_j + \sum_{k=1}^{t} \lambda_k \alpha_{ik} Y_{jk} + \grave{\varepsilon}_{ij}$

Where; $\bar{Y}_{ij}$ = mean yield of genotype i in environment j

μ_j= mean yield in environment j (where i = 1……g (16) and j = 1…… e (12))

t = number of principal components used in the model with $t \leq \min(e, g - 1)$

λ_k = model constraint where $\lambda_1 \geq \lambda_2 \geq \ldots\ldots \lambda_t \geq 0$

α_{ik} = orthonormality constraints where $\sum_{i=1}^{g} \alpha_{ik}\alpha_{ik'} = 1$ if k = k' and $\sum_{i=1}^{g} \alpha_{ik}\alpha_{ik'} = 0$ if $k \neq k'$

Y_{jk} = orthonormality constraints where $\sum_{j=1}^{e} Y_{jk}Y_{jk'} = 1$ if k = k' and $\sum_{j=1}^{e} Y_{jk}Y_{jk'} = 0$ if $k \neq k'$

$\grave{\varepsilon}_{ij}$= error tem, assuming NID($0,\sigma^2/r$) where r is number of replications in an environment.

Cultivar superiority (P_i): is an indicator of general performance of an experimental genotype in relation to the optimum response averaged over all locations (Lin and Binns, 1988). Genstat Stability coefficient macro was used to calculate cultivar superiority using the formula by Lin and Binns (1988).

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

Where: P_i = cultivar superiority

n = number of environments (n =1 …….12)

X_{ij}= yield of ith genotype at environment j (i =1….16)

M_j = maximum response among all genotypes in the jth location

Interpretation: Experimental genotype superiority is shown by a small mean square.

Mean ranks, $S^{(1)}_i$: mean of the absolute rank differences of a genotype over N environments (Nassar and Huhn, 1987). A genotype with maximum stability is the one where $S^{(1)}_i = 0$.

8.5. Results

8.5.1. AMMI ANOVA results for anthesis, leaf rust coefficient of infection, test weight, grain protein content and wet gluten

Only combined mean data for these traits was presented in this study as shown in Tables 8.4 and 8.5. Table 8.14 (Appendix 9) summaries the sources of variation for variables (a) to (f).

(a) Days to anthesis: Differences due to genotypes and environments were highly significant ($P<0.001$). The earliest genotype flowered at 80 days (G15) with G11 being the latest to flower (91 days) (Table 8.4). Environment E9 recorded the earliest flowering (71 days) while E5 was late at 99 days (Table 8.5).

(b) LRCI: Leaf rust was observed at 10 of the 12 sites with disease pressure being highest at E4 (16.8%) while there was no disease at E8 and E12, respectively (Table 8.5). Among the genotypes (Table 8.4), LRCI values ranged from 0.3 (G2) to 47.8 (G15). There were four genotypes namely G3, G9, G15 and G16 with LRCI values that were ≥ 19.

(c) Test weight (kg/hl): The minimum acceptable test weight in Zimbabwe is 75 kg/hl. Test weight was significantly ($P<0.05$) affected by environment with E12 recording the lowest test weight (75 kg/hl) and E4 the highest test weight (83 kg/hl) (Table 8.5). All 16 genotypes had test weights that were above the minimum required value (Table 8.4).

(d) Grain protein content (%) at 12.5% mb: Effects of genotypes and environments were highly significant ($P<0.001$) for grain protein content. All 16 genotypes had average grain protein content that was above the minimum accepted for premium wheat in Zimbabwe of ≥ 11% (Table 8.4). Highest values were obtained for G5, G11 and G15 with grain protein content values that were ≥ 13%. As for the environments, E3 and E10 had recorded values that were below 11% (Table 8.5).

(e) Starch content (%): A trait that is normally not considered in Zimbabwe during wheat trade. Genotypes (Table 8.4) ranged from 67.7% (G5) to 75.8% (G10) while environments (Table 8.5) ranged from 67% (E12) to 74.8% (E4).

(f) Wet gluten content (%): A wet gluten value of ≥ 25% is desirable in Zimbabwe. All genotypes were above this limit except for G12 that had a value of 24.7% (Table 8.4).

Some of the best genotypes were G11 (29.4%), G9 (29.2), G5 (29), G15 (28.6) and G10 (28.6). Only two environments, E3 and E10 had mean wet gluten content values that were below 25% (Table 8.5).

Table 8.4: Combined results for days to anthesis, leaf rust coefficient of infection, test weight, grain protein content, starch content and wet gluten for 16 wheat genotypes grown across all locations in Zimbabwe during the 2014 and 2015 seasons

Genotype	Anthesis (Days)	LRCI	Test Weight (kg/hl)	Grain Protein (%)	Starch content (%)	Wet Gluten (%)
G1	87.0	1.1	78.4	12.2	68.3	26.6
G2	88.1	0.3	78.4	12.3	69.4	27.0
G3	84.0	20.8	80.3	12.1	68.9	26.3
G4	86.3	0.7	78.5	12.6	68.7	27.7
G5	84.3	0.5	77.4	13.2	67.7	29.0
G6	86.9	0.4	77.1	11.9	68.8	25.7
G7	87.8	0.8	77.8	11.7	69.0	25.4
G8	85.5	4.8	77.6	11.5	69.5	25.2
G9	85.1	19.3	78.6	12.2	68.2	29.2
G10	87.5	0.8	78.8	12.1	75.8	28.6
G11	91.2	1.6	78.6	13.3	68.9	29.4
G12	89.9	1.0	77.8	11.4	68.8	24.7
G13	87.8	1.3	78.0	12.2	68.2	26.7
G14	88.0	1.4	83.9	11.9	69.1	25.7
G15	80.1	47.8	79.2	13.0	68.3	28.6
G16	82.4	41.0	78.5	12.2	69.2	26.5
Mean	86.4	9.0	78.7	12.2	69.2	27.0
Min	80.1	0.3	77.1	11.4	67.7	24.7
Max	91.2	47.8	83.9	13.3	75.8	29.4

G1 - 16=genotypes 1 - 16, LRCI=leaf rust coefficient of infection, Grain protein content at 12.5% moisture basis, Mean=combined average value for each trait over two seasons across 12 environments, Min=the lowest value recorded for each trait and Max=the highest value recorded for each trait.

Table 8.5: Environment acronyms, type of management, environment code and combined mean results on days to anthesis, leaf rust coefficient of infection, test weight, grain protein content, starch content and wet gluten recorded for 12 environments (2014 and 2015)

Environment	Management	Code	Anth	LRCI	TW	GP	Starch	WG
RARSN	Normal	E1	91.0	13.7	79.0	12.5	68.9	27.2
ARTN	Normal	E2	94.1	11.5	80.8	12.0	69.0	26.1
SISALN	Normal	E3	77.7	10.8	78.1	10.7	69.4	22.8
PANN	Normal	E4	72.8	16.8	82.6	11.2	74.8	27.7
SRCN	Normal	E5	98.7	11.2	78.5	12.3	68.6	26.7
CHZN	Normal	E6	85.2	8.6	77.0	11.3	69.6	25.0
ARTD	Deficit	E7	93.2	5.5	81.4	11.8	68.9	25.6
KRCN	Normal	E8	80.4	0.0	76.5	13.8	67.6	30.2
SAVEN	Normal	E9	70.6	10.2	77.8	14.7	67.3	32.9
RARSL	Late	E10	94.0	14.5	77.9	10.6	69.8	22.8
ARTL	Late	E11	88.5	5.1	79.4	11.6	69.1	25.2
SRCL	Late	E12	90.3	0.0	75.2	14.3	67.0	32.0
		Mean	86.4	9.0	78.7	12.2	69.2	27.0
		Min	70.6	0.0	75.2	10.6	67.0	22.8
		Max	98.7	16.8	82.6	14.7	74.8	32.9

RARSN=Rattray Arnold Research Station under normal planting (N), ARTN=Agricultural Research Trust farm under normal (N) planting, SISALN=Sisal farm under normal (N) planting), PANN=Panmure Experiment Station under normal planting (N), SRCN=Stapleford Research Centre under normal planting (N), CHZN=Chiredzi Research Station under normal planting (N), ARTD=Agricultural Research Trust farm under normal planting with deficit irrigation management (D), KRCN=Kadoma Research Centre under normal planting (N), SAVEN=Save Valley Experiment Station under normal planting (N), RARSL=Rattray Arnold Research Station under late planting (L), ARTL=Agricultural Research Trust farm under late planting (L), SRCL=Stapleford Research Centre under late planting (L). Normal= all plantings done before 1st of June. Late=all plantings done from 1st of June. E=environment code, Anth=days to anthesis, LRCI=leaf rust coefficient of infection (%), TW=test weight (kg/hl), GP=grain protein content (%) at 12.5% moisture basis, Starch=starch content (%), WG=wet gluten content (%).

8.5.2. Grain yield

8.5.2.1. AMMI ANOVA results for grain yield

ANOVA Table 8.6 showed that treatments were highly significant ($P<0.001$) accounting for 65.95% of the total sum of squares for yield. Environments had a significant effect ($P<0.001$) on treatment effects accounting for 53.03%, followed by variability among genotypes with 7.63% and interactions with 5.3% (Table 8.6). Environments explained 80.4% of the treatment sum of squares for grain yield followed by genotypes with 11.56% and lastly GEI with 8.03%. This indicated that environments accounted for the greater part of the model followed by genotypes then GEI.

Principal component analysis is used to identify patterns within the data, indicating similarities and dissimilarities among variables utilizing ordination techniques of multivariate methods (Bose *et al*., 2013). The first three Interaction Principle Component Axes (IPCA1, IPCA2 and IPCA3) were significant at $P<0.001$, 0.01 and 0.05, respectively. The three accounted for 73.21% of the total interaction effects in the model in the order IPCA1 (36%), IPCA2 (20.65%) and IPCA3 (16.56%). The three IPCAs accounted for 41.82% of the interaction total degrees of freedom.

Table 8.6: Full AMMI ANOVA table for grain yield showing how sum of squares and mean squares were partitioned to different sources of variation for 16 wheat varieties grown across 12 Zimbabwean environments during 2014 and 2015 seasons

				% Total explained		
Source	df	SS	MS	Total SS	Trt SS	Inter SS
Total	1535	5099271692	3322001			
Treatments	191	3362934725	17606988***	65.95		
Genotypes	15	388914547	25927636***	7.63	11.56	
Environments	11	2703892295	245808390***	53.03	80.40	
Block*	36	110608255	3072452***			
Interactions (G*E)	165	270127883	1637139**	5.30	8.03	
IPCA1	25	97234971	3889399***			36.00
IPCA2	23	55791068	2425699**			20.65
IPCA3	21	44743497	2130643*			16.56
IPCA4	19	28101304	1479016			10.40
IPCA5	17	17765577	1045034			6.58
IPCA6	15	10458609	697241			3.87
IPCA7	13	6128168	471398			2.27
IPCA8	11	5846430	531494			2.16
IPCA9	9	2776941	308549			1.03
Residuals	12	1281319	106777	0.03		
Error	1308	1625728712	1242912			

*, **, *** indicates that the term is significant at P<0.05, P<0.01 and P<0.001; IPCA 1 – 9 interaction principle component axes 1 to 9; df = degrees of freedom; SS = sum of squares; MS = mean square; Trt = treatments; Inter = interactions. *: the block source of variation refers to blocks within environments.

8.5.2.2. AMMI model signal to noise ratio

The model showed high levels of noise (76%) that were three fold compared to the signal level (24%) (Table 8.7). The best AMMI model is one that will be in a ratio of 1:1 with sum of squares for noise, 205080480. The relative contribution of each AMMI model to the total sum of squares for interactions given in Table 8.8 were used to calculate the signal to noise ratio.

Results showed that the sum of squares for noise lie between AMMI1 (172892912) and AMMI2 (214336815) but closer to AMMI2, making AMMI2 the best suitable model. IPCA1 was used in this study to plot three biplots against genotype means, environment means and combined genotype and environment means (Figures 8.3, 8.4 and 8.5) because it was significant at P<0.001 and it captured more signal (36%) compared to IPCA2 (20.65%) (Table 8.6) as recommended by Gauch (1992). Piepho (1997) acknowledged that total multiplicative models used for predictions must be lower than the total that shows significance with F tests. IPCA2 was used to plot the same type of graphs (Appendices 10, 11 and 12).

Table 8.7: Partitioned level of noise and signal in grain yield total sum of squares

Source	% level	Sum of squares
Noise	75.9198	205080480
Signal	24.0802	65047403
Total	100	270127883

Table 8.8: Contributions of each AMMI model to residual sum of squares and their calculated signal to noise ratios

AMMI model	RSS	NSS	NSS/RSS	SSS	SSS/RSS
AMMI1	172892912	205080480	1.19	65047403	0.38
AMMI2	214336815	205080480	0.96	65047403	0.30
AMMI3	225384386	205080480	0.91	65047403	0.29
AMMI4	242026579	205080480	0.85	65047403	0.27
AMMI5	252362306	205080480	0.81	65047403	0.26
AMMI6	259669274	205080480	0.79	65047403	0.25
AMMI7	263999715	205080480	0.78	65047403	0.25
AMMI8	264281453	205080480	0.78	65047403	0.25
AMMI9	267350942	205080480	0.77	65047403	0.24

AMMI 1 to 9 = additive main effects and multiplicative interaction 1 to 9; RSS =residual sum of squares; NSS = noise sum of squares; SSS = signal sum of squares.

8.5.2.3. MET genotype and environment grain yield rankings according to AMMI model

Genotypes showed alternating rankings from one environment to another as shown in Table 8.9. Across all 12 environments, the highest ranked genotype was G12 with mean yield of 7602 kg/ha while G11 was the lowest ranked with 5834 kg/ha. The top five ranked genotypes were G12, G2, G8, G7 and G6. Mean yields for the best genotype at each environment ranged from 4794 kg/ha (G10) at E6 to 9813 kg/ha (G12) at E2. The best yielding environment was E5 with mean yield of 8791 kg/ha while E6 was the lowest with 4302 kg/ha (Table 8.9). The top five yielding environments were E5, E2, E1, E8 and E3. Environments E1, E2 and E5 are located at ≥ 1200 masl (high potential sites) while E3 and E8 are between 800 – 1200 masl (medium potential sites).

Study results could also be classified into three groups depending on time of planting and irrigation management. At the same location, normal (N) referred to a trial that was planted before first of June, late (L) referred to trials planted after 31st of May while deficit (D) irrigation was practiced at ART farm (E7). Mean grain yield for late planted environments E10, E11 and E12 were lower than the mean for normal planted environments E1, E2 and E5. The average yield reductions due to late planting were 11.7% at RARS (E1 and E10), 22.8% at ART farm (E2 and E11) and 21% at SRC (E5 and E12). Mean yield loss among all genotypes due to late planting was 18.7% (Table 8.10). Genotype G15 had the lowest yield loss (8.6%) while the highest losses were for genotype G14 (21.9%).

Deficit irrigation at ART farm was practiced during the vegetative growth stage of the crop between tillering and booting stages (water stress can be tolerated). MacRobert (1993) reported that deficit irrigation must maximize returns per unit of irrigation water and unit wheat area grown. The aim in this case was to save on water and electricity costs by reducing irrigation cycles during this period but at the same time without affecting crop growth. Only G11 recorded a yield increase of 11.5% under deficit compared to normal irrigation practice while all other genotypes recorded yield losses. Overall, a 10.7% yield loss was recorded due to deficit irrigation (Table 8.10).

8.5.2.4. AMMI model top four genotype rankings per environment

A total of 12 genotypes appeared at least once in the top four rankings of at least one environment (Table 8.11). Genotypes G7 (E1, E4 and E8) and G12 (E2, E9 and E11) were ranked first at three environments each and G8 was best at two environments (E7 and E12). Under normal planting, G10 appeared six times among the top four, G4 appeared four times under late planted environments and G2, G4, G5 and G8 were the top four under deficit irrigation management (Figure 8.2).

Table 8.9: Mean grain yield (kg/ha), IPCA1 score, genotype rank and mean yield of 16 genotypes (G1 - G16) grown across 12 Zimbabwean environments (E1 - E12) during 2014 and 2015 seasons

				RARSN	ARTN	SISALN	PANN	SRCN	CHZN	ARTD	KRCN	SAVEN	RARSL	ARTL	SRCL
Code	Mean	IPCA1	Rank	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
G1	7417	-0.84	9	7881	8961	**8599**	7694	9211	4481	8216	8236	5043	6983	7084	6611
G2	7594	-6.95	2	8660	9447	7973	7751	8882	4379	8520	8479	5284	**7616**	7040	7103
G3	6989	-2.28	13	7702	8003	8054	7200	8558	4121	7310	8025	4684	6890	7123	6202
G4	7485	-11.98	7	8294	9028	8077	7525	8801	4332	8249	8751	4781	7281	7461	7245
G5	7398	-13.97	10	7730	9712	7500	7216	**9374**	4485	8344	8160	5094	7127	7262	6773
G6	7519	-4.80	5	8484	9277	7776	7915	9370	4725	7806	8763	4823	7237	6784	7269
G7	7549	3.53	4	**8785**	8486	7941	**8569**	8912	4593	7752	**8860**	5021	7584	7054	7034
G8	7563	13.68	3	7998	9392	8502	8518	9113	4608	**8726**	7402	4869	6931	6665	**8038**
G9	7012	3.65	12	7739	8386	7766	7213	8806	4071	8242	7544	4669	6574	6508	6628
G10	7491	9.47	6	8546	8748	8130	7973	9354	**4794**	8093	7950	5066	7106	6770	7364
G11	5834	45.85	16	6589	5786	6856	6824	7871	3595	6451	4900	4441	6119	4649	5922
G12	7602	-4.44	1	8613	**9813**	8368	7951	8828	4567	8111	7377	**5331**	7234	**7974**	7057
G13	7281	-11.37	11	8217	9083	7782	7149	8867	4307	7823	8368	4742	7076	7051	6906
G14	7425	-2.04	8	8535	9495	7823	8345	8855	4429	7717	8144	4753	7077	6880	7049
G15	6195	-22.16	15	6533	8180	6664	4849	7306	3210	6508	6910	4053	6925	6548	6651
G16	6866	4.66	14	7931	8120	7656	6334	8541	4144	7132	7961	4753	7446	5178	7198
Mean	7201			8015	8745	7842	7439	8791	4302	7812	7864	4838	7075	6752	6941
Min	5834			6533	5786	6664	4849	7306	3210	6451	4900	4053	6119	4649	5922
Max	7602			8785	9813	8599	8569	9374	4794	8726	8860	5331	7616	7974	8038

RARSN = Rattray Arnold Research Station under normal, ARTN = Agricultural Research Trust farm normal, SISALN=Sisal farm normal, PANN = Panmure Experiment Station normal, SRCN = Stapleford Research Centre normal, CHZN = Chiredzi Research Station normal, ARTD = Agricultural Research Trust farm deficit irrigation management, KRCN = Kadoma Research Centre normal, SAVEN = Save Valley Experiment Station normal, RARSL=RARS late, ARTL=ART late, SRCL=SRC late, Normal= planting done before 1st of June. Late=planting done from 1st of June. IPCA1=Interactive principal component axes 1. Best yield values/environment (bold), indicating cross-over GEI.

Table 8.10: Effects of planting time and irrigation management on grain yield (kg/ha) of 16 wheat genotypes grown in Zimbabwe during 2014 and 2015 seasons

	RARSN	ARTN	SRCN	MeanN	ARTD	RARSL	ARTL	SRCL	MeanL	% yield loss due to:	
G	E1	E2	E5		E7	E10	E11	E12		Planting time	Irrigation management
G1	7881	8961	9211	8684	8216	6983	7084	6611	6893	20.6	8.3
G2	8660	9447	8882	8996	8520	7616	7040	7103	7253	19.4	9.8
G3	7702	8003	8558	8088	7310	6890	7123	6202	6738	16.7	8.7
G4	8294	9028	8801	8708	8249	7281	7461	7245	7329	15.8	8.6
G5	7730	9712	9374	8939	8344	7127	7262	6773	7054	21.1	14.1
G6	8484	9277	9370	9044	7806	7237	6784	7269	7097	21.5	15.9
G7	8785	8486	8912	8728	7752	7584	7054	7034	7224	17.2	8.6
G8	7998	9392	9113	8834	8726	6931	6665	8038	7211	18.4	7.1
G9	7739	8386	8806	8310	8242	6574	6508	6628	6570	20.9	1.7
G10	8546	8748	9354	8883	8093	7106	6770	7364	7080	20.3	7.5
G11	6589	5786	7871	6749	6451	6119	4649	5922	5563	17.6	-11.5
G12	8613	9813	8828	9085	8111	7234	7974	7057	7422	18.3	17.3
G13	8217	9083	8867	8722	7823	7076	7051	6906	7011	19.6	13.9
G14	8535	9495	8855	8962	7717	7077	6880	7049	7002	21.9	18.7
G15	6533	8180	7306	7340	6508	6925	6548	6651	6708	8.6	20.4
G16	7931	8120	8541	8197	7132	7446	5178	7198	6607	19.4	12.2
Mean	8015	8745	8791	8517	7812	7075	6752	6941	6923	18.7	10.7

G1 to G16=Genotype 1 to 16, E1,2,5,7,10,11, and 12=Environments 1, 2, 5, 7, 10, 11 and 12, RARSN=normal planted trial at Rattray Arnold Research Station, ARTN=normal planted trial at Agricultural Research Station farm, SRCN=normal planted trial at Stapleford Research Centre, MeanN=Mean of the three (E1, E2 and E5) normal planted trials, ARTD=deficit irrigation trial at Agricultural Research Trust farm, RARSL=late planted trial at Rattray Arnold Research Station, ARTL=late planted trial at Agricultural Research Trust farm, SRCL=late planted trial at Stapleford Research Centre, MeanL=mean of late planted trials (E10,E11 and E12). % yield loss due to planting time = ((MeanN-MeanL)/MeanN)*100, % yield loss due to irrigation management = ((E2 – E7)/E7)*100.

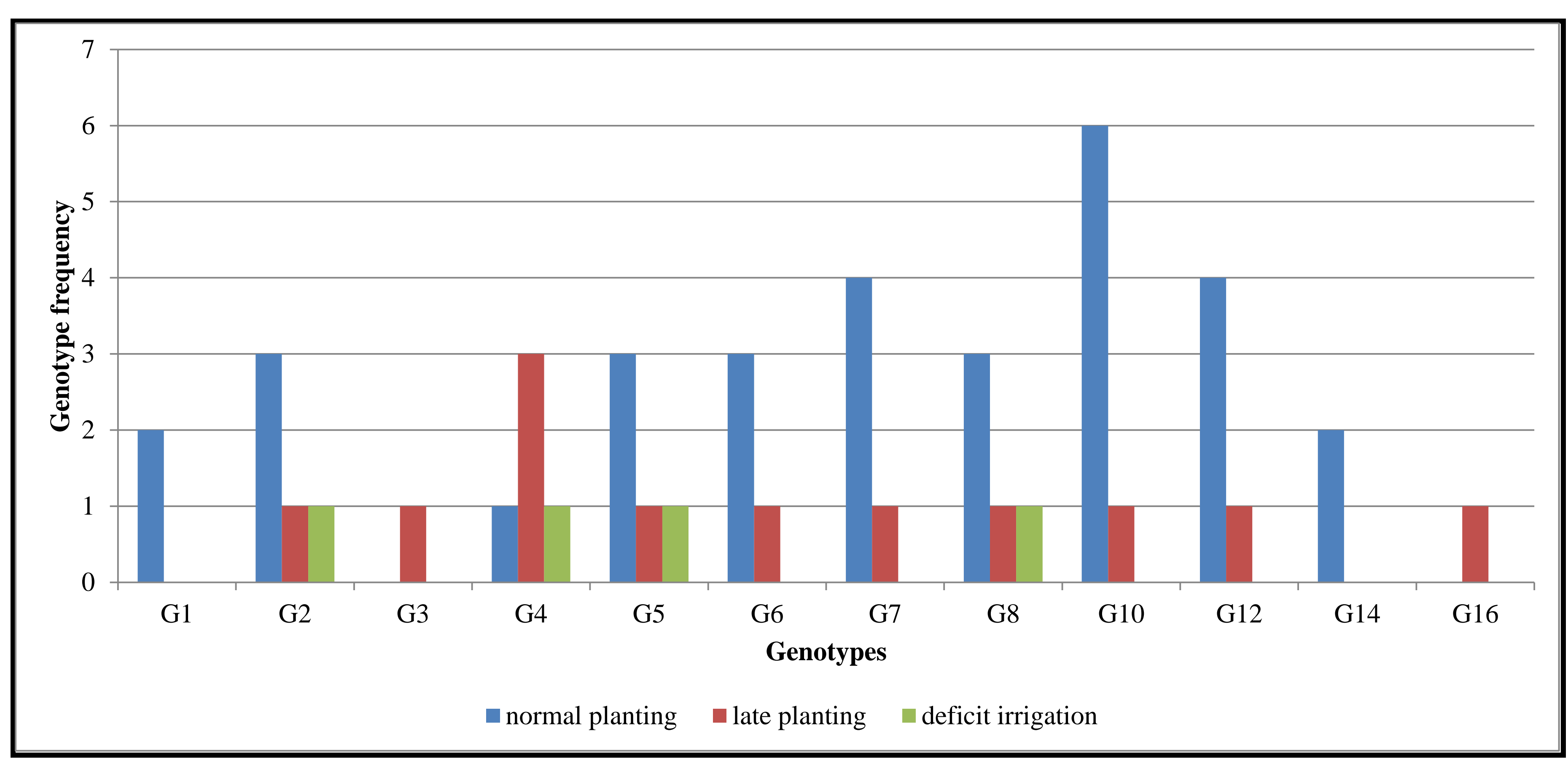

Figure 8.2: Genotype performances under normal planting, late planting and deficit irrigation.

Genotype frequencies by AMMI model top four rankings per environment according to time of planting and irrigation management practice.

Table 8.11: Environments, mean yield (kg/ha), IPCA1 score, environment rank (based on mean yield) and top four recommendations per environment according to AMMI model

Environment information						**Top 4/environment**			
Abbrev.	M/ment	Code	Yield	IPCA1 Score	Rank	1	2	3	4
RARSN	Normal	E1	8015	2.65	3	G7	G2	G12	G10
ARTN	Normal	E2	8745	-30.23	2	G12	G5	G14	G2
SISALN	Normal	E3	7842	10.76	5	G1	G8	G12	G10
PANN	Normal	E4	7439	26.14	7	G7	G8	G14	G10
SRCN	Normal	E5	8791	9.79	1	G5	G6	G10	G1
CHZN	Normal	E6	4302	11.56	12	G10	G6	G8	G7
ARTD	Deficit	E7	7812	2.53	6	G8	G2	G5	G4
KRCN	Normal	E8	7864	-28.29	4	G7	G6	G4	G2
SAVEN	Normal	E9	4838	11.96	11	G12	G2	G5	G10
RARSL	Late	E10	7075	-0.42	8	G2	G7	G16	G4
ARTL	Late	E11	6752	-23.26	10	G12	G4	G5	G3
SRCL	Late	E12	6941	6.80	9	G8	G10	G6	G4

RARSN = Rattray Arnold Research Station under normal, ARTN = Agricultural Research Trust farm normal, SISALN=Sisal farm normal, PANN = Panmure Experiment Station normal, SRCN = Stapleford Research Centre normal, CHZN = Chiredzi Research Station normal, ARTD = Agricultural Research Trust farm deficit irrigation management, KRCN = Kadoma Research Centre normal, SAVEN = Save Valley Experiment Station normal, RARSL=RARS late, ARTL=ART late, SRCL=SRC late, Normal= planting done before 1st of June. Late=planting done from 1st of June. M/ment=management practice where Normal include all plantings done before 1st June, Late cover all plantings done after 31st May and Deficit irrigation at ART, Yield=site mean yield in kg/ha, IPCA1=Interactive principal component axes 1. E = environment code, G = genotype code.

8.5.2.5. Grain yield AMMI biplots

Both genotypes and environments had both negative and positive IPCA1 values (Tables 8.9 and 8.11, respectively), indicating a cross over GEI due to disproportionate genotype responses. This means genotypes with a positive IPCA value in one environment will have a negative value in the other environment. A total of six genotypes (G7, G8, G9, G10, G11 and

G16) had positive IPCA1 values that ranged from 3.53 (G7) to 45.85 (G11). These had a positive interaction with environments. The rest of the genotypes had negative interactions with the environments with IPCA values ranging from -22.16 (G15) to -0.84 (G1). Genotypes G7, G8 and G10 were high yielding but relatively stable (Figure 8.3). Genotypes G16, G9 and G11 were low yielding and G9 and G16 were relatively stable. Low yielding and most stable genotype was G15 while G5 was the least stable among the high yielding genotypes. G1 was the best genotype because it combined high yield and was the most stable genotype.

Among environments, eight had positive IPCAs that ranged from 2.65 (E1) to 26.14 (E4). Environments, E1, E3, E4, E5 and E7 were above average while E6 and E9 were below average. Environments with negative IPCAs were E2 (-30.23), E8 (-28.29), E11 (-23.26) and E10 (-0.42). Among these E11 was low yielding and very unstable while E2 was high yielding and unstable (Figure 8.4). The best environment was E10 because it is equal to the mean of all environments and it was very stable since it is found at the origin. When plotted on the same graph with genotypes and environment on the abscissa and IPCA1 scores as ordinates, the best environment and genotype in terms of average yield and excellent stability were E10 and G1, respectively (Figure 8.5).

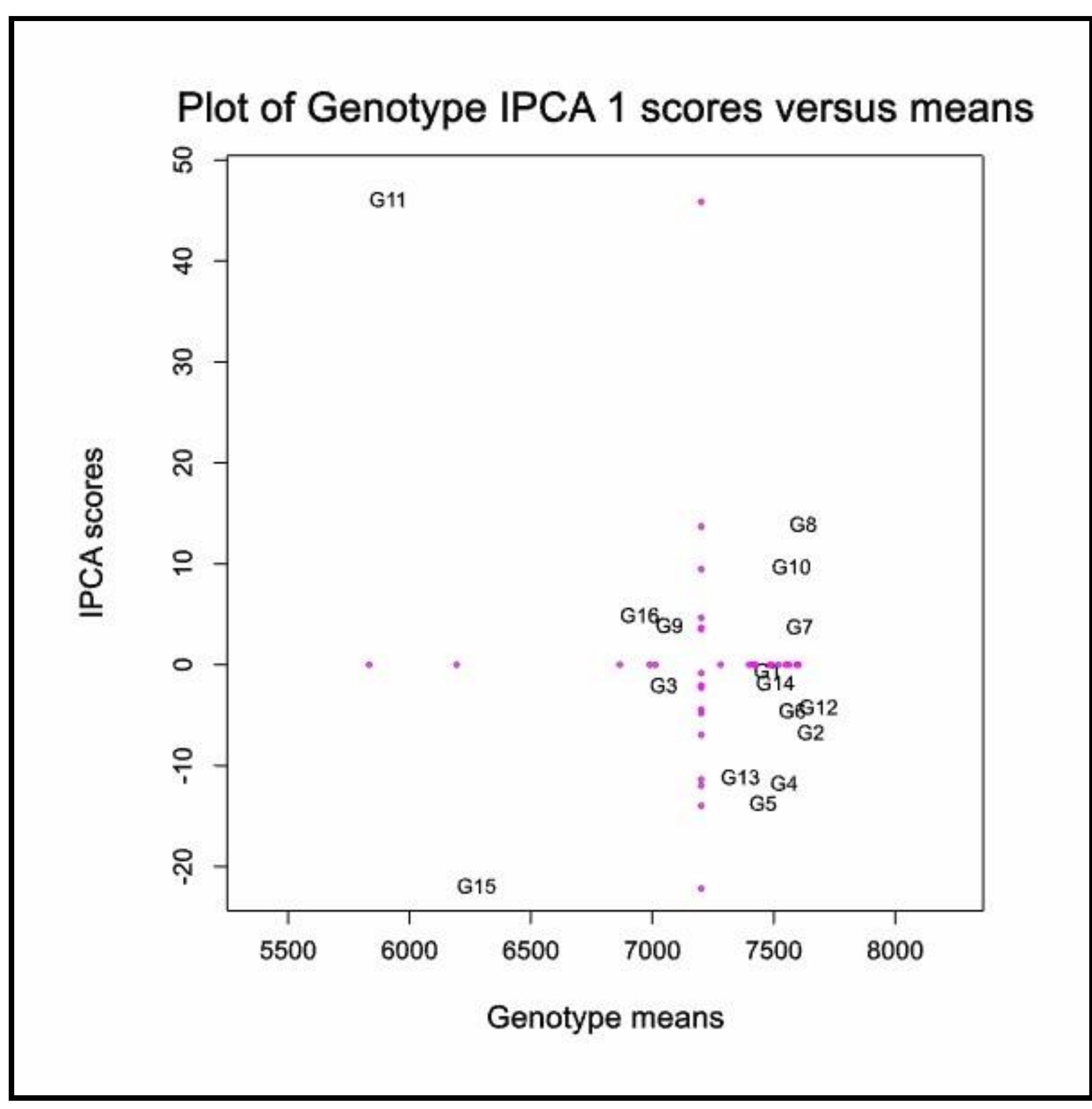

Figure 8.3: AMMI biplot with genotype means plotted against IPCA1 scores.

AMMI biplot for multiple environment trial of 16 wheat genotypes grown across 12 environments in Zimbabwe in 2014 and 2015 seasons. Genotype means (kg/ha) are shown on the abscissa and IPCA1 on the ordinate. G1 to G16 represents genotype codes.

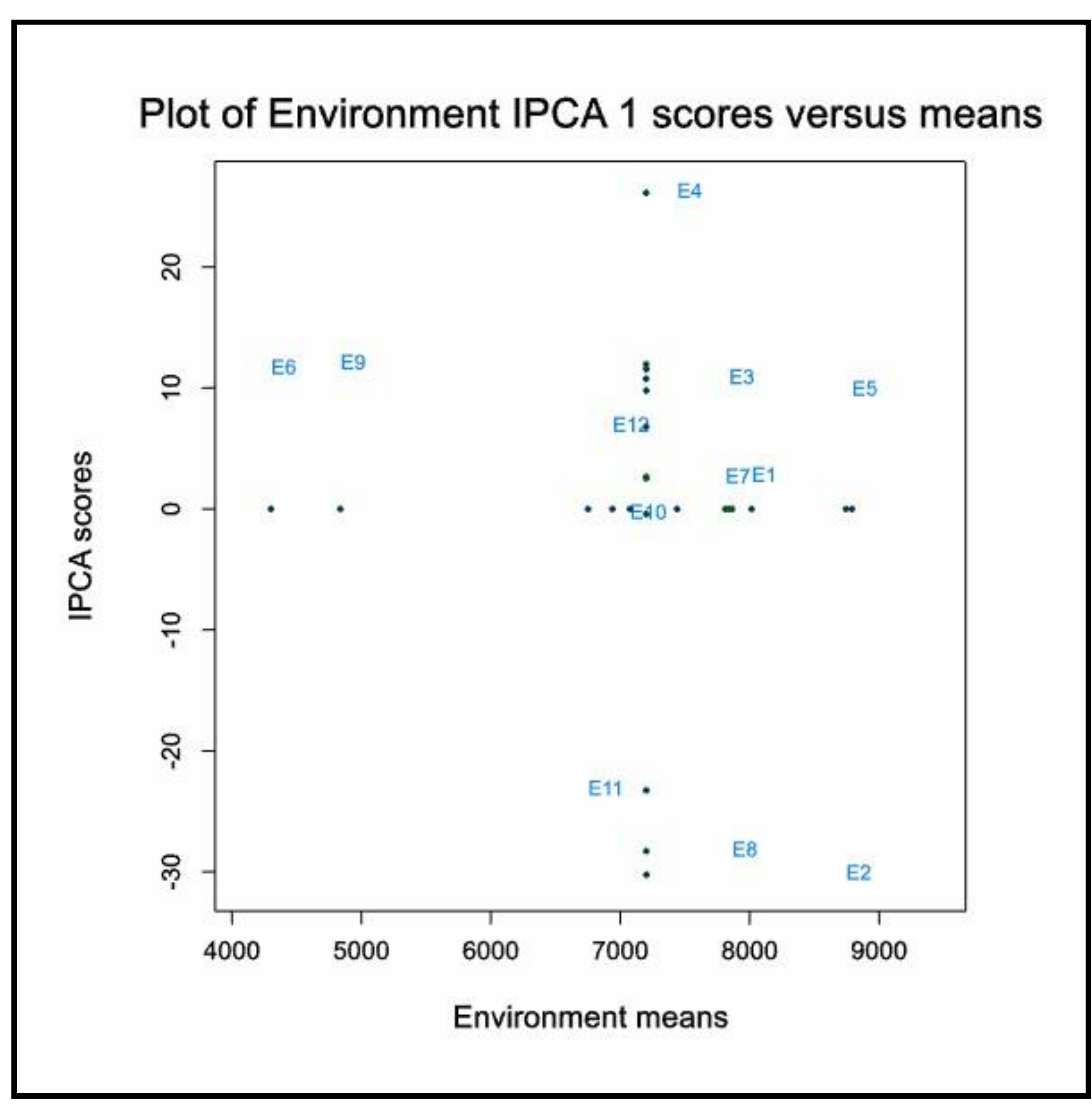

Figure 8.4: AMMI biplot with environment means plotted against IPCA1 scores.

AMMI biplot of multiple environment trial of 16 wheat genotypes grown across 12 environments in Zimbabwe during 2014 and 2015 seasons. Environment means (kg/ha) on the abscissa and IPCA1 on ordinates. E1 to E12 represents environment code where E1=RARSN, E2=ARTN, E3=SISALN, E4=PANN, E5=SRCN, E6=CHZN, E7=ARTD, E8=KRCN, E9=SAVEN, E10=RARSL, E11=ARTL and E12=SRCL.

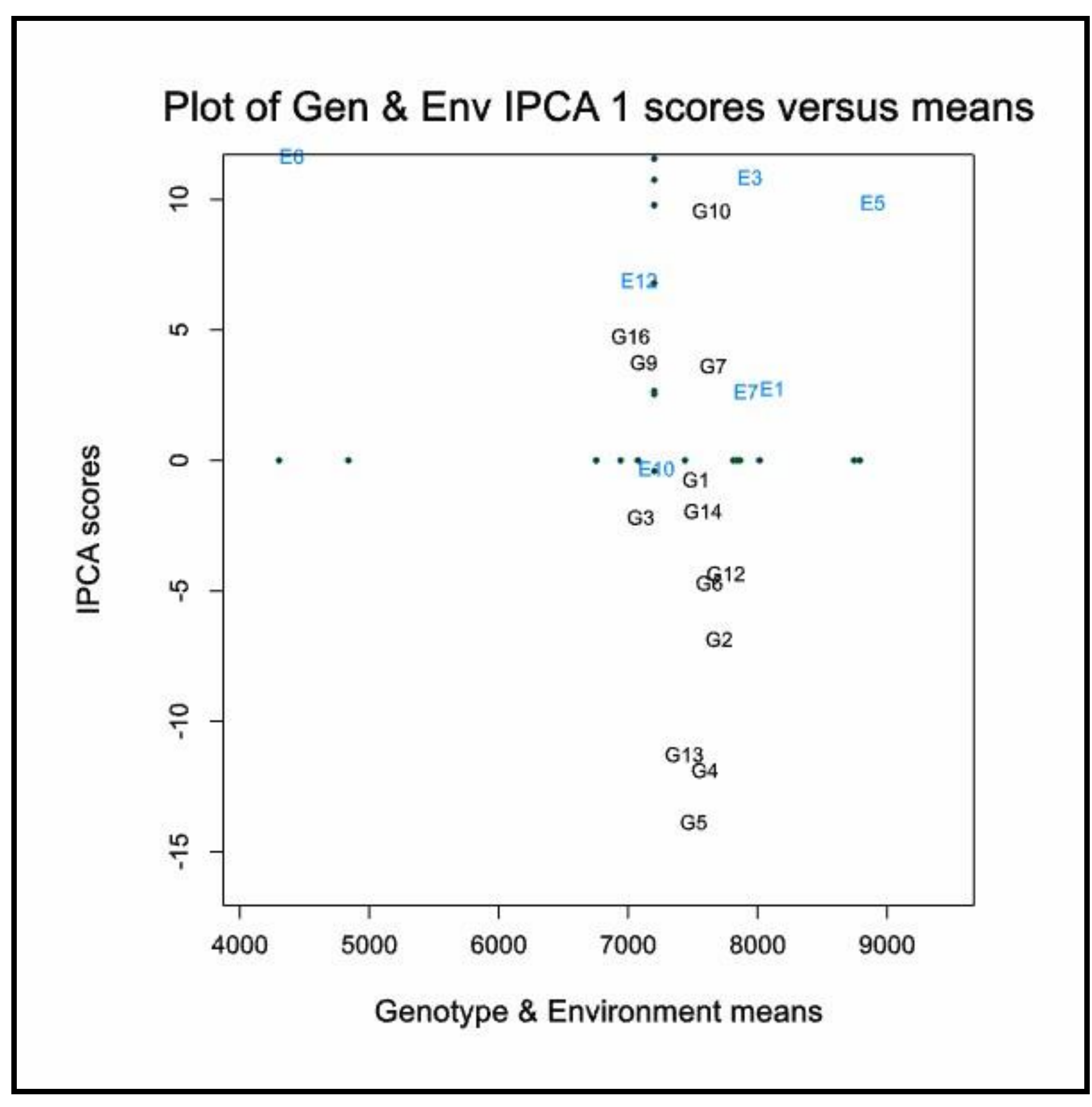

Figure 8.5: AMMI biplot when genotype and environment means are plotted against IPCA1 scores.

AMMI biplot of multiple environment trial of 16 wheat genotypes grown across 12 environments in Zimbabwe during 2014 and 2015 seasons. Combined genotype and environment means are shown on abscissa (kg/ha) and IPCA1 on ordinate. G1 to G16 represents genotypes. E1 to E12 represents environment code where E1=RARSN, E2=ARTN, E3=SISALN, E4=PANN, E5=SRCN, E6=CHZN, E7=ARTD, E8=KRCN, E9=SAVEN, E10=RARSL, E11=ARTL and E12=SRCL.

8.5.3. GGE Biplots

The GGE biplots were used to analyse mega-environments among the test environments, to evaluate the test environments and to evaluate the genotypes. A scatter plot with PC1 on the abscissa and PC2 on the ordinates was used analyse mega-environments. The scatter plot (Figure 8.6) accounted for 80.69% of the GEI effects with PC1 contributing 67.19% while PC2 accounted for 13.49%.

8.5.3.1. Mega-environment analysis (Which-won-where)

Performance of genotypes was represented by a heptagon with most responsive genotypes in each of the seven sectors being at the vertex. These genotypes are the most responsive and they can either be the best or the poorest at one or all of the locations (Yan and Rajcan, 2002). Genotype G4 was the winner in sector 1, G2 in sector 2, G12 in sector 3 and G8 in sector 4 (Figure 8.6). The presence of this cross-over GE indicates that the test environments could be delineated into mega-environments (ME) (Yan *et al.*, 2007). The scatter plot produced seven sectors but all 12 test environments were distributed into four sectors. GGE biplots demarcated environments into three major MEs namely, ME1 with E2 and E8, ME2 with E10 and E11, and ME3 with the rest of the test environments (E1, E3, E4, E5, E6, E7, E9 and E12). Test environment E10 has some characteristics that are similar to those in ME3 while E12 is a unique environment within ME3, with some characteristics that are not shown by other environments within the same ME3. E4 is also a unique environment within ME3 being the furthest among environments in ME3 (Figure 8.6).

8.5.3.2. Comparison of genotypes to the "ideal genotype"

A GGE comparison biplot was used to rank and compare performance of genotypes in relation to the ideal genotype as represented by the average environment coordination view (AEC) (Figure 8.7). The ideal genotype was defined as a genotype with higher mean performance and stability across test environments within a mega-environment. According to Figure 8.7, the best ranked genotypes from the study were G12 followed by G2, G6, G7 and G14 while G11 was the lowest ranked genotype.

8.5.3.3. Comparison of test environments to the "ideal environment"

The comparison indicated that E1 was the closest test environment to the ideal as indicated by AEC view that shows the ideal environment. Environment E4 was furthest from the "ideal environment" among all the 12 test environments (Figure 8.8).

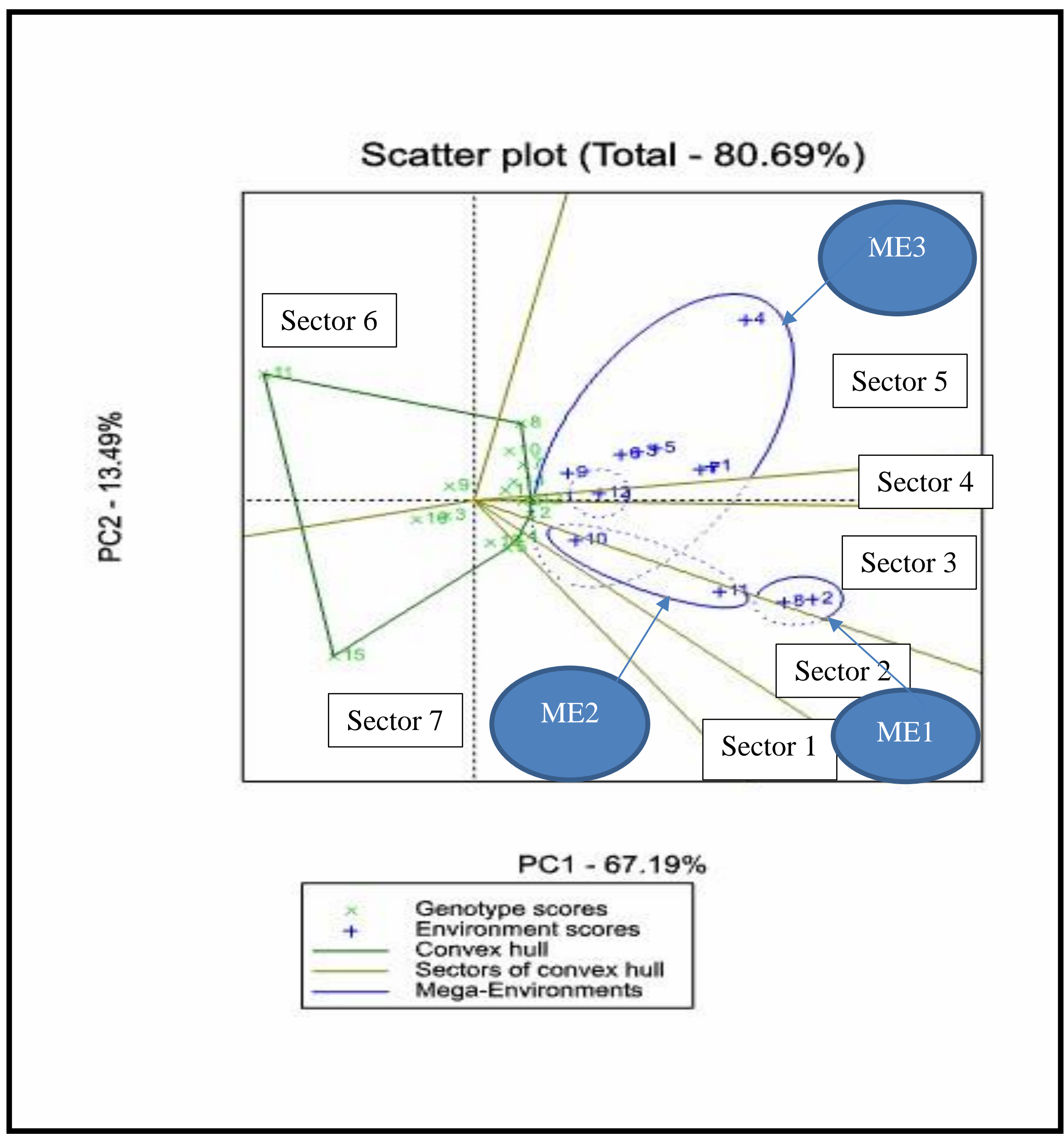

Figure 8.6: GGE scatter plot when PC1 is plotted against PC2.

GGE scatter plot (which-won-where) of multiple environment trials of 16 genotypes grown across 12 environments in Zimbabwe during the 2014 and 2015 seasons. PC1 accounts for 67.19% of the variability on the abscissa and PC2 (13.49%) on ordinates. GGE biplot scaling is based on environment means. Three Mega-environments and seven sectors are also shown.

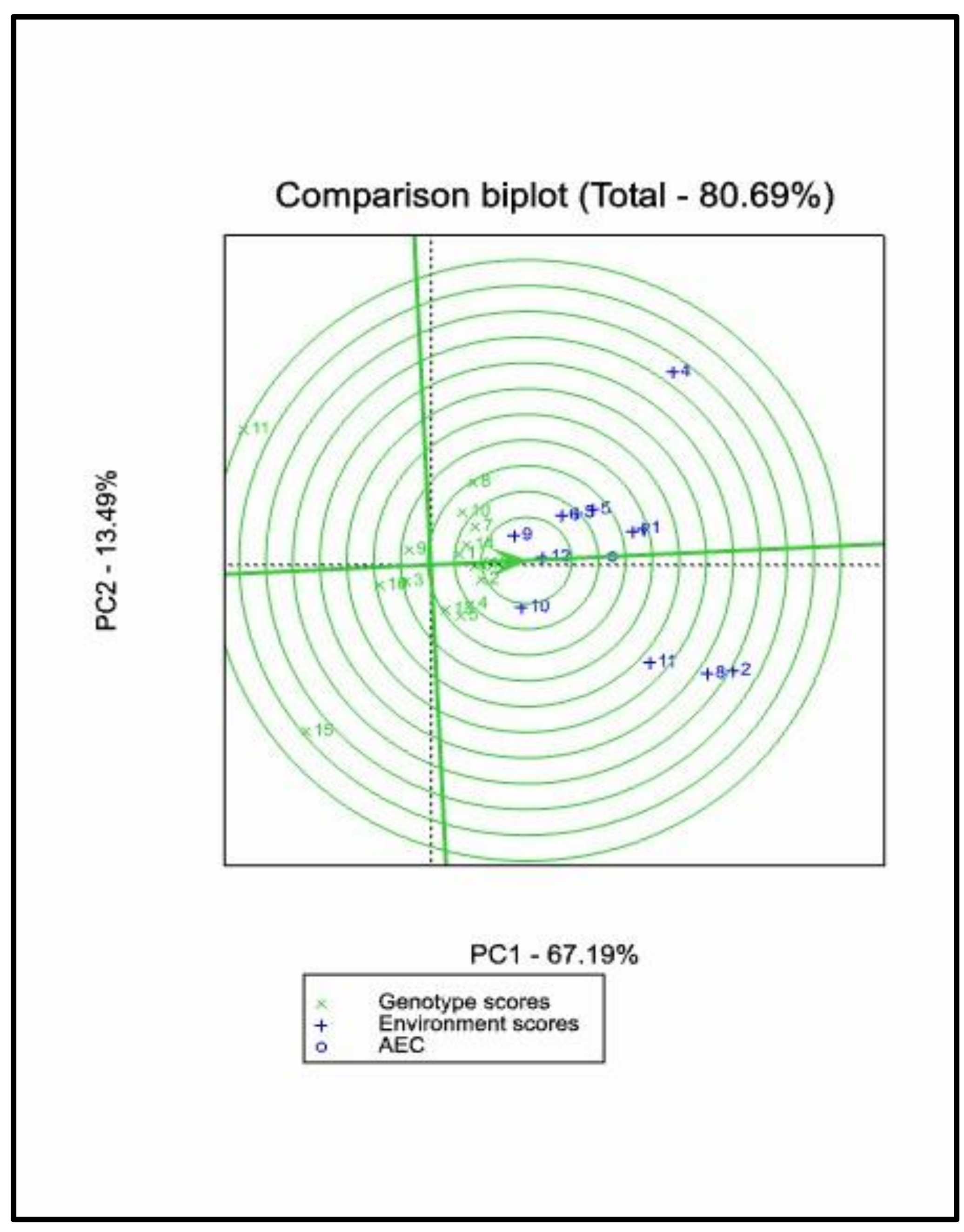

Figure 8.7: GGE genotype comparison biplot when PC1 is plotted against PC2.

GGE genotype comparison biplot when PC1 is plotted against PC2. GGE genotype comparison biplot for 16 wheat genotypes grown across 12 environments in Zimbabwe during 2014 and 2015 seasons. The biplot compares means and stability of 16 wheat genotypes based on average environment coordination (AEC).The plot accounts for 80.69% of variability with PC1 (67.19%) on abscissa and PC2 (13.49%) on ordinates. Scaling is based on environments.

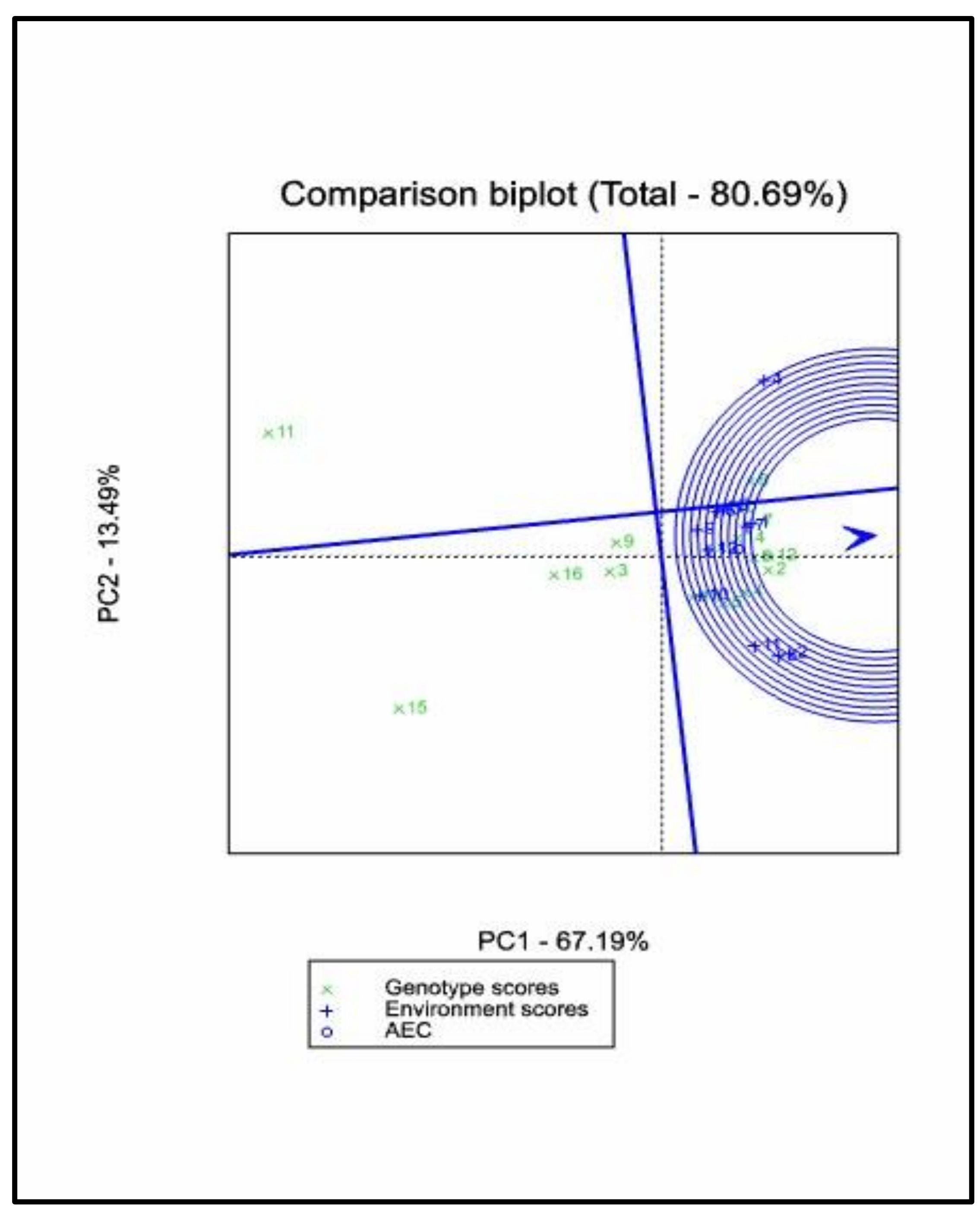

Figure 8.8: GGE environment comparison biplot when PC1 is plotted against PC2.

GGE environment comparison biplot for 12 environments used to test 16 wheat genotypes in Zimbabwe during 2014 and 2015 seasons. Environment means were ranked according to their mean yield performance and stability compared to average environment coordination (AEC). The plot accounts for 80.69% of variability with PC1 (67.19%) on abscissa and PC2 (13.49%) on ordinates. Scaling is based on genotypes.

8.5.4.1. Cultivar superiority and mean rank values

Based on cultivar superiority index, the top five best genotypes were G2, G4, G12, G6 and G10 (Table 8.12). These had a yield advantage that was between four to six percent over grand mean. Mean rank values have G2, G12, G7, G10 and G6 in top five stable genotypes.

Table 8.12: AMMI model stability analysis using cultivar superiority and mean rank values

Yield	Genotype	Mean yield	% of Mean	Cultivar Superiority (P_i)		Mean rank value	
Rank	Code	kg/ha	yield	Value	Rank	Value	Rank
1	G12	7602	105.6	188011	3	5.3	2
2	G2	7594	105.5	146451	1	5.1	1
3	G8	7563	105	227036	7	6	6
4	G7	7549	104.8	224483	6	5.5	3
5	G6	7519	104.4	200215	4	5.9	5
6	G10	7491	104	217217	5	5.6	4
7	G4	7485	103.9	182933	2	6.3	7
8	G14	7425	103.1	231225	8	7.5	10
9	G1	7417	103	264983	9	7.3	9
10	G5	7398	97.3	306058	10	7.2	8
11	G13	7281	101.1	327642	11	8.9	11
12	G9	7012	97.4	588452	12	12.1	14
13	G3	6989	97.1	648325	13	11.7	13
14	G16	6866	95.3	953945	14	11.6	12
15	G15	6195	86	1953994	15	14.8	15
16	G11	5834	81	2924287	16	15.4	16
	Mean	7201					

G=genotype code. The lower the values for both cultivar superiority (P_i) and mean rank the more stable is the genotype.

8.5.4.2. Environment stability analysis

Environments with the lowest variances estimated over the 16 genotypes grown in both years at all locations were considered to be more stable. The five most stable environments according

to variances were E9, E6, E10, E5 and E3. The most unstable environment was E8 (Table 8.13).

Table 8.13: AMMI model environment stability analysis based on variances

Environment	Code	Mean yield (kg/ha)	Variance
SAVEN	E9	4838	659303
CHZN	E6	4302	758356
RARSL	E10	7075	771720
SRCN	E5	8791	944003
SISALN	E3	7842	1005955
RARSN	E1	8015	1220269
ARTL	E11	6752	1494304
ARTD	E7	7812	1566836
ARTN	E2	8745	1862299
SRCL	E12	6941	2180223
PANN	E4	7439	2898606
KRCN	E8	7864	3499382
Mean		7201	3322001

RARSN = Rattray Arnold Research Station under normal, ARTN = Agricultural Research Trust farm normal, SISALN=Sisal farm normal, PANN = Panmure Experiment Station normal, SRCN = Stapleford Research Centre normal, CHZN = Chiredzi Research Station normal, ARTD = Agricultural Research Trust farm deficit irrigation management, KRCN = Kadoma Research Centre normal, SAVEN = Save Valley Experiment Station normal, RARSL=RARS late, ARTL=ART late, SRCL=SRC late, E = environment code.

8.6. Discussion

The influence of temperature on performance of wheat genotypes in Zimbabwe was significant. Environments at high altitude (≥ 1200 masl) had cooler conditions that resulted in high yields as compared to environments at low altitude confirming results from earlier studies (Havazvidi, 2008; Mtisi and Mashiringwani, 1988; Mugabe and Nyakatawa, 2000). SRC (E5) at 1466 masl had the highest environmental yield (8791 kg/ha) compared to the lowest yield (4302 kg/ha) at CHZ (E6) at 428 masl. Flowering was also influenced by temperature (altitude) and this

resulted in a short crop growing period in the low altitude environments. The length of the growing period has a positive influence on grain yield. Late planting results in a yield penalty because it shortens the growing period. Late planting also exposes the crop to high disease pressure because leaf rust tends to increase during the warmer months of August onwards. At that time the late planted crop will still be at the vegetative stage and disease can affect yield especially in susceptible genotypes. Late planting resulted in an average 18.7% yield reduction with G14 loosing 22% (Table 10).

Leaf rust is the major disease constraint to wheat production in Zimbabwe. Races SCDS (Pretorius and Purchase, 1990) TCPS, MCDS and FBPT were detected in Zimbabwe with TCPS being the most virulent affecting 72% of Zimbabwean commercial wheat varieties at seedling stage in the greenhouse (Pretorius *et al*., 2015). Recently, a fourth race CFPS (coded 3SA10) that is closely related to South African race CCPS was detected from a sample collected during 2016 winter season in Zimbabwe (unpublished). Genotypes G9 (SC1), G3 (Pan2), G16 (CBI6) and G15 (CBI5) were the most affected with LRCI scores of 19.3%, 20.8%, 41% and 47.8%, respectively. CBI genotypes G15 (CBI5), G16 (CBI6) and Seed-Co's G9 (SC1) have shown susceptibility to leaf rust race TCPS with seedling infection type of 33C, 4 and 4, respectively (Pretorius *et al*., 2015). Environments in Mashonaland East province namely RARSN (E1), RARSL (E10) and PANN (E4) recorded the highest LRCI scores of 13.7%, 14.5% and 16.8%, respectively and the district (Goromonzi) where TCPS was detected is within this province.

The significant ($P<0.01$) interactions of genotype x environment suggest that genotype performance varied from environment to environment. Highly significant differences ($P<0.001$) due to genotypes and environments, respectively, indicate the significance of genotype variability and environment differences in GEI. This gives an opportunity to select stable genotypes, evaluate environments and delineate environments into mega-environments. Environments contributed 53.03% of the total treatment effects (65.95%). This was due to high variability among the environments caused by differences in time of planting, non-uniformity of irrigation, application of chemicals, differences in leaf rust disease pressure, heterogeneous soils found in different environments and imprecision in field operations, as reported by Crossa *et al*. (1991). Total treatment effects (G, E, and GEI) of 65.95% are within the expected of ≥ 60 % (Gauch and Zobel, 1996).

The delineation of mega-environments was influenced by altitude and crop management practices implemented at various environments. ME1 was made up of environments that are both at high elevation (high potential) but that were late planted. ME2 consists of a medium elevation environment (KRCN) that usually has high yield on average and rarely experiences any disease pressure while E11 (ARTN) is a high elevation environment but there is usually disease pressure in this environment. ME3 is of interest because environments with rather diverse characteristics clustered into this single ME3. Environments with similar characteristics were found closer to one another. RARSN (E1) and ARTD (E7) normally have similar yield potential, both at high elevation and were geographically close to one another. Environments E6 and E9 are located at low elevation where conditions are very warm with a short growing period that routinely results in yields that are below six tonnes per hectare. These were close to one another within ME3. Environment E12 (SRCL) is unique as compared to other environments in ME3 and possesses characteristics that are not found in others. It is a high elevation site that was planted late but the yields were higher than some of the normal planted sites. It is an environment that can be considered of first priority if resources are limiting and few sites are required to represent ME3. PANN (E4) is also a unique site within ME3 given its distant position from the rest of the group. The AMMI biplot indicated E10 as the most stable environment followed by E7 and E1 while environment stability analysis variance values ranked environments in the order E9, E6 and E10, respectively. A comparison with the ideal environment as determined from Figure 8.6, E1 followed by E7 are the most ideal environments based on the gene pool used in the study.

Fentaw *et al*. (2015) defined an ideal genotype as one with high grain yield and less GEI (high stability). Comparison biplots, cultivar superiority index and mean rank value all indicate G2 as the most ideal and stable genotype with a six percent yield advantage over the grand mean. G11 was the lowest ranked genotype in the study. Genotype analysis results indicate an influence of germplasm source. Most of the genotypes evaluated are medium-late maturing except for CBI5 (G15) which is the earliest to mature. Genotype G11 had the lowest yield across all environments and was the latest genotype to flower. It is a Limagrain line, indicating a different gene pool from the Zimbabwean genotypes. G11 was unique compared to the rest of the genotypes. GGE scatter plot resulted in a heptagon, indicating G8 to be the best for ME3, G2 for ME2 and G4 for ME1. The study reveals that GEI was of a cross over type (Crossa, 1990) with interactions between genotypes and environments resulting in rank

changes, which demands subsets of genotypes to be recommended for certain environments or mega-environments.

An additional challenge that has to be addressed by breeders is to strike a balance between high yield, good agronomics, good quality and disease resistance based on resistance gene stewardship. A genotype that is recommended for commercial production must satisfy all the various stakeholders. A high yielding variety such as G12 is not always outstanding in terms of milling and baking quality. There is always a trade-off. Early maturity, as demanded by farmers because of the wheat cultivation window, has a penalty on yield since the growing period is very short. G15 combines early maturity and excellent qualities but fall short on yield. Therefore breeding programmes have to understand the needs of their targeted markets so as to ensure that the most appropriate products are released and adopted for commercial production by farmers.

8.7. Recommendations and conclusions

AMMI and GGE biplots were used to effectively in analyse data from MET trials conducted across Zimbabwe during the two seasons of study. Environment demarcation provides valuable information to the breeding programme and other stakeholders. The breeding programme can realign its breeding objectives given the need to develop genotypes that target a specific mega-environment rather than expecting one genotype to be grown across all the three mega-environments. The GGE scatter plot grouped environments into three mega-environments where G4 was the best genotype for ME1, G2 for ME2 and G8 for ME3. Environment analysis presents an opportunity to reduce the number of environments when several environments provide the same information. This is useful in case of resource constraints. E10 can represent both ME2 and ME3 while E12 is a good representative for ME3 because it has ME3 characteristics plus other traits that are not exhibited by the rest of the environments. The programme can choose to keep one of the two environments in ME1 (either E2 or E8) in case of resource constraints. E4 is an important environment given its uniqueness in ME3.

Genotype analysis was performed indicating the outstanding yield performance of G12 and identifying G2 as a high yielding and stable genotype. Specific genotype adaptation was also exhibited with G7 suitable for E1, E4 and E8; G12 for E2, E9 and E11; G1 for E3; G5 for E5; G10 for E6, G2 for E10 and G8 suitable for both E7 and E12. The analysis was able to show

that there are significant differences in genotype performance due to variability among genotypes (P<0.001), environments and GEI, resulting in cross-over GEI. Experimental genotypes such as G2 (SC40), G4 (SC41), G8 (SC18) and G1 (SC35) can be evaluated further and used as parents in the breeding programme.

The effect of late planting on wheat yield is a key point that needs consideration when one ventures into wheat production. This empowers extension staff to encourage farmers to plant early so as to realise high yields. There is a tendency by farmers to plant late due to circumstances such as delays in accessing inputs, shortage of land preparation machinery and delayed harvesting of a previous summer crop. There is a significant yield penalty and possibility of a crop right-off due to early summer rains that may cause pre-harvest sprouting. It is always critical to have a cropping programme that is well planned to allow timely execution of cropping programmes for both summer and winter.

The three objectives of the study were met as the AMMI model and GGE biplots managed to (a) analyse genotypes and identify most appropriate genotypes within and across environments, (b) evaluate environments based on genotype performance, and (c) delineate environments into three mega-environments that can be used to make future strategic decisions for the programme.

9. General recommendations and conclusions

Information on genetic composition of SC and other Zimbabwean lines was unknown till 2012 when the first genotyping exercise was done by CenGen laboratories. SC wheat breeding programme relied on conventional breeding methods that are time consuming delaying variety releases (reducing speed to market), use more land and financial resources, test both good and junk material (with or without disease resistance genes) for generations only to discard junk later in the breeding cycle. Decision to discard was based on phenotypic performance observed at non-Ug99 sites. Breeding material had to be sent to Kenya's CIMMYT-KALRO Njoro screening site for exposure to natural Ug99 infection. The SC programme relied on race specific genes with minimum use of backcross breeding method due to difficulties in tracking genes without knowledge on molecular markers. It was the endeavour of this study to:

(a) establish all stage and adult plant resistance responses of Zimbabwean wheat germplasm to the four Ug99 races that are prevalent in Southern Africa;
(b) determine the genetic basis of stem rust resistance in Zimbabwe `s most popular wheat variety (SC1);
(c) use MAS to widen the SC genetic base by introgressing *Sr25*, *Sr26* and *Sr39* genes;
(d) quantify yield losses that can be observed in Southern Africa wheat varieties when affected by Ug99; and
(e) identify promising experimental lines that can be targeted for commercialization or use as parents in variety improvement based on MET data.

The study revealed that gene complexity (race specific and non-race specific genes) and their pleiotropic interactions make it difficult to predict actual plant phenotypic responses. It is therefore recommended for breeders to utilize all tools available in determining plant responses to various races of Ug99. Molecular markers, phenotypic markers (such as PBC and chlorotic/flecking symptoms for *Sr2*) and characterization data such as generated by this study could be utilized by breeders as proven in this study. Seedling bioassays and genotyping of breeding materials must be a continuous process as they can be used as aids in tracking the number of genes and their mode of inheritance in a breeding programme in light of rapid changes in both the plant and pathogen environments due to climate change.

Over-reliance on race-specific stem rust resistance genes such as *Sr31* in SC1 by the Zimbabwean wheat programmes, left it vulnerable to Ug99. There is need to breed for durable resistance to stem rust using targeted approaches and modifications to traditional breeding

methods. Breeding for APR has been proven to be the right way to go, with *Sr2* providing the foundation for resistance. Elite *Sr2* sources such as SC13, are already present in Zimbabwean germplasm and can be used. Breeding is a game of intelligent numbers and this has to be maintained in order to increase frequency of selecting minor genes while MAB can be practised to keep track of both deployed resistant genes and disease pathotypes that exist in a targeted market. Zimbabwean breeding programmes have to incorporate marker assisted breeding, explore diverse sources of resistant genes, and use *Sr2* as the backbone for pyramiding resistant genes. There is an urgent need to replace all susceptible commercial varieties such as SC20, SC3, SC1, CBI5 and CBI6 to ensure that they do not act as host for inoculum build up.

Human resource development in areas of rust surveillance, phenotyping and information dissemination has to be enhanced. This might call for an independent centre of excellence that will provide phenotyping services to all breeding programmes and maintaining a database on all commercial varieties under production in the country. Resistance gene stewardship must be practiced by all breeding programmes to ensure longevity of resistant genes.

The level of protection against Ug99 stem rust is improved with increased number of effective resistant genes. ASR offered better protection against Ug99 compared to APR as indicated by the better performance of SC8 compared to the three APR lines (Kingbird, W1406 and W6979). However, this study was only done at one site where disease pressure was very high due to repeated inoculation; it will be ideal to test the same germplasm under natural conditions in multi-location farmers' fields to verify findings. The study revealed that several genes increase the level of protection and breeders are encouraged to stack several resistant genes. Examples of resistance genes that can be used for stacking include *Sr22*, *Sr25*, *Sr26*, *Sr32*, *Sr35*, *Sr39*, *Sr40* and *Sr50.* It is also advisable for the Zimbabwean wheat breeding programmes to pyramid several effective stem rust resistance genes so as to enhance the level of protection. Either breeding for APR which has shown better protection than the single race-specific genes in susceptible varieties, or breeding for ASR as shown by SC8 can be the way to go for Zimbabwean wheat breeding programmes.

AMMI and GGE biplots were effective in analysing data from MET trials conducted across Zimbabwe during the two seasons of study. Environment demarcation provides valuable information to the breeding programme and other stakeholders. The breeding programme can realign its objectives given that there is need to develop genotypes that target a specific mega-

environment rather than expecting one genotype to be grown across all the three mega-environments. The GGE scatter plot grouped environments into three mega-environments and G4 was best genotype for ME1, G2 for ME2 and G8 for ME3. Environment analysis presents an opportunity to reduce the number of environments where several environments provide the same information. This is useful in case of resource constraints. E10 can represent both ME2 and ME3 while E12 is a good representative for ME3 because it has ME3 characteristics plus other traits that are not exhibited by the rest of the environments. The programme can choose to keep one of the two environments in ME1 (either E2 or E8) in case of resource constraints. E4 is an important environment given its uniqueness in ME3.

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Effect of late planting on wheat yield is a key point that needs consideration when one ventures into wheat production. This empowers extension staff to encourage farmers to plant early so as to realise high yields. There is a tendency by farmers to plant late due to circumstances such as delays in accessing inputs, shortage of land preparation machinery and delayed harvesting of previous summer crop. There is a significant yield penalty and possibility of a crop right-off due to early summer rains that may cause pre-harvest sprouting. It is always critical to have a cropping programme that is well planned to allow timeous execution of cropping programmes for both summer and winter.

AMMI model and GGE biplots managed to (a) analyse genotypes that were used, (b) evaluate environments based on genotype performance, and (c) delineate environments into three mega-environments that can be used to make future strategic decisions for the programme. The use of these two biplots improves data interpretation and decision making as compared to standard statistical analysis. Use of these models result in economic use of limited resources, identification of widely adapted lines that can be grown across mega-environments,

identification of lines for targeted niche markets and allow for delineation of testing environments so as to focus on very informative sites.

The five research projects were able to answer all objectives of the study. Wheat lines with potential for possible commercialization or continued use in the breeding programme as parental material in the crossing block were also identified and above all important information that can contribute to the Zimbabwe wheat database was generated.

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Table 7. 10: Appendix 1 - 2014 and 2015 thousand kernel weight (g) for seven wheat varieties when sprayed, non-sprayed and calculated losses

	2014				**2015**			
Variety	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**
SC1	37.7	32.7	35.2	13.3	36.0	22.3	29.2	38.0
SC3	40.7	33.7	37.2	17.2	34.3	24.7	29.5	28.1
SC8	40.3	38.7	39.5	4.1	35.0	28.7	31.8	18.1
Line 37	42.7	30.7	36.7	28.1	39.0	20.0	29.5	48.7
W1406	37.3	33.0	35.2	11.6	36.0	25.3	30.7	29.6
W6979	48.3	45.0	46.7	6.9	44.3	35.0	39.7	21.1
Kingbird	40.7	39.0	39.8	4.1	36.0	28.7	32.3	20.4
Mean	**41.1**	**36.1**	**38.6**	**12.2**	**37.2**	**26.4**	**31.8**	**29.2**
Summary statistics for thousand kernel weight (g) during 2014 and 2015 seasons								
Source	**M**	**S**	**M*S**	**DL.M**	**M**	**S**	**M*S**	**DL.M**
se	0.9	1.3	1.9	1.8	0.6	0.7	1.1	1.0
sed	1.2	1.8	2.7	2.5	0.9	1.0	1.6	1.4
LSD (5%)	5.4	3.7	5.5	5.2	3.7	2.1	3.4	3.0
F. Sign.	NS	**	NS		**	***	***	

M=Main plot factor (Fungicide treatment) S=Subplot factor (varieties)

M*S=Interaction between main (fungicide treatment) and subplot (variety) factors

DL.M=Differences of means when comparing means with the same level of; main (M)

s.e= standard errors of means sed= standard errors of differences of means

LSD (5%)=Least significant differences of means at 5% level

F. Sign.=Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001

% Loss=((Sprayed-Non-sprayed)/Sprayed)*100

Table 7. 11: Appendix 2 - Mean thousand kernel weight (g) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

Variety	Sprayed	Non-sprayed	Mean	% Loss			
SC1	36.8	27.5	32.2	25.3			
SC3	37.5	29.2	33.3	22.2			
SC8	37.7	33.7	35.7	10.6			
Line 37	40.8	25.3	33.1	38.0			
W1406	36.7	29.2	32.9	20.5			
W6979	46.3	40.0	43.2	13.7			
Kingbird	38.3	33.8	36.1	11.7			
Mean	**39.2**	**31.2**	**35.2**	**20.3**			
Mean Summary statistics for thousand kernel weight (g)							
Source	**M**	**S**	**Y**	**M*S**	**M*Y**	**S*Y**	**M*S*Y**
s.e	0.7	0.8	0.4	1.3	0.8	1.1	1.6
s.e.d	1.0	1.2	0.5	1.9	1.1	1.5	2.3
LSD (5%)	4.2	2.5	1.0	3.9	3.4	3.1	4.6
F. Sign.	*	***	***	**	***	NS	NS
Except when comparing means within the same level of:							
		M		**S**	**M*S**	**M*Y**	
Source	**M*S**	**M*Y**	**M*S*Y**	**S*Y**	**M*S*Y**	**M*S*Y**	
se	1.2	0.5	1.5	0.9	1.3	1.5	
sed	1.7	0.7	2.2	1.3	1.9	2.2	
LSD (5%)	3.5	1.5	4.3	2.7	3.8	4.3	

M=Main plot factor (Fungicide treatment). S=Subplot factor (varieties). M*S, M*Y, M*S*Y, S*Y, M*S*Y represents interaction effects where M=fungicide treatment, S=variety and Y = year. s.e= standard errors of means. s.e.d = standard errors of differences of means.

LSD (5%) = Least significant differences of means at 5% level.

F. Sign.= Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, *** = highly significant at <0.001.

% Loss = ((Sprayed-Non-sprayed)/Sprayed)*100.

Table 7. 12: Appendix 3 - 2014 and 2015 hectolitre mass (kg/hl) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

	2014				2015			
Variety	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**
SC1	79.0	73.0	76.0	7.6	71.0	57.0	64.0	19.7
SC3	79.0	74.0	76.5	6.3	69.0	60.0	64.5	13.0
SC8	78.0	76.0	77.0	2.6	70.0	61.0	65.5	12.9
Line 37	81.0	71.0	76.0	12.4	70.0	49.0	59.5	30.0
W1406	74.0	70.0	72.0	5.4	67.0	56.0	61.5	16.4
W6979	80.0	77.0	78.5	3.8	71.0	66.0	68.5	7.0
Kingbird	78.0	78.0	78.0	0.0	67.0	60.0	63.5	10.5
Mean	78.0	74.0	76.0	5.1	69.0	59.0	64.0	14.5
Summary statistics for hectolitre mass (kg/hl) during 2014 and 2015 seasons								
Source	**M**	**S**	**M*S**	**DL.M**	**M**	**S**	**M*S**	**DL.M**
se	0.1	0.9	1.2	1.3	0.1	0.9	1.2	1.3
sed	0.2	1.3	1.7	1.8	0.2	1.3	1.7	1.8
LSD	0.9	2.6	3.4	3.7	0.9	2.6	3.4	3.7
F.Sig	**	***	*		**	***	*	

M=Main plot factor (Fungicide treatment)   S=Subplot factor (varieties)

M*S=Interaction between main (fungicide treatment) and subplot (variety) factors

DL.M=Differences of means when comparing means with the same level of; main (M)

s.e= standard errors of means   sed= standard errors of differences of means

LSD (5%) = Least significant differences of means at 5% level

F. Sign.= Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001

% Loss = ((Sprayed-Non-sprayed)/Sprayed)*100

Table 7. 13: Appendix 4 - Mean hectolitre mass (kg/hl) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

Variety	Sprayed	Non-sprayed	Mean	% Loss			
SC1	75.0	65.0	70.0	13.3			
SC3	74.0	67.0	70.5	9.5			
SC8	74.0	68.0	71.0	8.1			
Line 37	75.0	60.0	67.5	20.0			
W1406	71.0	63.0	67.0	11.3			
W6979	75.0	72.0	73.5	4.0			
Kingbird	72.0	69.0	70.5	4.2			
Mean	**74.0**	**66.0**	**70.0**	**10.8**			
		Mean Summary statistics for hectolitre mass (kg/hl)					
Source	**Main**	**S**	**Y**	**M*S**	**M*Y**	**S*Y**	**M*S*Y**
se	0.5	0.9	0.3	1.3	0.6	1.0	1.5
sed	0.7	1.3	0.4	1.8	0.8	1.4	2.1
LSD (5%)	3.1	2.6	0.7	3.8	2.5	2.9	4.2
F.Sig	**	***	***	**	***	***	NS
	Except when comparing means within the same level of:						
	M			**S**	**M*S**	**M*Y**	
Source	**M*S**	**M*Y**	**M*S*Y**	**S*Y**	**M*S*Y**	**M*S*Y**	
s.e	1.3	0.4	1.4	0.7	0.9	1.4	
s.e.d	1.8	0.5	2.0	0.9	1.3	2.0	
LSD (5%)	3.7	1.0	4.1	1.9	2.7	4.1	

M=Main plot factor (Fungicide treatment). S=Subplot factor (varieties). M*S, M*Y, M*S*Y, S*Y, M*S*Y represents interaction effects where M=fungicide treatment, S=variety and Y=year. s.e= standard errors of means. Sed = standard errors of differences of means.

LSD (5%) = Least significant differences of means at 5% level.

F. Sign.= Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001.

% Loss = ((Sprayed-Non-sprayed)/Sprayed)*100.

Table 7. 14: Appendix 5 - 2014 and 2015 grain protein content (%) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

	2014				2015			
Variety	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**
SC1	11.6	11.9	11.7	-2.8	12.9	12.6	12.8	2.5
SC3	11.2	11.2	11.2	-0.2	13.1	11.7	12.4	10.3
SC8	10.6	11.0	10.8	-3.5	12.8	13.2	13.0	-2.8
Line 37	11.3	10.0	10.6	11.6	13.8	11.5	12.7	16.5
W1406	11.5	12.8	12.1	-11.1	13.4	13.4	13.4	0.4
W6979	12.2	11.9	12.0	2.0	14.6	12.9	13.7	12.1
Kingbird	11.2	12.1	11.6	-8.1	14.0	13.3	13.6	4.9
Mean	**11.4**	**11.5**	**11.4**	**-1.6**	**13.5**	**12.7**	**13.1**	**6.5**

Summary statistics for Grain protein content (%) during 2014 and 2015 seasons

Source	**M**	**S**	**M*S**	**DL.M**	**M**	**S**	**M*S**	**DL.M**
se	0.2	0.2	0.3	0.3	0.1	0.3	0.4	0.4
sed	0.2	0.3	0.4	0.4	0.1	0.4	0.5	0.5
LSD	0.9	0.6	0.9	0.9	0.6	0.8	1.0	1.1
F.Sign.	NS	**	**		*	**	*	

M=Main plot factor (Fungicide treatment) S=Subplot factor (varieties)

M*S=Interaction between main (fungicide treatment) and subplot (variety) factors

DL.M=Differences of means when comparing means with the same level of; main (M)

s.e = standard errors of means sed = standard errors of differences of means

LSD (5%) = Least significant differences of means at 5% level

F. Sign.= Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001

% Loss = ((Sprayed-Non-sprayed)/Sprayed)*100

Table 7. 15: Appendix 6 - Mean grain protein content (%) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

Variety	Sprayed	Non-sprayed	Mean	% Loss
SC1	12.25	12.26	12.26	-0.08
SC3	12.12	11.46	11.79	5.45
SC8	11.71	12.07	11.89	-3.07
Line 37	12.52	10.73	11.63	14.03
W1406	12.46	13.08	12.77	-4.98
W6979	13.39	12.38	12.89	7.54
Kingbird	12.59	12.69	12.64	-0.79
Mean	12.44	12.10	12.27	2.73

Mean Summary statistics for grain protein content (%)

Source	M	S	Y	M*S	M*Y	S*Y	M*S*Y
se	0.0	0.2	0.089	0.2	0.1	0.2	0.3
sed	0.1	0.3	0.126	0.3	0.1	0.3	0.5
LSD	0.3	0.5	0.258	0.7	0.3	0.7	1.0
F.Sig	*	***	***	***	***	NS	NS

Except when comparing means within the same level of:

		M		**S**	**M*S**	**M*Y**
Source	**M*S**	**M*Y**	**M*S*Y**	**S*Y**	**M*S*Y**	**M*S*Y**
s.e	0.3	0.1	0.3	0.2	0.3	0.3
s.e.d	0.4	0.2	0.5	0.3	0.5	0.5
LSD (5%)	0.7	0.4	1.0	0.7	1.0	1.0

M=Main plot factor (Fungicide treatment). S=Subplot factor (varieties). M*S, M*Y, M*S*Y, S*Y, M*S*Y represents interaction effects where M=fungicide treatment, S=variety and Y=year. s.e= standard errors of means. s.e.d = standard errors of differences of means.

LSD (5%) = Least significant differences of means at 5% level.

F. Sign.= Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001.

% Loss = ((Sprayed-Non-sprayed)/Sprayed)*100.

Table 7. 16: Appendix 7 - 2014 and 2015 yield (kg/ha) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

	2014				2015			
Variety	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**
SC1	6512.0	4691.0	5601.5	28.0	5164.0	3618.0	4391.0	29.9
SC3	6617.0	5484.0	6050.5	17.1	5132.0	3838.0	4485.0	25.2
SC8	7157.0	6411.0	6784.0	10.4	4934.0	5000.0	4967.0	-1.3
Line 37	6263.0	3333.0	4798.0	46.8	5263.0	2675.0	3969.0	49.2
W1406	4538.0	3803.0	4170.5	16.2	3596.0	2747.0	3171.5	23.6
W6979	6106.0	4871.0	5488.5	20.2	4945.0	4485.0	4715.0	9.3
Kingbird	6249.0	5383.0	5816.0	13.9	4550.0	4326.0	4438.0	4.9
Mean	**6220.0**	**4854.0**	**5537.0**	**22.0**	**4798.0**	**3813.0**	**4305.5**	**20.5**
Summary statistics for yield (kg/ha) during 2014 and 2015 seasons								
Source	**M**	**S**	**M*S**	**DL.M**	**M**	**S**	**M*S**	**DL.M**
se	489.5	244.7	585.0	346.1	132.4	133.9	219.7	189.4
sed	692.2	346.1	827.3	489.5	187.2	189.4	310.7	267.9
LSD	2978.3	714.3	2292.5	1010.2	805.6	390.9	676.6	552.8
F.Sign.	NS	**	*		*	***	***	

M=Main plot factor (Fungicide treatment) S=Subplot factor (varieties)

M*S=Interaction between main (fungicide treatment) and subplot (variety) factors

DL.M=Differences of means when comparing means with the same level of; main (M)

s.e= standard errors of means sed= standard errors of differences of means

LSD (5%)=Least significant differences of means at 5% level

F. Sign.=Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001

% Loss=((Sprayed-Non-sprayed)/Sprayed)*100

Table 7. 17: Appendix 8 - Mean yield (kg/ha) for seven wheat varieties when sprayed, non-sprayed and calculated losses.

Variety	**Sprayed**	**Non-sprayed**	**Mean**	**% Loss**			
SC1	5838.0	4155.0	4996.5	28.8			
SC3	5874.0	4661.0	5267.5	20.7			
SC8	6095.0	5705.0	5900.0	6.4			
Line 37	5763.0	3004.0	4383.5	47.9			
W1406	4067.0	3275.0	3671.0	19.5			
W6979	5526.0	4678.0	5102.0	15.4			
Kingbird	5400.0	4854.0	5127.0	10.1			
Mean	5509.0	4333.0	4921.0	21.4			
Mean summary statistics for yield (kg/ha)							
Source	**Main**	**S**	**Y**	**M*S**	**M*Y**	**S*Y**	**M*S*Y**
se	245.9	134.3	126.5	302.3	276.5	272.0	450.9
sed	347.7	189.9	178.8	427.5	391.0	384.7	637.7
LSD	1496.2	391.9	366.3	1139.2	1205.1	775.5	1340.5
F.Sig	NS	***	***	***	NS	NS	NS
Except when comparing means within the same level of:							
	M			**S**	**M*S**	**M*Y**	
Source	**M*S**	**M*Y**	**M*S*Y**	**S*Y**	**M*S*Y**	**M*S*Y**	
s.e	189.9	178.8	384.7	334.6	473.2	384.7	
s.e.d	268.6	252.9	544.1	473.2	669.1	544.1	
LSD (5%)	554.3	518.1	1096.7	969.2	1370.7	1096.7	

M=Main plot factor (Fungicide treatment). S=Subplot factor (varieties). M*S, M*Y, M*S*Y, S*Y, M*S*Y represents interaction effects where M=fungicide treatment, S=variety and Y=year. s.e= standard errors of means. s.e.d = standard errors of differences of means.

LSD (5%)=Least significant differences of means at 5% level.

F. Sign.= Significance of F probability value where NS=not significant, *= significant at 0.05%, **= significant at 0.01%, ***= highly significant at <0.001.

% Loss = ((Sprayed-Non-sprayed)/Sprayed)*100.

Table 8. 14: Appendix 9 - Sources of variation based on mean square values for days to anthesis, leaf rust coefficient of infection (LRCI), test weight, grain protein content, starch content and wet gluten.

Source	**Anthesis**	**LRCI**	**Test weight**	**G. protein**	**Starch**	Gluten
Total	110.2	459	300.5	2.98	267.6	85.8
Treatments	676.8***	2629***	313.5	16.87***	281.3	163.4***
Genotypes	730.6***	22521***	239.9	30.18***	318.6	226.2***
Environments	10518.5***	3702***	578.6*	241.75***	502.5*	1352.6***
Block	11.1	135	280.0	2.77***	274.9	73.6
Interactions	15.8	749***	302.5	0.67	263.1	78.4
IPCA1	58.9**	4199***	1981.5***	1.41	1712.9***	492.0***
IPCA2	20.0	616***	5.5	1.11	19.2	9.7
IPCA3	9.7	96	5.1	0.86	4.2	8.7
IPCA4	8.0	62	3.4	0.58	1.5	4.1
IPCA5	6.2	40	1.9	0.44	0.7	3.3
IPCA6	5.1	18	1.0	0.32	0.6	2.1
IPCA7	3.9	17	0.7	0.30	0.4	1.4
IPCA8	4.4	5	0.8	0.24	0.3	1.5
IPCA9	2.4	3	0.5	0.18	0.2	1.4
Residuals	1.3	1	0.2	0.08	0.2	0.7
Error	30.2	152	299.2	0.95	265.4	74.9

*, **, *** F. significance at 0.05%, 0.01% and 0.001% respectively.

LRCI=leaf rust coefficient of infection. G. protein=Grain protein content. Starch=starch content. Gluten=wet gluten content.

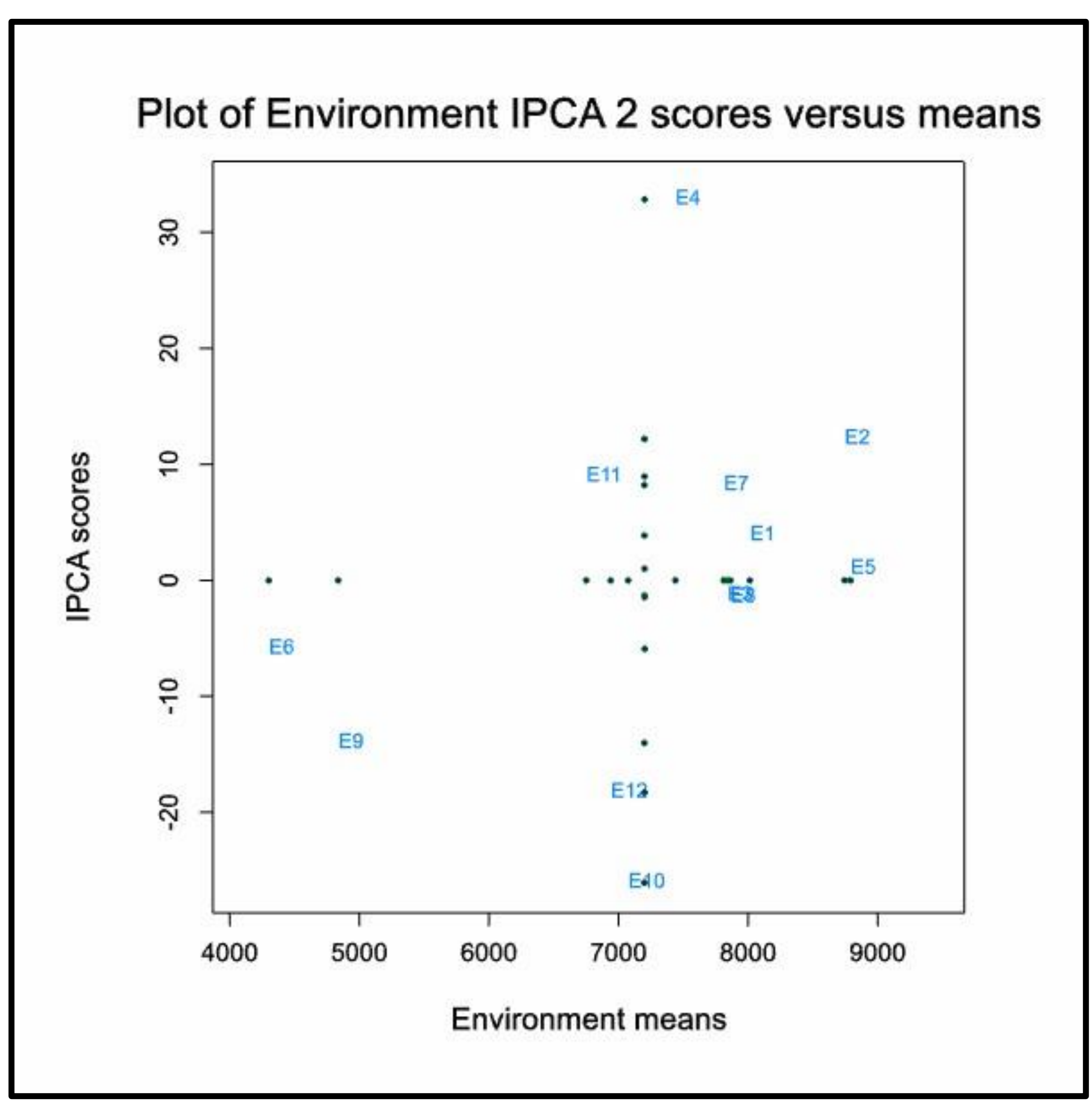

Figure 8. 9: Appendix 10 - AMMI biplot when environment means are plotted against IPCA2

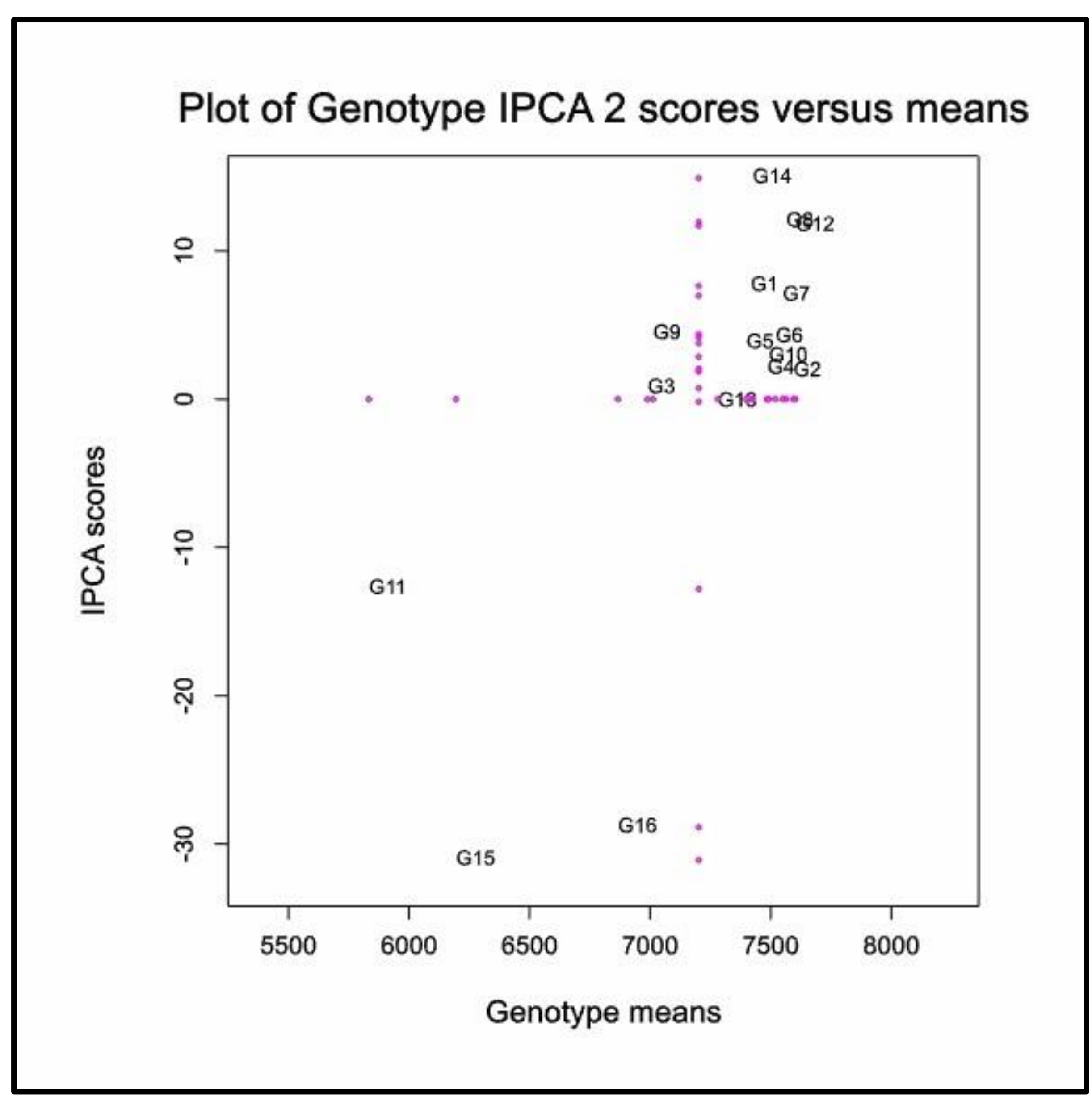

Figure 8. 10: Appendix 11 - AMMI biplot when genotype means are plotted against IPCA2

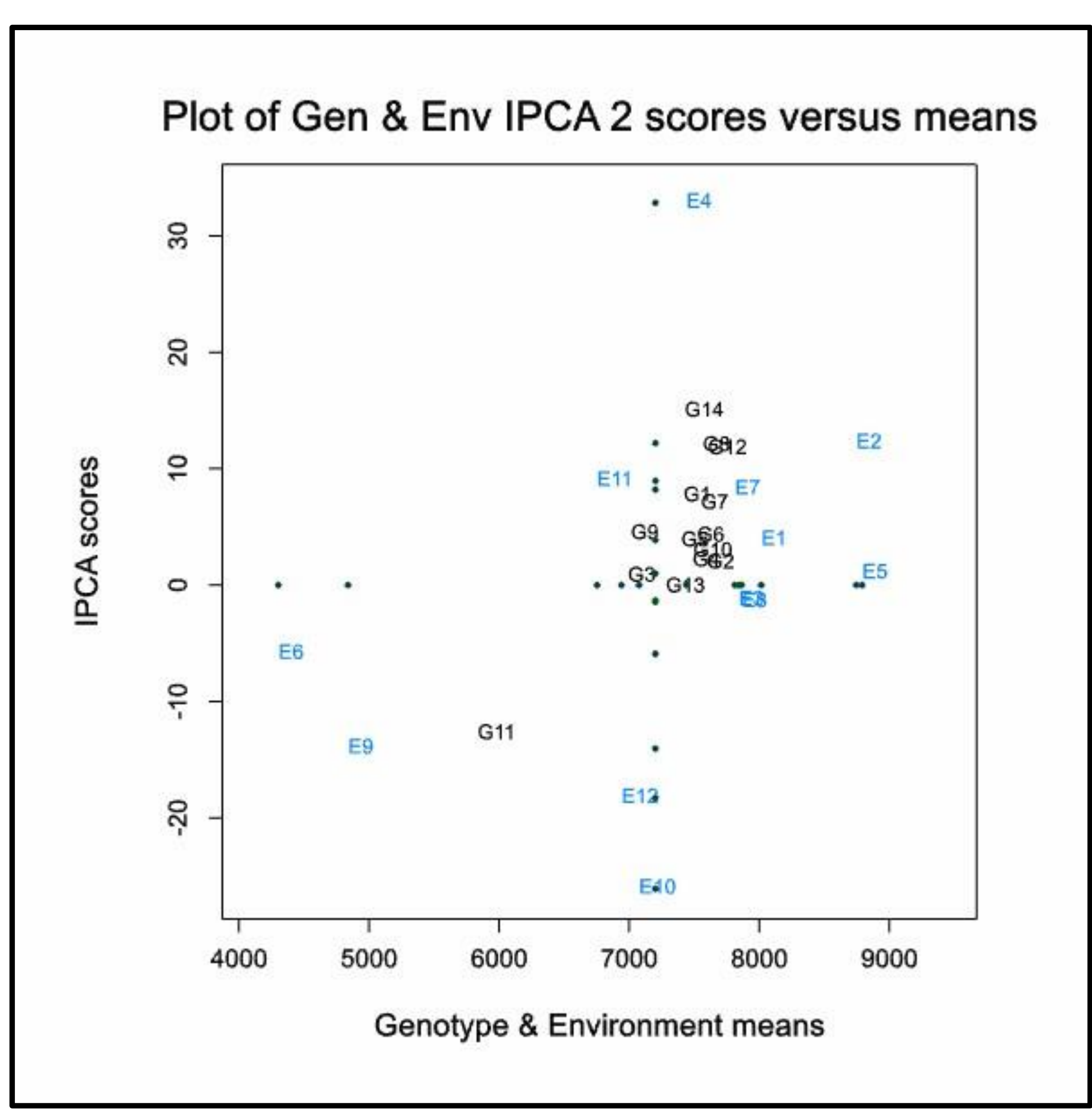

Figure 8. 11: Appendix 12 - AMMI biplot when genotype and environment means are plotted against IPCA2