INTROGRESSION AND QTL MAPPING OF STEM RUST RESISTANCE GENES USING BI-PARENTAL WHEAT POPULATIONS

BY

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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Declaration by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

To the God almighty, for I am who I am because of who he is, and without him this work would not have been possible.

I dedicate this work to my loving parents Peter and Emily Karumbi, you are my role models and mentors, and may you experience joy and fulfillment everlasting.

ABSTRACT

Rust diseases are a global challenge to wheat production, causing yield losses of up to 50% and even 100% in susceptible host cultivars. A virulent stem rust race named *Ug99* (TTKSK) was discovered in Uganda in 1999, and has since then continued to cause a significant threat to world wheat production and food security in turn. Kenya wheat production is particularly at risk as the Kenyan weather makes it a hotspot for stem rust to thrive. Among the best strategies to combating stem rust is host based disease resistance, made even more achievable with the advent of genomics and bioinformatics. This study's objective was to characterize identified mapping populations for resistance to stem rust and use molecular markers to track introgressed genes as well identify genomic regions potentially harboring resistance genes through QTL mapping. Two bi-parental mapping populations were used, an F_2 Robin/Kwale and an $F_{2:5}$ PBW343/Akuri population. Both populations were evaluated for stem rust resistance in the field in Njoro, Kenya for several seasons. F_2 population was evaluated at the F_2 and F_3 generation. Parental purity and uniformity of the parental genotypes used to make the cross were evaluated using ten SSR markers from the 1A and 6A chromosomes of the wheat genome. These revealed un-uniform banding patterns for both genotypes. Pearson's Chi square test with co-efficient of infection data fit the 13:3 (at *p=0.05)* gene ration revealing a dominant and a recessive gene underlying observed resistance. SSR markers *gwm533* and *xcfd49* were used to track the introgression of genes *Sr2* and *SrTmp* respectively. The parents and one hundred and forty eight lines of the $F_{2:5}$ recombinant inbred line (RIL) population were evaluated for three seasons under field conditions and genotyped using DArT markers. A frequency distribution of the disease severity data revealed a normal distribution, indicative of underlying quantitative resistance. Linkage mapping was done using Join Map v 4.1 revealing 44 linkage groups and a map spanning 2759.39 cMs with 910 markers. Composite interval mapping was implemented on Windows QTL Cartographer to detect QTLs at an LOD threshold of 2.5 revealing three QTL on 1BL, 2BL and 3B consistent in more than one season, and were designated as *QSr.cim-1BL*, *QSr.cim-2BL*, and *QSr.cim-3B*-. These QTL respectively explained ~7, 9, and 8% of the phenotypic variation. Results from these studies will go a long way in the efforts to enhance utilization of marker assisted selection in combating *Ug99* and boost food security.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DNA – Deoxyribonucleic Acid

EDTA - Ethylenediaminetetraacetic acid

EtBr – Ethidium Bromide

HR – Homozygous Resistant

HS – Homozygous Susceptible

$MgCl_2$ – Magnesium Chloride

mM - milimole

PCR – Polymersase Chain Reaction

pmol – picamol

SEG – Segregating

TBE – Tris Borate EDTA

UK – United Kingdom

µl - micro liter

QTL – Quantitative trait Loci

LOD – Logarithm of Odds

CIM – Composite Interval Mapping

PVE – Phenotypic Variance Expected

KALRO – Kenya Agricultural Livestock Research Organization

NIAB- National Institute for Agricultural Botany

BBSRC – Biotechnology and Biological Sciences Research Council

DRRW –Durable Rust Resistance in Wheat

SSR – Simple Sequence Repeats

µg – micrograms

TBE – Tris Borate EDTA

Ltd - Limited

PIC – Polymorphism Information Content

λ- Lamda

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Wheat (*Triticum aestivum*) is grown on more land than any other crop worldwide (FAOSTAT, 2018). In Kenya wheat is the second most important grain after maize. The world wheat demand is projected to be around 1 billion tonnes by 2020.

Stem rust (*Puccinia graminis* f. sp. *tritici*), also known as black rust of wheat has had a history of causing severe devastation periodically and was once the most dreaded disease in various countries in all continents where wheat is grown (Hibbett, 2012). The fear of stem rust is understandable because apparently a healthy looking crop three weeks prior to harvest could be reduced to a black tangle of broken stems and shriveled grain by harvest. According to Saari and Prescott (1985), stem rust was historically a major problem in all of Africa, the Middle East, all of Asia except Central Asia, Australia and Newzealand, Europe, and both North and South America.

Wheat stem rust had only been at a nuisance level in most major wheat production areas, hence breeding for stem rust resistance as a priority trait, and had declined substantially. This was until 1998 when stem rust infections were observed on wheat in Uganda and the race, with virulence on *Sr31*, was identified and designated as *Ug99* (Pretorius *et al*., 2000). This race was later designated as TTKS in 2006 using the Northern American nomenclature system (Roelfs and Martens 1988). Later stem rust race *Ug99* was detected in Kenya and Ethiopia in 2005 (Wanyera *et al*., 2006) and in Sudan and Yemen in 2006 (Jin *et al.*, 2007). A new variant of this race with virulence to *Sr24* was detected in

Kenya, India and South Africa in 2006. (Jin *et al.*, 2007). The race is changing rapidly and thirteen known variants have been identified within the *Ug99* lineage (CIMMYT, 2016). They pose a significant threat to food security, as they already have in Eastern Africa and to the rest of the world unless strategies to incorporate effective resistance against stem rust are implemented without delay.

Many traits of interest including that for rust resistance show quantitative mode of inheritance. This complicates the breeding process since phenotypic performance only partially reflects the genetic values of individuals. The genetic variation of a quantitative trait is controlled by the collective effects of quantitative trait loci (QTLs), epistasis (interaction between QTLs), the environment, and interaction between QTL and environment (Semagn *et al.*, 2010). The most efficient and environmentally friendly method to reduce losses due to rusts is to use resistant wheat cultivars (Knott, 1989). Host based resistance provides a cost-effective strategy to reduce and prevent losses in wheat from attack by rust pathogens (Speilmeyer *et al.*, 2005). About 50 stem rust resistance genes have been characterized and of these, only a few are effective against *Ug99 (*Singh *et al.*, 2006).

Rust resistance in wheat is categorized into race-specific (vertical, major) and race non-specific (horizontal, minor) resistance. Race-specific genes are associated with seedling or all stage resistance and provide protection at all stages of plant growth. The downside has been made known to be that the pathogen usually overcomes major genes in a few years sometimes leading to 'boom and bust cycles' (Parlevliet 2002). Race non-specific resistance is associated with adult plant resistance (APR) and is detected at post-seedling

stages. Consequently, several QTLs can regulate the expression of a single phenotypic trait as is the case with race-nonspecific rust resistance.

Combining of major resistance genes, multiple minor genes or both major and minor genes and pyramiding them into a similar genetic background to develop resistant cultivars is among the preeminent strategies for sustainable control of wheat stem rust (Singh et al., 2014). Gene pyramiding through conventional breeding methods is cumbersome, needs large populations and is time consuming.

Advancements in biotechnology involving molecular marker techniques offer powerful tools to characterize quantitative traits such as those conferring partial resistance to stem rust (William et al., 2005). Different molecular marker platforms are available for use in genotyping. Amongst them, microsatellite markers are markers of choice in most molecular genetic studies as they are highly polymorphic even between closely related lines, require low amount of DNA and can be easily automated for high throughput screening (Gupta *et al.,* 1999).

A number of statistical methods have been developed and are available for detection of QTLs and estimation of their effects. Composite interval mapping combines interval mapping for a single QTL in a given interval with multiple regression analysis on marker associated with other QTL. This method was improved by Huihui *et al*., (2007) to inclusive composite interval mapping (ICIM) and this method increases detection power, reduces false detection rate and biased estimated QTL effects (Li *et al*., 2007).

1.2 Statement of the Problem

Stem or black rust of wheat, caused by *Puccinia graminis* f. sp. *tritici,* continues to poss a major threat to world wheat production, including in Kenya, causing documented yield losses of up to 50% where conditions are favorable (Roelfs et al., 1992) and upto 100% losses on susceptible varieties (Leonard and Szabo, 2005). Stem rust race, *Ug99*, designated TTKSK, asserted itself as a versatile pathogen when it broke the historic *Sr31* gene resistance and mutated quite fast to acquire virulence to notable resistance genes including *Sr24*, *Sr36, Sr21 and Sr9h*. Statistics and surveys done by the CIMMYT's Global Rust Monitoring Systems show that *Ug99* (TTKSK) now has 13 identified variants (CIMMYT, 2016). Annual surveys done in Kenya in 2010 revealed 100% of the samples collected were infected by stem rust race *Ug99*. The last five variants identified under the *Ug99* lineage of races were identified in Kenya. Movement out of Africa of stem rust and its various races is inevitable. Urediniospores of stem rust are relatively resistant to varying levels of light, temperature and humidity allowing them to remain viable even over long distances of wind dispersal, 100km to at times 2000 km (Luig 1985). Already it stem rust has been reported in Yemen and Iran(reference), and recently in Germany and United Kingdom (Olivera et al., 2017, Lewis et al., 2018) with even an outbreak of stem rust devastating bread and durum wheat for the first time in decades reported in Sicily, Europe (Bhattacharya, 2017). Development and deployment of resistant cultivars still remains one of the best strategies to control the stem rust dilemma, using genes that confer host based resistance. This is particularly true for resource constraint small-holder farmers who cannot afford continuous use of expensive fungicides as a method of control. Host-based resistance encompasses varieties with race-

specific or race non-specific genes either occurring singly or in combination. Identification of resistance genes and use of markers tightly linked to these genes to track them hastens the breeding process aiding in faster release of resistant varieties. The objective of this study therefore was to use molecular markers to track introgression of resistance genes into wheat mapping populations coupled with identifying QTLs conferring resistance. The results of this study will enhance the efforts of breeding for varieties with resistance to stem rust not just for Kenya but globally.

1.3 Justification

Development of resistant varieties using classical breeding strategies to combine several major and minor genes is tedious and takes an undesirably long time. Breeding for resistance to wheat rusts involves development of varieties with major genes alone or minor genes alone or their combination thereof in one genetic background. It also requires a large population for selection. Development of molecular procedures and tools has provided powerful tools for characterization of qualitative and quantitative traits and allows for manipulation of genotyping data to evaluations at a molecular level (Collard and Mackill, 2008). Molecular information about these genes is vital in developing markers for these genes to help identify them hence enable their accumulation in one background more rapidly through marker assisted breeding (Jiang, 2013). This would enhance the efforts of achieving “durable” stem rust resistance. Identifying new sources of resistance and genomic regions harboring these genes is key in efforts to breed for host based resistance to stem rust. As well, saturation of these genomic regions would assist in identifying markers more closely linked to these genes. This would enhance the

effectiveness of marker-assisted selection for faster release of resistant varieties not just in Kenya but for the global wheat community.

1.4 General Objective

To identify genes for stem rust resistance in bi-parental wheat mapping populations and use molecular genes to track introgression of rust resistance genes to support efforts to breed for host based disease resistance particularly through marker-assisted breeding.

1.5 Specific Objectives

1. To evaluate response of identified mapping population to stem rust
2. To genotypically characterize identified mapping populations using molecular markers

1.6 Alternate Hypotheses

1. Phenotypic variation within the selected mapping populations in relation to disease resistance to stem rust will be observed.
2. Genotypic variation within the selected mapping populations for stem rust resistance will be identified.
3. QTLs associated with adult plant resistance are present in identified mapping population.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Wheat Crop

Wheat (*Triticum aestivum*) is a grass from the family *Poaceae (*also referred to as *Gramineae).* It belongs to kingdom *Plantae*, genus *Triticum* and species *aestivum*. It is commonly known as wheat, common wheat or bread wheat. Wheat is a hexaploid (6x) that forms 21 pairs of chromosomes during the process of meiosis. It has three genomes A, B, and D each containing 7 pairs of chromosome (AABBDD) (Agropedia, 2009). Wheat is believed to have originated from South-western Asia and was first cultivated in the United States in 1602 on an island off the Massachusetts coast (Lance and Garren, 2002). Two main species of wheat commonly grown in the world are *T. aestivum*, and *T. turgidum ssp. durum*. *T. aestivum*, better known as bread wheat, forms the classes of hard red winter, hard red spring, a soft red winter and soft white wheat. Bread wheat accounts for 95% of world wheat production (Encyclopedia of Food and Culture, 2003). *T. turgidum ssp. durum* includes durum wheat classes, accounting for 5% of world wheat production. Bread wheats are the most common but there are other related species that make up the genus *Triticum* that include einkorn, emmer, and spelt wheat (Encyclopedia of Food and Culture, 2003).

Wheat is a national staple in many countries, supplying almost 20% of food calories for the world's population. (Lance and Garren, 2002). Wheat is grown on more land than any other cereal crop worldwide and is second after rice in total world production (FAOSTAT, 2018). Area harvested for wheat in 2016 was more than 220 million ha,

surpassing any other crop worldwide (FAOSTAT, 2018). Wheat use on the other hand has been increasing at a slightly higher rate, surpassing production, with shortcomings being met by world stocks. With little or no change in world consumption trends of wheat, a projection of 800 million tonnes of wheat will be required annually by 2020.

Wheat is an annual plant that flowers in spring and senesces as temperatures increase in late spring. It is adapted to a wide range of conditions and is an important global crop adapted to cultivation in many soil types with a short growth period and good yields, growing well in fairly dry and mild climates (Shumann and Leonard,200). Wheat does best at latitudes of between 30^{o} and 60^{o} N and 27^{o} and 40^{o}S but can be grown from within the arctic circle to higher elevations near the equator (Nuttonson, 1995). Optimum growth temperatures range from between 3^{o} to 4^{o}C minimum to about 30^{o} to 40^{o}C maximum. Warm conditions and high relative humidity in addition to encouraging most wheat diseases; generally do not promote wheat cultivation. Three-fourths of the area under wheat cultivation receives a precipitation of about 375 and 875 mm annually but by and large wheat does well in most areas where precipitation ranges from 250 to 1,750 mm annually (Leonard and Martin, 1963). Wheat requires an adequate amount of moisture in the growing season but too much moisture can result in yield losses and root problems. The best soils for wheat cultivation are well aerated, well drained deep soils with ample organic matter, and optimum soil pH of between 5.5 and 7.5. Wheat is harvested around the world at any given time except.

Wheat is used mainly for human consumption, primarily for bread manufacture. It provides high amounts of carbohydrates in addition to valuable proteins, minerals, and

vitamins (Saari and Prescott, 1985) Wheat grain is also used to manufacture alcoholic beverages, livestock feed where the by-products of flour-milling are used, straws and green forage can be grazed by livestock or used as hay or foliage (SciencAid, 2017).

The classes of wheat are determined by the growth type and also by their hardiness, color and shape of their kernels. Different classes of wheat are grown in different areas depending on rainfall, temperature, soil conditions and native traditions of an area. Wheat is classified as spring or winter depending on the season when it is grown. There are five major classes of wheat grown in the world including hard red winter wheat, hard red spring wheat, soft red winter wheat, white and durum wheat (Curtis, 2002). Hard red spring wheat contains the largest percentage of protein and gluten hence is an excellent bread wheat with superior milling and baking qualities. Soft red winter wheat is high yielding but with relatively low levels of protein and is thus used for flat breads, cakes, pastries and crackers (Curtis, 2002). Durum wheat is among hardest of all wheats and is used to make semolina flour for pasta production. White wheat is closely related to the red wheats except for its color and has a milder, sweeter flavor, with similar fiber and similar milling and baking qualities as red wheats (ScienceAid,2017). White wheat is used mainly for yeast breads, hard rolls, bulgur tortillas and oriental noodles.

In East Africa, wheat currently occupies second largest production figures after maize (*Zea mays* L.). In Kenya, wheat is the second most important grain after maize. The annual consumption for wheat in Kenya stands at almost two million metric tonnes annually, but the country is only able to produce only an eighth of its demand, with the deficit being met by wheat imports costing the country billions annually (Grain and Feed

Annual, 2018). Small-scale farmers who are the majority of the Kenyan wheat farmers produce only 20% of total wheat produced, the bulk of it (80%) is produced by a handful of large-scale famers.

Agriculture in Kenya is very diverse but the climate in most areas is suitable for wheat production. In ten years up to 2016, land area under wheat production in Kenya averaged 154,000 ha and production 350,000 metric tonnes (FAOSTAT, 2018), falling short of the increase in demand for wheat in the country. Population growing at a rate of 4% per annum and a distinct shift in food preferences towards wheat and its products increase wheat demand by a big percentage (The Business Daily, 2011).

Disease and pests cause up to 35% losses of potential harvest around the world. Wheat is attacked by a number of biotic factors mainly fungal diseases caused by both biotrophs (obligate parasites) and hemibiotrophs (facultative parasites). Obligate parasites include rusts, powdery mildew, the bunts and the smuts. Facultative parasites include *Septoria tritici*, *Septoria nodorum* blotch, spot blotch, *Fusarium* head blight (scab) and Tan spot diseases. Collectively these parasites cause great losses to the quality and quantity of wheat produce. Russian wheat aphid (RWA) is a major pest of wheat, causing documented losses of up to 35 to 60% (Du Toit and Walters, 1984). Abiotic factors like acid soils, copper deficiency, climate change and high costs of inputs also affect wheat production, particularly in developing countries.

Yellow rust, leaf rust and stem rust make up the rusts that infect wheat and are the most important diseases of wheat both in Kenya and globally. Rusts are evidently among the most economically important constraint in wheat production. This is attributed to several

factors including their wide distribution in wheat growing areas, a high capacity to form new races that can attack previously resistant cultivars, and the ability to move long distances and develop rapidly under optimal environmental conditions that can result in serious yield losses (Priyamvada *et. al.,* 2011). As obligate parasites, rusts are highly specialized and considerable variation exists in their population for virulence to specific resistance genes (Shumann and Leonard, 200). Evolution in rust populations for new virulence genes occurs through migration, mutation, recombination of existing genes and their selection.

2.2 Stem Rust Disease, Pathogen and Epidemiology

Stem (black) rust is caused by the fungus *Puccinia graminis f. sp*. Eriks & E. Henn that is in the order *Uredinale*s and family *Pucciniaceae* (Kirk *et al.,* 2001). Infection occurs mainly on leaf stems and leaf sheaths but also on leaves, glumes and awns of a wheat plant. Stem rust first presents as a small chlorotic fleck which appears a few days after infection. About 8-10 days' post infection it is seen as elongated blister-like diamond-shaped pustules or uredinia (Singh *et al.,* 2008; Kurt *et al.,* 2005). Powdery masses of urediniospores produced in pustules are brownish red in color. As infected plants mature, uredinia convert to telia that are firmly attached to plant tissue, changing color from red to black, hence the name black rust (Singh *et al*., 2008). The picture below shows a stem with severe stem rust infection.

Plate 2.1: A photo showing a stem with severe stem rust infection, Source; Wheat and small grains, 2012

The stem rust fungus is heteroecious and macrocyclic, with asexual reproduction on its gramineous hosts and sexual reproduction that begins in the resting spore stage and culminates on the alternate *Berberidaceae* host (Kurt *et al.,* 2005). Maximum temperatures for spore germination are between 15 to 24˚C and that for sporulation is 30˚C. *P. graminis* has a wide range of crop species hosts that include bread wheat, durum wheat, barley and triticale. Its most important alternate host is *Berberis vulgaris*, among other *Berberis* and *Mahonia* species (Roelfs, 1985).

As a biotroph the stem rust pathogen needs living wheat plants or other secondary hosts for survival in absence of alternate hosts (Singh *et al.,* 2008). Urediniospores are

produced in large numbers during the crop season and wind dispersion transmits these urediniospores onto same or new host plants in the vicinity or distantly.

Most spores are disposed close to the source but long distance dispersal is well documented. including single event extremely long distance (cross-continent) dispersal that results in colonization of new regions. This type of dispersion is rare and unpredictable. Several examples of long distance dispersal have been described including the introduction of sugarcane rust into the Americas from Cameroon in 1978 and a wheat stem rust introduction into Australia from Southern Africa in 1969 (Brown and Hovmoller, 2002). An enabling factor in this type of dispersion is the robust nature of spores ensuring protection against environmental damage (Roelfs *et al.*, 1985). Airborne spores are deposited in new territories through deposition into susceptible host through rain-scrubbing. Deposition in new areas is primarily through rain-scrubbing of air-borne spores onto susceptible hosts (CABI, 2018).

Assisted long-distance dispersal on travelers' clothes or infected plant material also contributes highly to colonization of new areas by pathogens. Even though phyto-sanitary restrictions have been beefed up in many countries, increase flight travel as well as globalization still posses the risk of pathogens being spread. Strong evidence supports the accidental introduction of yellow rust into Australia in 1979 (Steele *et al*., 2001), probably on travelers' clothing.

Stepwise range expansion, occurs over shorter distances and more often than not within a country or region. A good example is the spread of a *Yr9*-virulent race of *Puccinia striiformis* that evolved in Eastern Africa and migrated to South Asia through the Middle

East and West Asia in a stepwise manner over about ten years and caused severe epidemics along its path (Singh *et al.,* 2004)

The extinction and re-colonization dispersal model is considered a sub-mechanism of stepwise range expansion and occurs in areas that have unsuitable conditions for year round survival, typically the temperate regions where hosts are absent during winter or summer (Singh *et al.,* 2008). A well documented extinction re-colonization example is that of wheat stripe rust survival and spread from the mountains in Gansu province of China (Brown and Hovmoller, 2002) and wheat rusts in the Himalayas and Nilgiri Hills in the northern and southern India respectively (Nagarajan and Joshi, 1985) where susceptible hosts can be found year round and environmental conditions are favorable for the pathogen to survive. Urediniospores from these areas are then blown to wheat fields in other areas to initiate disease.

2.3 Economic importance of Stem Rust and the *Ug99* Race

Stem rust (*Puccinia graminis* f. sp. *tritici*), has had a history of causing severe epidemics and was once the most feared disease in all wheat growing countries worldwide, causing losses of up to 100% in susceptible cultivars. Stem rust is primarily a warm weather disease but it can cause great damage to susceptible wheat crops over broad geographical regions (Kurt *et al.,* 2005). The dread of stem rust is understandable because an apparently healthy looking crop about three weeks to harvest affected with severe rust infection, which interrupts nutrient flow to developing heads is reduced to shriveled grain and weakened stems more prone to lodging and further loss of grain in mere weeks (CABI, 2018).

Stem rust was historically a problem in all of Africa, the Middle East, all of North and South America, the whole of Asia except Central Asia, Australia, New Zealand and Europe. Apart from the last major stem rust epidemic that occurred in Ethiopia in 1993 and 1994 (Shank, 1994), the rest of the world remained unhurt from stem rust for over three decades. This was attributed in part to the eradication of common barberry in plants in North America and the deployment of cultivars with resistance genes such as *Sr31*, *Sr24* and *Sr26* (Mehmet and Nuh, 2012). Epidemics caused by the other two rusts, leaf rust caused by *Puccinia triticina* and yellow rust caused by *Puccinia striiformis* were more common in recent years causing a shift in priority and resources away from stem rust research and breeding so much that in some countries testing and breeding for stem rust had been suspended.

Race *Ug99* was subsequently detected in Kenya and Ethiopia in 2005 (Wanyera *et al*., 2006) and in Sudan and Yemen in 2006 (Jin *et al*, 2007). A race of *Ug99* lineage with virulence to *Sr24* was detected in Kenya in 2006 (Jin *et al*., 2007). In 2007 epidemics caused by race TTKST with combined virulence to *Sr24* and *Sr31* were reported (FAO, 2010). Figure 2 below shows the current distribution of *Ug99* and its derivatives. Countries under the FAO danger list include the leading wheat producing countries worldwide including China, India, Russia, United States, Canada, Pakistan, Australia and Ukraine. Statistics and surveys done by the Global Rust Monitoring Systems show that *Ug99* (TTKS) has 13 identified variants. A survey done in Kenya in 2010 revealed 100% of the samples collected were infected by the TTKST variant of stem rust race *Ug99*. Most evidence, albeit circumstantial, indicates that *Ug99* is likely to spread beyond the borders of the Eastern Africa where it has currently colonized.

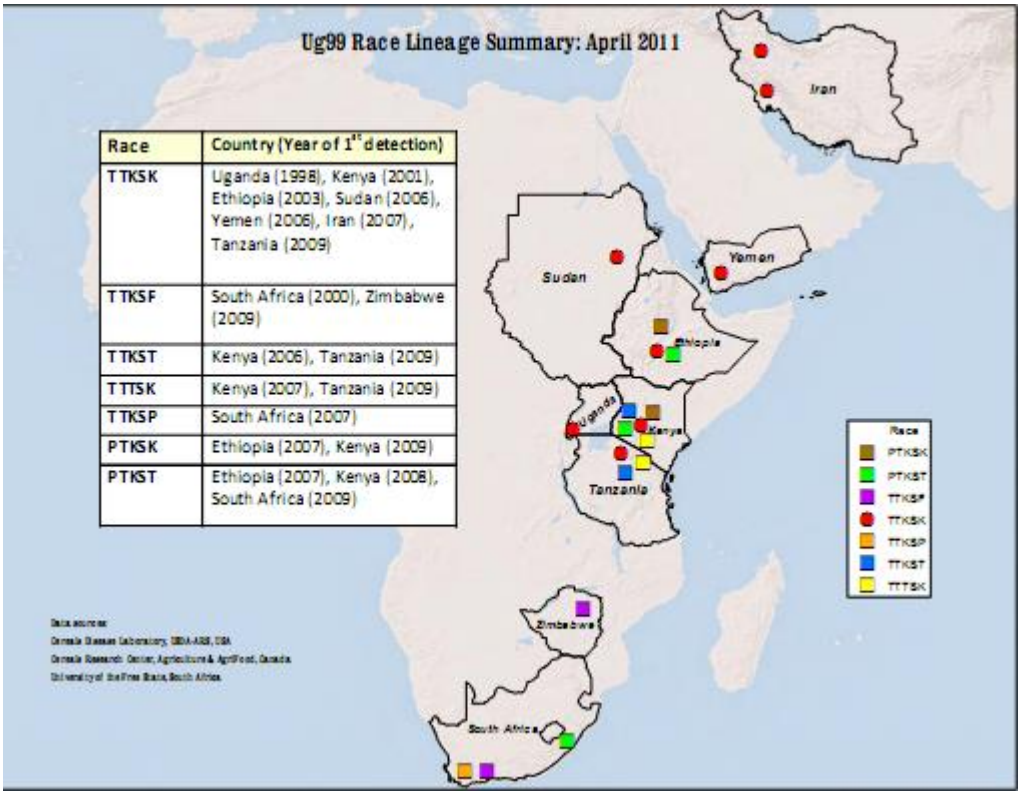


Figure 2.2 Distribution of *Ug99* and its derivatives. Source: FAO, 2010

2.4 Control and Management of Stem Rust

It is essential to understand the epidemiology of a disease before starting any control strategy, especially one involving cultural or chemical methods. A combination of cultural control practices with disease resistance and perhaps fungicide applications will be the most effective means of controlling the stem rust disease (Curtis, 2002). Quarantine measures against the pathogen can only delay and not prevent entry of disease owing to the airborne nature of the innoculum of stem rust.

2.4.1 Chemical (Fungicide) Control

Chemical control has been used successfully in Europe, permitting high yields of up to 6 to 7 tonnes/ha (Buchenauer, 1982). Fungicide control is effective when applied at early disease onset. Two active ingredients of fungicides are known to be effective for use against stem rust namely Propiconazole and Tebuconazole (Wanyera *et al.,* 2009). Studies done in Kenya in 2009 found foliar fungicides with these active ingredients to be effective in significantly reducing stem rust race *Ug99* severities in the field (Wanyera *et al.*, 2009).

2.4.2 Cultural Control

Cultural methods provide a method for partial management of rust epidemics. Although no single practice is effective under all conditions, using a series of cultural practices greatly enhances existing resistances. Use of early maturing cultivars marked initial success in controlling stem rust in Australia (McIntosh, 1992) while Mexican farmers learned to sow early to avoid stem rust prior to use of resistant cultivars (Borlaug, 1954). Removing the green-bridge with tillage or herbicides is an effective control measure for epidemics that would result from endogenous innoculum. Benefits of gene deployment can be obtained by a farmer if more than one cultivar is used that differs in resistance from those grown by immediate neighbors. On large farms it may help if fields are arranged so that early maturing cultivars are downwind from late maturing cultivars.

2.4.3 Eradication of Alternate Hosts

An alternate host eradication programme for stem rust was successful in northern Europe (Hermansen, 1968) and north-central states of United States (Roelfs, 1988). Except for Eastern Europe and north-western United States, no other areas of the world are yet known where alternate hosts play any role in stem rust epidemiology.

2.4.4 Genetic Control

Deployment of resistant cultivars as a strategy for control of stem rust enjoyed much success over the twentieth century. The *Sr2* resistance gene derived from Hope is the only catalogued adult plant stem rust resistance gene and results in reduced uredinia in the internode tissues (Hare and McIntosh, 1979). *Sr2* has been the most universally used *Sr* gene worldwide since the 1940s. The 1BL.1RS wheat-rye translocation associated with *Sr31, Lr26* and *Yr9* (Singh *et al.*, 2002) provided protection against stem rust for over 30 years prior to its breakdown by race *Ug99*. *Sr2* and *Sr31* are currently present in many high-yielding wheat cultivars grown around the world.

2.5 Seedling and Adult Plant Resistance Genes

Identification, deployment and stewardship of genetic sources of resistance are strategies intensively pursued today for the sustainable control of rusts. The recent breaches of some of the most widely deployed genetic defenses by *Ug99* underscores the ongoing need for new sources of resistance.

Rust resistance genes are classified as either being race-specific or race non-specific genes. Most resistance genes discovered and deployed against wheat stem rust are major genes or race-specific genes. They are mostly found at a single loci and confer effective

levels of resistance against specific physiologic races of the pathogen generally throughout the lifecycle of the host i.e. seedling resistance (Lowe *et al*., 2010). A race-specific resistance gene though effective against some races of the pathogen, is by definition vulnerable to at least one other race (Lowe *et al*., 2010). They are for this reason said to lack 'durability' that is the ability of a widely deployed resistance gene to provide an economic level of protection over an extended period of time (Johnson 1984). Race-specific resistance has commonly proved ephemeral due to evolution of virulent fungal isolates that negated the breeders' efforts leading to spectacular "boom and bust" cycles (Priyamvada *et al.*, 2011).

Breakdown of genetic resistance occurs due to the evolution of local pathogen population because of selection of mutants, recombinants or immigrants that are better adapted to the resistant cultivar due to increased pathogenicity (Singh et al., 2006). All pathogens are variable with respect to host resistance, but virulence in itself is a variable quality (Priyamvada *et al.,* 2011). Favorable environmental conditions for the pathogen play a role in reducing effectiveness of resistance, this coupled with size of pathogen population and increase in susceptible hosts affect the rate of pathogen evolution. Environmental factors could also include reduction in resistance in host cultivar due to changes in the conditions of cultivation like higher or lower fertility or moisture (Priyamvada *et al.,* 2011).

Adult plant resistance to stem rust describes a form of quantitative disease resistance that is detected in mature plants and is associated with non-race specific resistance and is quantitatively inherited (Knott, 1982). Sources of quantitative resistance in crop plants

have proven to be highly durable (Parlevliet, 2002) making APR genes attractive targets for long-term durable stem rust resistance. APR genes (also known as horizontal resistance or slow-rusting resistance genes) confer partial resistance and are expressed as slow rusting and are associated with longer latent periods, fewer and smaller uredinia and spore reduction when compared to susceptible checks. Uniform or race non-specific resistance is by definition permanent because any variety possessing it should be effective to the same degree against all the races of a pathogen regardless of differences in specific virulence on other varieties (Vanderplank, 1963; Robinson, 1973).

The most successful first catalogued APR gene to stem rust, *Sr2*, has provided partial resistance to all stem rust races since its deployment in the 1920s (McFadden, 1930; McIntosh *et al*., 1995). *Sr2* provides a degree of resistance expressed as slow rusting though not adequate under high disease pressure. It provides adequate levels of resistance in combination with other minor genes. Unfortunately, not much is known about the other genes in the *Sr*2 complex and their interactions but Knott (1988) revealed that adequate levels of stem rust resistance can be achieved by accumulating approximately four to five minor genes in the same genetic background, referred to as gene pyramiding. Gene pyramiding is likely to result in negligible disease levels at maturity under high disease pressure described as “near-immunity” by Singh *et al.,* (2000).

Traditionally partial resistance genes were difficult to isolate due to their relatively minor effects and were difficult to combine with major genes, but this has been made possible and now routinely addressed with quantitative genetic methods like QTL mapping (Lowe *et al*., 2010). Molecular markers can be used to tag resistance genes and further be used

in improving the efficiency of selection in plant breeding by marker-assisted selection (MAS). Marker assisted selection is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust resistance cultivars. Selection of genotypes with combinations of race-nospecific resistance genes defining durable resistance over years as well as race specific genes at seedling stage is a task of prime importance for molecular assisted selection (Parlevliet, 2002).

2.6 Current genetic diversity for Rust Resistance genes

A great majority of modern bread wheat varieties grown worldwide carry resistance genes *Sr2, Sr24, Sr30, Sr31* and *Sr36*, all of which are race-specific genes, that were effective either alone or in combinations prior to detection of *Ug99* and its derivatives (Bariana, 2008). Adoption of CIMMYT germplasm worldwide also promoted use of bread wheat germplasm carrying *Sr2, Sr8a, Sr17, Sr30* and *Sr31* in various combinations and also *Sr38,* widely distributed due to its linkage with *Lr37, Yr17*. (Bariana, 2008). *Cre5. Sr8b, Sr9e and Sr13* are present in different combinations in durum cultivars of which only *Sr13* is effective against *Ug99* (Bariana, 2008). Synthetic hexaploid germplasm have genes from durum, predominantly *Sr9e, Sr8b* and *Sr13* and the *Triticum tauschii* derived genes *Sr33, Sr45* and *Sr46* (Bariana, 2008).

2.7 Molecular Markers used in Mapping

A genetic marker can be defined as a chromosome landmark or allele that allows for the tracing of a specific region of DNA; or as a gene whose phenotypic expression is usually easily identifiable and used to identify an individual or cell that carries it or as a probe to mark a nucleus, chromosomes or locus (King and Stansfield, 1990). By learning where

markers occur in a chromosome and how close they are to specific genes, they can be used to create a genetic linkage maps. A genetic map can then serve several purposes including detailed analysis of associations between economically important traits and genes or quantitative trait loci (QTLs) and facilitate the introgression and pyramiding of desirable genes and traits into genotypes with favorable genetic backgrounds (Semagn *et al*., 2006).

Various molecular markers are classified into different groups based on mode of transmission (biparental nuclear inheritance, maternal nuclear inheritance, maternal organelle inheritance or parental organelle inheritance), mode of gene action (dominant or co-dominant markers) or method of analysis (hybridization-based or PCR-based markers) (Semagn *et al*., 2006).

Properties of a good molecular marker include high polymorphism, co-dominant inheritance, frequent occurrence and even distribution throughout the genome, selectively neutral behavior, easy access, easy and fast assay, low cost and high-throughput, high reproducibility and transferability within laboratories, populations and/or species (Semagn *et al*., 2006). No molecular marker yet fulfills all these requirements and hence several factors need to be considered in choosing one or more of the various molecular markers (Yang *et al.,* 1996; Rungis *et al*., 2005) including;

- Marker system availability
- Simplicity of the technique and time availability
- Anticipated level of polymorphism in the population
- Quality and quantity of DNA available
- Transferability between laboratories, populations, pedigrees and species

- The size and structure of population to be studied
- Availability of adequate skills and equipment
- Data generation costs and resources availability
- Marker inheritance (dominant or co-dominant) and the type of genetic information being sought.

There are different molecular platforms available for research that include but not limited to;

- random amplified polymorphic DNA (RAPDs),
- amplified fragment length polymorphism (AFLP),
- inter-simple sequence repeats (IS*SR*),
- restriction fragment length polymorphism (RFLP),
- microsatellites (SSRs),
- expressed sequence tags (ESTs),
- cleaved amplified polymorphic sequence (CAPS),
- sequence characterized amplified region (SCAR),
- sequence tagged sites (STS),
- single nucleotide polymorphism (SNP)
- and diversity array technology (DArT).

RFLP is the most widely used hybridization-based molecular marker and the first genetic maps were produced using RFLPs (Halentjaris *et al*., 1986). It is based on restriction enzymes that reveal a pattern difference between DNA fragment sizes in individual organisms. AFLP combines the power of RFLP with the flexibility of PCR-based technology by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh, 1998).

RAPDs use a single arbitrary oligonucleotide primer to amplify template DNA without prior knowledge of the target sequence. RAPD markers have been used to construct linkage maps in several species but has not found wide acceptance (e.g., Demeke *et al.*, 1997; Yang *et al.*, 1996). Non-reproducibility, co-migration of RAPD markers and their dominant inheritance has limited their use in mapping (Semagn *et al.*, 2006).

A scar marker is a genomic DNA fragment that is identified by PCR amplification using a pair of specific oligonucleotide primers (Paran and Michelmore, 1993) and are derived by cloning the two ends of RAPD markers that appeared to be diagnostic for specific purposes for example disease resistance. SCARs are advantageous over RAPD markers as they detect only a single locus, their amplification is less sensitive to reaction conditions and they can potentially be converted into co-dominant markers (Paran and Michelmore, 1993).

DNA fragments large enough for amplification occurring between two oppositely oriented SSRs are what are to as ISSRs. Using microsatellite as primers in a single primer reaction, multiple genomic loci are targeted to amplify mainly inter sequence repeats of different sizes (Semagn *et al*., 2006). ISSRs are highly specific and require sequence information for primer synthesis as it uses random markers; it is also quick and simple and shows high level of polymorphism (Collard et al., 2005).

Public accessibility to genome sequences of several organisms has enabled the study of sequence variations between individuals, cultivars, and subspecies (Semagn *et al.*, 2006). These studies have led to the revelation that single nucleotide polymorphisms (SNPs), insertions and deletions (InDels) are highly abundant and distributed thought the genome

of various species including plants (Vignal *et al.,* 2002). An SNP marker is a single base change in a DNA sequence with a usual alternative of two possible nucleotides at a given position

DArT enables use of a large number of polymorphic loci in a genome on genotypes from respective crop genotypes. DArT not needing prior sequence information for target study species provides a fast high throughput, and highly reproducible method of genotyping (Wenzl et al., 2004) It is also cost effective with the genetic scope of analysis being defined by the user and is easily expandable and it is not covered by exclusive patent rights but on the contrary is an open-source resource (Semagn *et al.,* 2006).

ESTs are derived from complimentary DNA (cDNA) that are synthesized by an enzyme called reverse transcriptase from functional messenger RNA sequences that serve as templates for protein synthesis. ESTs are quite instrumental in gene discovery, for obtaining data on gene expression and regulation, sequence determination and for developing highly valuable molecular markers such as EST-based RFLPs, SSRs, SNPs, and CAPs (Harushima *et al*., 1998). Genetic mapping with ESTs would enable a more rapid transfer of linkage information between species (Cato *et al.*, 2001), however, the scope of EST-derived marker development is limited to species for which sequencing databases already exist (Eujayl *et al.*, 2004).

STS is a short, unique sequence whose exact sequence is found nowhere else in the genome (Semagn *et al*., 2006). In plants STS is characterized by a pair of primers that are designed by sequencing either an RFLP probe representing a mapped low copy number sequence (Blake *et al*., 1996) or AFLP fragments. STS markers are able to distinguish

segregating populations are easy to reproduce and are PCR-automatable (Reamon-Buttner and Jung, 2000).

CAPs is a combination of PCR and RFLP, and the technique involves amplification of target DNA through PCR followed by digesting with restriction enzymes (Powell *et al.,* 1996). CAP markers rely on differences in restriction enzyme digestion patterns of PCR fragments caused by nucleotide polymorphism between samples. The ability of CAPs to detect DNA polymorphism is however not as high as SSRs and AFLPs because nucleotide changes affecting restriction sites are essential for the detection of DNA polymorphisms through CAPs (Semagn *et al*., 2006).

Microsatellites otherwise known as simple sequence repeats (SSRs) are the smallest class of simple repetitive DNA sequences (Armour *et al.,* 1999). SSR allelic differences are the results of variable numbers of repeat units within the microsatellite structure and these markers present high levels of inter- and intra-specific polymorphism, particularly with repeat numbers of ten or higher (Queller *et al*., 1993). Development of microsatellite markers involves microsatellite library construction, identification of unique microsatellite loci, identifying a suitable region for primer design, obtaining PCR products, evaluation and interpretation of banding patterns and assessing PCR products for polymorphism (Roder *et al*., 1998). SSRs are a marker of choice as they are highly polymorphic even between closely related lines, require low amount of DNA, can be easily automated for high-throughput screening and are highly reproducible (Gupta *et al*., 1999). SSRS also have high information content, co-dominant inheritance and locus specificity. High development costs and effort required to obtain working primers are a

major constraint of using SSRs from genomic libraries. With the availability of large numbers of ESTs and masses of DNA sequence data, development of EST-based S*SR* markers through data mining has become a fast, efficient, and relatively inexpensive in comparison to development of genomic SSRs (Gupta *et al*., 2003).

2.8 Types of Mapping Populations

The first step in producing a mapping population is selection of two genetically divergent parents showing clear genetic differences for the trait(s) of interest, but not too distant so as to cause sterility of progenies or show very high levels of segregation distortion during linkage analysis (Semagn *et al*., 2006). Choice of parents and mating designs to be used for development of mapping population as well as type of markers to be used depend largely on the objectives of the experiments, availability of markers and the molecular map, the timeframe as well as resources available for undertaking QTL analysis (Singh and Singh, 2015). Different types of mapping populations that are often used in mapping for self-pollinating species include F_2 populations, F_2 derived F_3 (F_2:F_3) populations, backcross populations (BCs), doubled haploids (DHs), recombinant inbred lines (RILs) and near-isogenic lines (NILs) (Burr *et al.*, 1988).

F_2 populations are products of a single meiotic cycle and are good for preliminary mapping as they require little time and minimum efforts to develop, however the one cycle of recombination limits their use for fine mapping and mapping of quantitative traits(Dhingani et al., 2015). This is because each individual is genetically different and cannot be evaluated in replicated trials over locations and years hence the effect of G X E

interaction on the expression of quantitative traits cannot be estimated precisely as it is also not a long term population making seed increase difficult (Semagn et al., 2010).

F_2 derived F_3 populations are obtained by selfing the F_2 individuals for a single generation and are suitable for mapping of quantitative traits or recessive genes. $F_{2:3}$ families can be used for reconstituting the genotype of respective F_2 plants by pooling the DNA from plants in the family but like the F_2 populations they are not a long term population.

RILs are produced by continuous selfing or sib mating the progeny of individual lines of an F_2 population until near complete homozygosity is attained and this is best achieved by single seed descent (SSD) (Keurentjes et al., 2011). The genetic segregation for both dominant and co-dominant markers would be 1:1. Once homozygosity is achieved RILs can be propagated indefinitely hence can be replicated over locations therefore are of immense value in mapping of quantitatively inherited traits i.e. QTL mapping. As RILs are obtained after several cycles of meiosis, they are especially useful in identifying tightly linked markers.

Doubled haploid plants are as a result of chromosome doubling of anther cultured derived haploid plants hence are also the products of one meiotic cycle (Santra et al., 2017). The expected ratio for markers is 1:1 irrespective of whether the marker is dominant or co-dominant. With production of DHs homozygosity is achieved instantly thus saving time and giving rise to a permanent mapping population that can be replicated over locations and years (Warwick Crop Centre, 2016). This makes DHs particularly useful in mapping of both qualitative and quantitative traits. However, depending on which gamete is used

for haploid production, recombination is accounted for from only one side (either male or female).

NILs are generated either by repeated selfing or backcrossing F_1 plants to the recurrent parents (Tanksley *et al.*, 1995). NILs developed through backcrossing are similar to the recurrent parent but for the gene of interest (Wellings et al., 2009) while those developed through selfing are similar in pair but for the gene of interest. Expected segregations for markers is 1:1 irrespective of their genetic nature. NILs are immortal mapping populations hence are suitable for tagging traits whenever such populations are available and are also quite useful in functional genomics.

Backcross populations are developed by crossing the F_1 with either of the parents, usually the recessive parent (test cross) for genetic analysis resulting in genotypes with more uniform backgrounds for clarity of mapping results(Septiningsih et al., 2003). Backcross populations require little time to develop but are not permanent and the recombination information is based on only one parent (Babu *et al.,* 2004).

2.9 Statistical Approaches to QTL Mapping

After generation of appropriate genotyping and phenotypic data, the next step is to test the two hypotheses in QTL analysis. One being that the null hypothesis (H_0) that no QTL is present or a QTL is present but is not linked to the markers and the other being the alternate hypothesis (H_A) that a QTL is present and is linked to the markers. Several approaches have been developed for determining QTL/trait associations.

I. Single Marker Approach

This method, also known as the single factor analysis of variance (SF-ANOVA) is done for each marker locus independent of information from other loci. The test statistic underlying the ANOVA test, the F-tests, are used to test the significance of marker –locus genotype differences. This approach is quite simple to undertake owing to the fact that it does not need prior construction of a genetic map, but it has a few disadvantages. This include (i) the likelihood of QTL detection decreases significantly as the distances between marker and QTL increases, (ii) the method cannot determine whether the markers are associated with one or more QTLS and (iii) the effects of QTL are likely to be underestimated because they are confounded with recombination frequencies (Semagn *et al.*, 2010).

II. Simple Interval Mapping (SIM)

This method was developed by Lander and Botstein (1989) and requires prior construction of a linkage map. SIM uses one marker-interval at a time to search for a hypothetical target QTL by performing a likelihood ratio test at every position within the interval. Simple interval QTL mapping algorithim test is nam improvement of single factor QTL analysis in that it test for putative QTLs between every two adjacent markers(Plant and Soil Science eLibrary, 2018). The chromosomal location of the maximum LOD score surpassing significant threshold is taken as the position of the QTL (Semagn *et al*., 2010). When multiple QTLs are present in a segregating cross however, SIM fails to take into account genetic variance caused by other QTLs (Haley and Knott 1992).

III. Composite Interval Mapping (CIM)

Jansen (1993) and Zeng (1993) independently proposed combining SIM with multiple regression analysis in mapping termed as ‘composite interval mapping’ (CIM). CIM evaluates the possible presence of a target QTL at multiple analysis points across each inter-marker interval and it also includes, at each point, the effect of one or more background markers, often referred to as cofactors (Semagn *et al.,* 2010). CIM has several advantages; (i) that mapping of several QTLs can be achieved by the search in one dimension, (ii) by using linked markers as cofactors, the test is not affected by QTL outside the region thus increasing the precision of QTL mapping and (iii) by eliminating much of the genetic variance by other QTL, the residual variance is reduced thereby increasing the power of detection of QTL(Plant and Soil Science eLibrary, 2018).

IV. Multiple Interval Mapping (MIM)

To address the limitations of CIM, Kao *et al.*, (1999) proposed and implemented multiple interval mapping (MIM). The principle behind MIM is to fit multiple putative QTL and effects associated epistatic effects directly in a model to facilitate the search, test and estimation positions, effects and interactions of multiple QTLs (Semagn *et al.*, 2010).

CHAPTER THREE

Use of marker assisted selection (MAS) for the characterization and introgression of rust resistance genes using an F_2 Robin/Kwale population

Abstract

Breeding for durably resistant varieties is among the best strategies to exterminate the threat posed by stem rust race *Ug99* of wheat. This race has continued to spread and ascertain itself as a danger to global wheat production and consequently food security. The continuous swift development of new DNA marker technologies has proved invaluable in breeding for durable rust resistance to stem rust race *Ug99* and its lineage of races. This study aimed to evaluate introgression of stem rust resistance genes from variety Robin to Kwale using a bi-parental population. To ascertain purity of seed lots used to develop the population, five seeds of each parental genotype (Kwale and Robin) were sowed and nucleic acids isolated from their leaves. They were screened with a panel of ten microsatellite markers to evaluate genetic purity. Results revealed differences in banding patterns among samples for each of the parents. For the introgression studies, 315 F_2 progeny lines of the Robin /Kwale bi-parental population were evaluated from their response to stem rust under field conditions. Simple sequence repeat markers were used to evaluate introgression of genes Sr2 and SrTmp for stem rust resistance against race Ug99.Frequency distribution tables of co-efficient of infection data for 315 F_2 plants revealed that segregation leaned more towards resistance. Chi square analysis using F_2 plants data revealed an expected 13:3 ratio of one dominant and one recessive gene conferring resistance to stem rust, revealing that introgression was indeed achieved.

Results of this study underscore the importance of maintaining varietal purity, and proved to embolden use of molecular markers as an efficient tool for selection of genotypes with the desired traits/genes in breeding populations.

3.1 Introduction

Rust diseases of wheat cause notable losses particularly of grain production in cereals. Stem or black rust of wheat, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), has recently gained significance after the discovery of the broadly virulent African strain *Ug99* (FAO, 2010). This race and its lineage of races continue to cause a stir in the minds of breeders, a race against time to find practical and sustainable solutions to combat the threat they pose to global wheat production and food security. Two major strategies for combating rusts are chemical and genetic control, the latter being the preferred strategy as it is environmentally and economically feasible particularly to financially constrained farmers in developing countries.

Earlier efforts to combat *Ug99* included screening and identification of sources of resistance from available germplasm. The original *Ug99* race virulent to stem rust resistance gene *Sr31* was designated as TTKSK by North American nomenclature system (Wanyera et al 2006). Several documented resistance genes namely *Sr23, Sr22, Sr24, Sr25, Sr26, Sr27, Sr28, Sr33, Sr35, Sr36, Sr37, Sr39, Sr40, Sr44* and *SrTmp* revealed low infection types to *Ug99* race TTKSK in green house tests (Jin et al 2007). As the *Ug99* race continued to evolve and conquer new lands, variants with combined virulence to *Sr31+ Sr24* were detected in 2006 first in Kenya then in Tanzania (2009), Eritrea (2010) and Uganda (2012) (CIMMYT, 2016). Again variants with combined virulence to

Sr31+Sr36 were detected first in Kenya in 2007 and then in Tanzania (2009), Ethiopia (2010), Uganda (2012) and Rwanda (2014) (CIMMYT, 2016). *Ug99* continues to evolve, believed to be through single step mutations, and currently 13 variants of this potent race are known (CIMMYT, 2016).

Wheat varieties with genetically controlled resistance provide breeders with a proficient means to control stem rust, being simple, practical and economical in addition to saving time, energy and money spent on other measures of control (Burdon et al 2014). To achieve this conventionally through traditional breeding techniques takes extended periods of time that farmers do not have.

Biotechnology thorough the use of DNA molecular markers presents a viable and feasible option to breed for resistant varieties in shorter periods by improving the efficiency of selection hence cutting the breeding time to almost half. The objective of this study was to improve the popular Kenyan wheat variety Kwale with the introgression of genes from the resistant variety Robin. The success of introgression was assessed by use of microsatellite markers polymorphic on the two parents. Superior transgressive segregants were identified and recommended for use in the breeding programme in breeding for resistance to stem rust race *Ug99*.

3.2 Materials and Methods

3.2.1 The site

The study was carried out at the KALRO-Njoro International Stem Rust Screening Fields. This area is a hot spot for rust development due to its prevalent weather conditions and the presence of wheat in the fields the whole year through providing the green-bridge needed for the rust fungus to survive from season to season. The nursery site is located at 0º20'S, 35º56E, and 2,185 meters above sea level.

3.2.2 Plant Materials

An F_2 population was used in this study. One thousand F_2 seeds were generously provided by Dr. Peter Njau, from the Kenya Agricultural Research Institute-National Plant Breeding Center for Wheat at Njoro. This population was a cross between the popular wheat varieties Kwale and Robin. This cross was made intentionally to introgress the stem rust resistance gene *SrTmp* among other traits present in Robin (Babax/Lr 42//Babax*2/3Tukuru) into the variety 'Kwale'. Kwale (Kavkaz/Tanori-71/3/Maya/-74(SIB)/Bluebird/Inia-66) was bred at KARI-Njoro, (now KALRO-Njoro), and released in 1974. It is a late maturing semi-dwarf variety very popular among Kenyan wheat farmers for its high yields averaging 7 tonnes/ha, producing flour with high protein content and good milling and baking qualities. It also posses the earliest catalogued adult plant resistance gene, *Sr2*. Introgressing *SrTmp* into Kwale was expected to generate progeny exhibiting higher levels of resistance.

3.2.3 Field Experiment

Experiments for evaluation of Adult Plant Resistance to stem rust on the F_2 population were conducted in the main season of 2013 between May and October. A single F_2 seed was sowed individually in a 2.4M by 1.6M block, 96 seeds in eight rows and twelve columns per block with a distance of 20cm between the seeds. There were ten such blocks hence a total of 960 individual F_2 seeds were sowed. The parents Kwale and Robin were planted in each block at the 95 and 96 position. Two continuous rows of spreader plants (a mixture of cultivars Thatcher, Morroco, and Cacuke, known to be highly susceptible to stem rust) were planted around all the ten blocks to facilitate uniform disease infection. Another spreader row was planted between two adjacent rows of plants within the individual blocks.

To initiate artificial stem rust epidemic, spreader rows were inoculated using a solution of fresh stem rust urediniospores collected from the KALRO-Njoro trap nurseries that were predominantly of the race TTKSK (race *Ug99*). Urediniospores were suspended in water then injected into individual spreader plants prior to booting (growth stage Z35-37; Zadoks et al, 1974). To further boost disease innoculum, spreader plants were also sprayed with a suspension of urediniospores suspended in a light weight mineral oil Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX) twice during stem elongation.

Nitrogen and Phosphorus fertilizers were applied during planting at the rate of 22.5 kg N ha^{-1} and 25.3 kg P ha^{-1}, respectively. A post emergence herbicide, Buctril MC at 225 g $L^{-1,}$ was sprayed at tillering stage at the rate of 7 ml L^{-1} of water to control broad-leafed

weeds. Insect pests were controlled by the use of Buldock Duo (225 g L^{-1} Beta-Cyfluthrin) sprayed at the rate of 10 ml L^{-1} of water. Weeding was carried twice manually twice, between stem elongation and booting stages to eradicate grasses.

At booting stage, 500 well established plants were tagged and numbered (from 1 to 500). Disease notes were recorded as described by Peterson et al in the modified Cobb's scale twice on the plants (described below) after which the plants were sprayed with Folicur, a foliar fungicide used for the control of stem rust. This was done to enable enough seed to be harvested for phenotyping studies to be done on the next generation of $F_{2:3}$ families.

$F_{2:3}$ families were descendants of an individual F_2 plant. 315 entries were selected from the 500 plants tagged and harvested at the F_2 stage to provide $F_{2:3}$ populations. Twenty seeds of each $F_{2:3}$ family were planted in hill plots 50cm between rows and 30cm between entries. Two rows of spreader plants were sowed around the block and between every two rows of entries. Artificial stem rust epidemics were initiated as described above. Respective agronomic practices were adhered to as described above.

3.2.4 Phenotyping

Disease infection on developed plants was scored twice on the 500 F_2 plants and thrice on 315 $F_{2:3}$ families using the modified Cobb Scale described by Peterson et al., 1948 when the susceptible check had reached maximum severity. Severity was scored as % infestation of disease on the plant (total area of stem covered by the disease) and host plant response recorded as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) as described by Knott, 1989.

The parents and their families were also evaluated for the presence of the pseudo black chaff (PBC) phenotype, generally used a morphological marker of the stem rust gene *Sr2* (Mishra et al., 2005)

Plants and families were scored and categorized as either homozygous resistant or homozygous susceptible based on the comparison with the infection type of the parents Robin and Kwale. Robin has an observed infection of 'moderately resistant (*MR*) to moderately susceptible (*MRMS* or simply *M*)', and progeny lines with this infection type at the F_2 generation were considered as resistant. The parent Kwale has an observed moderately susceptible to susceptible ('*MS-MSS*') infection, and progeny lines with this infection were considered susceptible.

3.2.5 Statistical Analysis

A coefficient of infection (CI) was computed by combining disease severity and host response data into a single value. Thus, severity was multiplied with a constant for host response (Yu et al. 2011) where immune = 0.0, R = 0.2, MR = 0.4, M = 0.6, MS = 0.8 and S = 1.0. For example, If a plant had a score of 40MR,its CI value would be 8, i.e 40(disease severity)*0.2(constant for host response). The CI was used to plot a frequency distribution histogram of mean disease severity in the cropping season.

Pearson's Chi Square goodness of fit test (McDonald, 2014) was computed using *"chisq.test"* function in R software (R Development Core Team, 2011) to estimate the number of genes present conditioning resistance to stem rust on our study population.

This test uses the formula;

$X^2 = \sum [(O-E)^2 /E]$

Where X^2 = Chi Square statistic

O = Observed Value

E = Expected Value

The Chi-square analysis was used to test the goodness of fit of observed ratios of resistance and susceptibility to expected Mendelian genetic ratios explaining various gene action theories.

3.2.6 Molecular Marker Assays

3.2.6.1 Verification of Parental Purity

Five seeds of each of the parental genotypes of Robin and Kwale were planted as a single seed, and named Robin one (Robin 1) to Robin five (Robin 5) and Kwale one (Kwale 1) to Kwale five (Kwale 5) respectively. Although wheat is primarily a self-pollinating crop, at heading stage the heads were covered with glycine bags to ensure that no cross contamination took place during pollination. Genomic DNA was extracted from young leaves of the parental genotypes and 500 F_2 plants using a modified Doyle and Doyle 1990 protocol.

Quantification and assessment of the quality of isolated DNA was done by comparing DNA samples with known concentrations of uncut, unmethylated lambda (λ) DNA (0.3 µg/ µl, Thermoscientific) standards in a 0.8% agarose (Sigma, UK) gel in 1x TBE buffer

(89.2mM Tris, 89.0mM Boric acid, 1.25mM EDTA pH 8.0). Lamda DNA of different concentrations and the isolated DNA samples were mixed with 2 µl of 6X loading dye (Thermoscientific) and resolved on agarose gels at 80 volts for 40 minutes.

The parental genotypes were analyzed for genetic purity and uniformity using microsatellite markers from the wheat genome. 20µl Aliquots of isolated DNA of parents (Kwale and Robin) were sent to CENGEN (PTY) Ltd, Worcester, Western Cape, South Africa for genetic analysis of parental purity using microsatellite markers. Five markers were used from chromosome 1D and five from chromosome 6A. The markers were chosen because of good marker profile and reproducibility. A list of the markers used is given in Table 3.1.

Table 3.1: Identities of SSR markers used for determination of genetic purity of parental genotypes

	Markers from chromosome 1D	***Markers from Chromosome 6A***
1	cfd48	Gwm427
2	Gdm111	Psp3152
3	Psp3000	Wmc179
4	Wmc147	Barc113
5	Wmc216	Wmc243

The mean number of alleles per locus (MNA), polymorphism information content (PIC), observed heterozygosity (HO) and expected heterozygosity (HE) were obtained across

different loci and populations using Excel Microsatellite Toolkit v. 3.1.1 Add-in utility for Microsoft Excel (Park, 2001). The genetic diversity statistic H_s was calculated per population, as described by Nei, 1978.

3.2.6.2 Polymorphism Screening of SSR Markers

Polymorphism screening of SSR markers was done using the parents, homozygous resistant (HR), homozygous susceptible (HS) and segregating bulks. Markers used were determined by looking at the pedigrees of the parents and the potential genes present in them as researched in literature. Sequences of markers for identified genes were sent to Inqaba Biotechnical Industries (Pty) Ltd, Muckleneuk Pretoria, South Africa for synthesis. The markers used are listed in Table 3.2.

Table 3.2; List of markers for stem rust resistance genes to evaluate presence of these genes in the population, determined from the pedigree of the parents Robin and Kwale

Gene	*Marker name*	*Chr[a]*	*Expected band size in bp[b]*	*Tm[c]*	*Primer Sequence*	*Reference*
Sr2	gwm533	3BS	120	60	5'AAGGCGAATCAAACGGAATA3' 5' GTTGCTTTAGGGGAAAAGCC 3'	Speilmeyer et al., 2001
Sr28	wmc332	2BL	169	50	Fwd; cATTTAcAAAgcgcATgAAgcc Rev; gAAAAcTTTgggAAcAAgAgcA	http://maswheat.ucdavis.edu
Sr31	SCM9	1B	207	60	5'TGACAACCCCCTTTCCCTCGT 3' TCATCGACGCTAAGGAGGACCC	Olson et al, 2010
SrTmp	xcfd49	6D	214	60	5' TGAGTTCTTCTGGTGAGGCA 3' 5' GAATCGGTTCACAAGGGAAA 3'	http://maswheat.ucdavis.edu
Sr42	xbarc183	6D	151	58	5' CCCGGGACCACCAGTAAGT 3' 5' GGATGGGGAATTGGAGATACAGAG 3'	Ghazvini et al, 2012
Lr34	csLV34	7D	150 (+ves) 229(–ves)	55	F 5'- GTT GGT TAA GAC TGG TGA TGG -3' R 5'- TGC TTG CTA TTG CTG AAT AGT -3'	http://maswheat.ucdavis.edu
Lr42	wmc432	1D	189	51	Fwd;ATgAcAccAgATcTAgcAc Rev; AATATTggcATgATTAcAcA	Liu et al, 2013

Chr[a] – Chromosome

Bp[b] – basepairs

Tm[c] – Annealing temperature

3.2.6.3 Molecular Characterization of F_2 Robin/Kwale Population

Genomic DNA was isolated from 4-6 week old seedlings of 500 F_2 plants of the Robin/Kwale population using a modified Doyle and Doyle 1990 protocol. Quantification of isolated DNA was done as previously described above.

Parents Kwale and Robin, HR, SEG and HS bulks were screened for polymorphism with SSR markers for genes *Sr2, Sr31, Lr34, Lr42, Sr42* and *SrTmp.* 19 homozygous resistant, 19 segregating and 19 homozygous susceptible lines were selected based on their phenotype scores at F_2 and F_3 generations (data in Table 3.4) and their DNA was used for the genotype studies. The two genotypes of the each of the parents were included in the assays as different samples, and named Robin a (Ra), Robin b (Rb), Kwale a (Ka) and Kwale b (Kb) respectively. Where available the positive control for the gene marker was included in the PCR assay. PCR was done as described below.

Polymerase chain reaction was performed using *Taq* PCR Master Mix Kit (250 U) from Qiagen. The final solution consisted of 6.25µl *Taq* PCR Master Mix, 0.25µl each of forward and reverse primers (10pmol), 0.75µl of 25mM $MgCl_2$, 4µl of double distilled de-ionized water (ddH_2O) and 2µl of template DNA to make a final volume of 12.5µl. The PCR was performed on a thermocycler from Applied Biosystems (model number 2720) at 94ºC for 5 minutes' initial denaturation, 45 cycles of 94ºC for 30 seconds, annealing temperatures and time ranging from 44-60ºC depending on the primer (shown in figure 3.2 on previous page), 72ºC for 60 seconds, and a final extension at 72ºC for 7 minutes.

Products of PCR amplification were resolved on 2% agarose gels (2 grams of agarose powder in 100 ml of 1X TBE; Tris-Boric-EDTA buffer). The gel solution was stained using 3µl of 10mg/ml EtBr (Ethidium bromide). Products and 3.5µl of a 100base pair molecular ladder (0.1µg/µl, from ThermoSCIENTIFIC) were resolved in an electrophoresis gel set (C.B.S Scientific) at 80 volts for eighty minutes. The gel was visualized under Ultra violet (UV) light trans-illuminator (E-Box VX5, Vilber Lourmat).

3.3 Results

3.3.1 Phenotyping

Sufficient disease pressure was observed in the cropping seasons during which the populations were tested in the field. Significant variation in disease severity and infection type was observed within the population as shown in the data in appendix 1.

Disease severity ranged from 1 to 60 with all infection types being observed. The distribution of disease severity leaned highly towards resistance, as observed in the frequency distribution histogram in *figure 3.1* below;

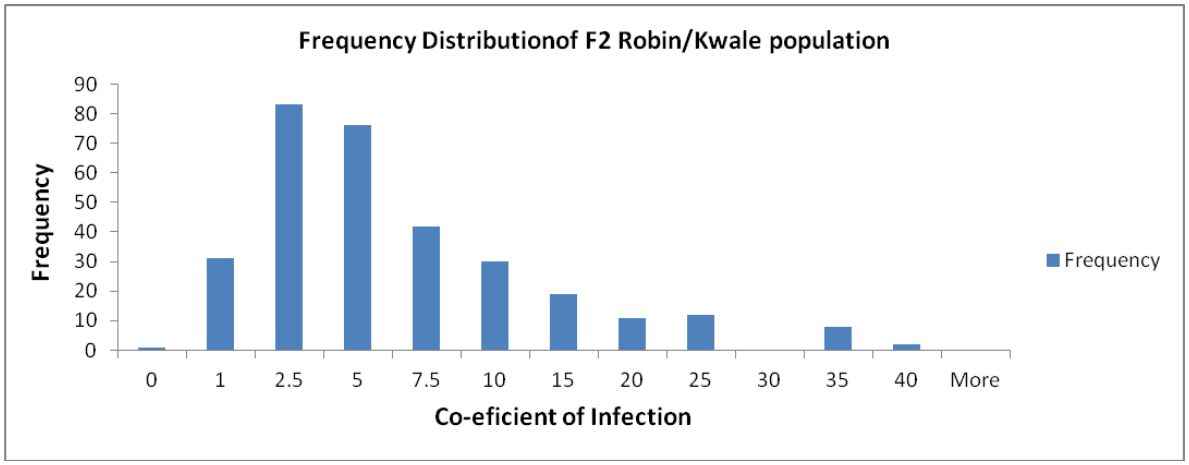


Figure 3.1: Frequency Distribution table of 315 lines selected for $F_{2:3}$ generations, using the Co-efficient of Infection (shown in Table 3.3) for the lines scored as F_2 plants.

Chi square analysis was performed to test the Mendelian gene ratios of 13:3, 9:7, 12:4 and 15:1, at a P-value of 0.05.

Table 3.3; Segregation data of F_2 lines, tested with different Expected Chi Square ratios to determine the underlying gene action

No. of Seg. F_3 Families	**F_2 Genetic Ratios 13:3**		**9:7**		**12:4**		**15:1**	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
Resistant	255.125	257	176.625	257	235.5	257	294.375	257
Susceptible	58.875	57	137.375	57	78.5	57	19.625	57
Total	314	314	314	314	314	314	314	314
Chi square and P value	x^2 = 0.0735* p-value = 0.7863		x^2 = 83.6011 p-value = <2.2e-16		x^2 = 7.85114 p-value = 0.005078		x^2 = 75.9244 p-value = <2.2e-16	

P value = 0.05

Among the ratios tested, the ratio of 13 (257) resistant to 3 (57) susceptible is the only one that fit the data observed, as shown in Table 3.4. This indicated that resistance exhibited by the population was conferred by one dominant gene and one recessive gene.

3.3.2 Verification of Parental Purity

Isolated DNA from parents was found to be intact and of high quality as is seen in Plate 3.1 below. The DNA was of good quality for subsequent SSR analysis that followed.

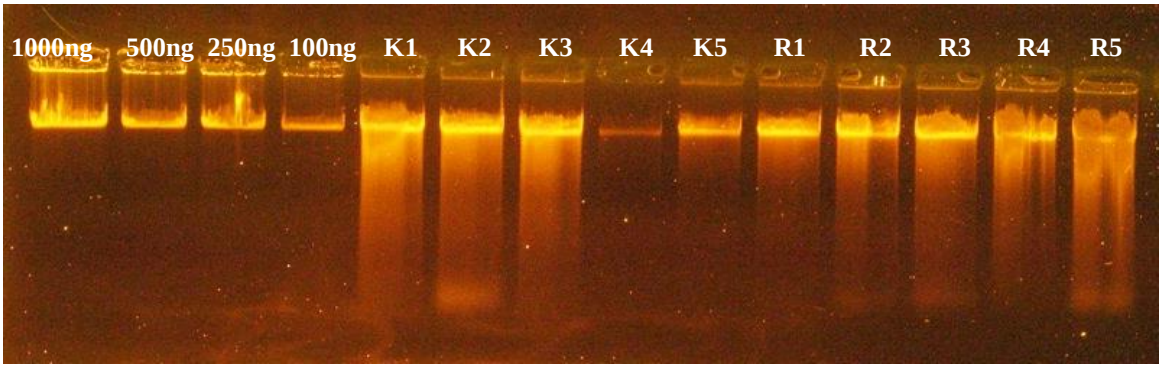


Plate 3.1; Gel picture showing results of quantification of DNA isolated from the parental lines Kwale (K1 to K5) and Robin (R1 to R5). Resolved on a 0.8% agarose gel stained with 2 µl of Ethidium Bromide.The first four samples are different concentration of lambda (λ) DNA.

Each marker produced an average of two alleles per loci. Different allele profiles were observed from both Robin and Kwale genotypes as shown in Table 3.5. Genotypes one and two for both Kwale and Robin amplified alleles of the same size, that differed from the allele sizes amplified from genotypes three, four and five. This revealed that the seeds of Robin and Kwale were mixed and not genetically pure. The genetic diversity indices calculated for this populations, albeit small, confirmed these results as the indices for each were quite high as seen in Table 3.6 below.

The highest PIC was observed for marker *cfd48* on Kwale and *wmc243* on Robin, indicating that with further testing and validation these two markers could be used effectively to differentiate the two different genotypes within the Kwale and Robin populations.

Table 3.4 Allele sizes amplified from the different genotypes using selected SSR markers to access for genetic purity

	cfd48		gdm111	psp3000	wmc147	wmc216		barc113		gwm427	psp3152	wmc179		wmc243	
Kwale_01	223	256	199	244	151	89	126	122		213	222	198		160	172
Kwale_02	223	256	192	244	151	93	126	110		198	240	235		160	172
Kwale_03	227	260	203		148	89	130	116		213	224	200	233	160	172
Kwale_04	227	260	203		148	89	130	110	116	213	224	200	233	160	172
Kwale_05	227	260	203		148	89	130	116		213	224	200	233	160	172
Robin_01	239	260	199	267		89	130	131		198	249	200		162	174
Robin_02	239	260	199	267	151	89	130	131		198	249	200		162	174
Robin_03	227	260	203		148	89	130	116	120	213	224	200	233	160	172
Robin_04	227	260	203		148	89	130	116		213	224	200	233	160	172
Robin_05	227	260	203		148	89	130								

From the above table, the parental genotypes were observed to prodeuce alleles of different sizes for the different markers used, as depicted by different colors used to highlght them. For Kwale the two genotypes highlighed in blue produced differernt allele sizes from those highlighted in green. While for Robin, different allele sizes were amplified from the the first two genotypes highlighted in orange, as opposed to those highlighted in yellow.

Table 3.5; Polymorphism information content (PIC) values for SSR markers used to evaluate genetic purity, and their respective Genetic diversity indices of the among the Kwale and Robin genotypes used.

PIC values		
	Populations....	
Locus	***Kwale***	***Robin***
cfd48	0.6918	0.5478
gdm111		
psp3000		
wmc147		
wmc216	0.6454	0.375
barc113	0.375	0.375
gwm427		
psp3152		
wmc179	0.375	0.375
wmc243	0.375	0.703125

Genetic Diversity				
Population	*Sample size*	*Loci typed*	*Unbiased Hs*	
Kwale	5	5	0.7511	
Robin	5	5	0.7537	

The PIC informed how effective the markers used were at differentiating the genotypes. This gave an insight into how useful they would be in differentiating the observed differences among the parental genotypes of Robin and Kwale. Marker cfd48 had the highest PIC for Kwale while marker wmc243 had the highest PIC for Robin. The

Unbiased *Hs* value was an indication of how different the genotypes were. This is revealed high indices suggesting genotypic differences among the parental genotypes used for both Kwale and Robin.

The differences observed genotypically from the parental genotypes prompted a closer evaluation of their phenotype in the field. The ear density was observed to be a distinct character that differentiated the genotypes for both Kwale and Robin. Pictures plates displaying the differences observed in the parental germplasm head types are below.

Plate 3.2; Different head (ear) types of Robin as observed at the KALRO-Njoro Fields.

As can be seen in the photos above, the heads in photo A are longer, the ear density is laxed and the heads appear to be coloured as compared to the heads in photo B that are more plump with a denser ear that appears white.

Plate 3.3; Different head types of Kwale as observed at the KALRO-Njoro Fields.

The above photos illustrate the differences aboserved between the two disparate head types of kwale, A and B. In photo A the heads are long, have a dark shade and are lax in dnsity. The heads in photo B are more dense, and have a darker shade as copmared to those in photo A.

3.3.3 Polymorphism Screening of Parents and Progeny lines with SSR markers

All the markers after the PCR assay had good products that were visible and could be scored and interpreted. Marker Xgwm533 for stem rust resistance gene *Sr2* amplified a 120 base pair (bp) product in positive samples. Thus the 120bp band was observed in all the parents and bulks included in the assay.

The stem rust resistance gene *Sr31* was assayed by using the SSR marker SCM9. The expected band size in positive samples for this marker is of 207 bp, observed in the second genotypes of both parents, Kb and Rb. There was no amplification for Ka, and Ra had multiple bands of approximately 190, 450 and 460 bp in reference with the molecular ladder. The HR and HS bulk samples had 207 bp products while the SEG bulk sample had two bands at 207bp and 450bp.

Marker csLv34 for the pleiotropic gene *Lr34* amplified a positive 150bp band only on the first Kwale genotype, Ka. DNA of the wheat variety Frontanna was used as a positive control in this assay. All other parental genotypes and bulks produced a characteristic 229bp present in negative samples.

Gene *Sr42* was assayed for by the SSR marker Xbarc183, and has an expected band size of 169bp in positive samples. For this assay we had no known positive control. All parental genotypes and bulks amplified the expected 169bp except for the second Robin genotype, Rb.

The gene in Robin *SrTmp* was assayed for by marker Xcfd49. This marker had an observed 214bp band amplified only in the second genotype of Robin Rb, while all other parental genotypes and bulks amplified a smaller band approximately 160bp in reference to the molecular ladder. The positive control in this assay also produced a 160 band in this assay.

Lr42 is a gene of which its carrier line is in the pedigree of Robin, and we assayed for it using the marker wmc432. The expected band size for this marker was a band of 189 bp. Both genotypes of Kwale were negative for this marker as no visible amplification was

observed. The first Robin genotype Ra, amplified two bands, a 189bp and a 300bp band. The second Robin genotype amplified only one band of 189 bp. The bulks HR, SEG and HS amplified all two bands of 189 and 300bp.

A summary of the results of the polymorphism screening follows in *table 3.7.* Following also is a table displaying the results of the screening of the individual HR, SEG and HS lines with the respective molecular markers *(table 3.8).* The table also displays the terminal disease severity of the lines in response to evaluating them for resistance to stem rust (Ug99).

Table 3.6 Summary table of polymorphism tests done on the different genotypes of Kwale and of Robin, together with the bulks. The table shows the different alleles amplified from testing the parents and the bulks with the markers intended to test the progeny lines from the F_2Robin/Kwale population.

Gene	Marker	Expected Band Size	KWALE A	KWALE B	ROBIN A	ROBIN B	HR	SEG	HS
Sr2	X3BO42G11	172bp	172	172	172	-	172	172	172
Sr2	Xgmw533	120bp	120	120	120	120	120	120	120
Sr31	SCM9	207bp	-	207	190,450,460	207	207	207,450	207
Sr42	Xbarc183	169bp	169	169	169	169	169	169	169
SrTmp	Xcfd49	160bp	160	160	160	214	160	160	160
Lr34	XcsLv34	150bp (+ves) 229bp (–ves)	150	229	229	229	229	229	229
Lr42	Xwmc432	189bp	NA	NA	189,300	189	189/300	189/300	189/300

Table 3.7; Terminal disease severity (TDS) data and observed molecular data for the selected HR, SEG and HS individual lines used for genotyping

	Terminal Disease Score				Molecular Marker Data					
F_2 No.	**F_2 TDS[a]**	**F_3 TDS[a]**			***Sr2***	***Sr31***	***Lr34***	***Lr42***	***SrTmp***	***Sr42***
	13/05/13	**31/102013**	**PBC[b]**	**Category[c]**	**Xgwm533**	**SCM9**	**csLv34**	**Xwmc432**	**Xcfd49**	**Xbarc183**
HOMOZYGOUS RESISTANT (HR)										
27	30MR	15MR		HR	120	207	229	NA[d]	160	169
35	10MR	10RMR		HR	NA[d]	NA[d]	NA[d]	NA[d]	160	NA[d]
41	15MR	10RMR		HR	120	NA[d]	229	200	160	169
53	15MR	15RMR		HR	NA[d]	207	229	200	160	169
74	15MR	10RMR	+	HR	120	207	229	200	160	169
97	10MR	15RMR	+	HR	120	NA[d]	229	200	160	169, 200
102	5MR	10RMR	+	HR	120	207	229	200	160	169
117	5MR	10RMR		HR	120	207	229	200	160	169, 500
194	10MR	10RMR		HR	120	207	229	200	160	169
208	5MR	10RMR	+	HR	120	207	229	200	160	169
230	10MR	15RMR	+	HR	120	207	229	200	160	169
246	5MR	5RMR	+	HR	120	207, 450, 460	229	200	160	169, 500
308	5MR	15RMR	+	HR	120	207, 450, 460	229	200	160	169, 500
337	5MR	10RMR	+	HR	120	207	229	200	160	169
372	5MR	15RMR	+	HR	120	207	229	200	160	169
373	R	15RMR	+	HR	120	207	229	200	160	169
381	5RMR	10RMR		HR	120	207, 450, 460	229	200	160	169, 500
413	5MR	10RMR		HR	120	207, 450, 460	NA[d]	200	160	169, 413
479	10MR	20RMR		HR	120	207	NA[d]	200	160	169

SEGREGATING (SEG)										
18	5MR	5RMR;10MSMR		SEG	120	450, 460	229	200	160	169
42	15MR	10RMR;10M		SEG	120	NA[d]	229	200	160	169
78	5MR	10RMR 10MSMR	+	SEG	120	207	229	200	160	169, 500
147	40MSS	20MSS 20RMR	+	SEG	120	207, 450, 460	229	200	160	169, 500
227	10M	20M 15RMR	+	SEG	120	207	229	200	160	169
229	20MR	10RMR 10M		SEG	120	207	229	200	160	169, 500
245	5MR	5RMR 15MSMR		SEG	120	207	229	200	160	169, 500
268	10MR	10RMR 10MSS	+	SEG	120	NA[d]	229	200	160	169
302	10MR	15RMR 20MS MR		SEG	120	207, 450, 460	229	200	160	169, 500
319	15MSS	10RMR 15M		SEG	120	207	229	200	160	169
351	5MR	10RMR 15M		SEG	120	207	229	200	160	169, 500
394	5MR	15M	+	SEG	120	207, 460	229	200	160	169
395	15MR	10RMR 10MSS		SEG	120	207, 450, 460	229	200	160	169, 500
411	5MR	15RMR 20MS	+	SEG	120	207	229	200	160	169
432	15M	10RMR TMS	+	SEG	120	207, 460	229	200	160	169, 500
471	15M	15RMR 15M	+	SEG	120	207	229	200	214	169
473	5MR	10RMR 10M	+	SEG	120	207	229	200	160	169
494	20MR	10MSS 15M		SEG	120	207	229	200	160	169
499	10MR	20M	+	SEG	120	207	229	200	160	169
HOMOZYGOUS SUSCEPTIBLE (HS)										
14	50MSS	15MSSMR		HS	120	450	229	200	160	169, 500
29	30MSS	20MSS		HS	NA[d]	207	229	200	160	169
58	25MSS	20MSS		HS	120	NA[d]	NA[d]	200	160	169
79	20MSS	15MSS		HS	120	NA[d]	229	200	160	169, 500
89	30MSS	30MSS	+	HS	120	207, 450, 460	229	200	160	NA[d]
92	40MSS	30MSS 10M		HS	120	207, 450, 460	229	200	160	169
176	40MSS	30MSS		HS	120	207, 450, 460	229	200	160	169, 500
277	30MSS	20MSS MR	+	HS	120	207, 450, 460	229	200	160	169, 277

279	20MSS	20MSS MR		HS	120	207	229	200	160	169
349	10MSS	30MSS		HS	120	207	229	200	160	169
374	10MS	30MSS		HS	120	207, 450, 460	229	200	160	169, 500
430	30MSS	20MSS	+	HS	120	207, 450, 460	229	200	160	169
448	15MSS	30MSS	+	HS	120	450, 460	NA[d]	200	160	169
455	40MSS	40MSS		HS	120	207	229	200	160	169
467	20MSS	20MSS 5M	+	HS	120	207	229	200	160	NA[d]

The TDS is the last stem rust score recorded during evaluation at the F2 and F3 generation. The marker data is a summary of the results recorded after screening the selected lines with the respective markers

(**R** – resistant; **RMR** – resistant to moderately resistant; **MR** - moderately resistant; **MS** - moderately susceptible; **MSS** – moderately susceptible to susceptible; **S** - susceptible as described by Knott, 1989)

a - TDS; terminal Disease score

b - PBC; Psuedoblack chaff

c – Category; category of line either homozygous resistant, susceptible or segregating bulk

d – NA; no amplification

3.4 Discussion

Genetic purity is herein defined as "trueness to type of plants/seeds conforming to the characteristics of the variety or the level of contamination of seeds/seed lots instigated by presence undesired varieties or species"(Eurofins Bio-Diagnonstics, 2018). To evaluate the genetic purity of the seeds used to make the cross (Robin/Kwale), seed lots of both parents were tested using a select panel of microsatellite markers. This is important in any mapping study to ensure parental genotypes are genetically uniform. Nei 's (1978) Gene Diversity statistic $\boldsymbol{H_e}$ was used to measure the genetic purity of genotypes, revealing high values of 0.7511 for Kwale and 0.7537 for Robin. This study revealed that both parental seed lots were genetically not similar.. Each of the parents were found to have differences among the genotypes used, as the allele profiles of the samples tested were not similar. Deterioriation of genetic purity in varieties is documented to be affected by several factors including effects of disease, mutation, cross breeding, minor variations in the genetic makeup and mechanical mixing among others (World Agriculture, 2018).

Two main genes were expected to segregate in this population, *Sr2* known to be in both Kwale and Robin, and *SrTmp* from the parent Robin, as depicted by the phenotyping results.

Robin is known to carry *SrTmp*, a major gene, as well as also *Sr2* (GRIS, 2017). Kwale on the other hand carries *Sr2*, a minor gene expressed only in its homozygous recessive state. In light of this, it was expected that the underlying genetic action observed would depict action of a dominant gene and a recessive gene, depicting introgression of *SrTmp* and *Sr2* genes in to the progeny of the cross. A frequency distribution of co-efficient of

infection values revealed that the frequency leaned towards the left, i.e. towards resistance. Chi square analysis done to test the underlying gene action in the population revealed that the ratios conformed to a 13:3 ratio, explained by Douglas Knott in 1989 to be expected when one dominant and one recessive gene condition stem rust resistance. The results of this study showed this to be true.

Sr2 was first described in the variety Hope developed by (McFaden 1930) from a cross between Marquis and Yaraslav. It was the first race non-specific resistance gene to be genetically defined and has been providing resistance in commercial wheat varieties for more than 10 decades (McIntosh et al., 1995; Ellis et al., 2014). It is known to be located the short arm of the 3B chromosome of wheat. Marker Xgwm533 (Spielmeyer et al., 2001) was used to screen for *Sr2* in this study. The *Sr2* gene was amplified as expected in all the parents and the bulks for both markers, confirming what was expected.

The other gene expected to segregate in the test population was the *SrTmp* gene from the parent Robin. *SrTmp* was derived from the wheat cultivar 'Triumph 64' (hence the name Tmp) (Hiebert et al., 2011). *SrTmp* conferred race specific (major gene) resistance to stem rust race *Ug99* until late 2015 (after the phenotyping studies for this study had already been completed) when races known to be virulent to it were discovered in Kenya (Singh et al., 2015). The gene *SrTmp* has been documented to be mapped to the short arm of chromosome 6D (Lopez-vera et al., 2014).

In this study, the microsatellite marker Xcfd49 was used to screen for this gene, as used by Lopez-vera et al 2014 in an elaborate study involving six mapping populations. Observed results from this study correspond to results observed by Lopez-vera et. al 2014

in his Cacuke/Pfunye study population where the resistant parent Pfunye and the resistant Cacuke/Pfunye bulk produced a 160pb band. The susceptible bulk and susceptible parent from his population produced a 214 bp fragment as observed in this study from the second genotype of Robin (Rb). This was an indication of the gene from the parent Robin (i.e. *SrTmp*) being introgressed into the progeny of the cross.

Several genes for stem rust race *Ug99* resistance are known to be on chromosome 6DS including *SrCard* (Hiebert et al., 2016), and *Sr42* (Ghazvini et al., 2012, Gao et al., 2015). It is still not certain whether *SrCard* and *Sr42* are different genes or alleles of the same gene. The marker Xbarc183 was used to screen for gene *Sr42* (Ghazvini et al., 2012). Lopez-vera et al, 2014 in his study postulated that *SrCard* and *Sr42* are alleles of the same gene and that *SrTmp* is closely linked to these genes or possibly an allele of the same gene. If so, this would explain why both Xcfd49 and Xbarc183 amplified relatively same band sizes respectively in both the Kwale and the Robin genotypes. From its pedigree, Kwale has the parents Kavkaz and Bluebird , known to carry the *Sr42* gene (GRIS, 2017). Hence, the assertion that *Sr42, SrCard and SrTmp* are allelic is re-asserted in this study.

The *Sr31* gene from the 1BL.1RS rye translocation chromosome translocation (Pretorius et al., 2000) was assayed in this study using the rye specific molecular marker Xscm9 (Saal and Wricke 1999). This marker produced a 207 bp fragment in the positive control, the second genotypes of Kwale and Robin (Ka and Rb) and the HR and HS bulks. The first Kwale genotype (Ka) did not amplify any band and the first Robin genotype (Ra) produced three fragments of 190, 450 and 460 bp. The segregating bulk amplified two

fragments at 207 and 450bp. Weng et. al., in 2007 also reported a 207bp fragment with this marker to be an indication of the presence of the *Sr31* gene. In the screening of the individual HR, SEG and HS lines there was varied fragment sizes in all the groups, probably because the parental genotypes were also quite varied.

The gene *Lr34* has a pleiotropic effect with gene *Yr18* and was first described in cultivar Frontana (Dyck et. al., 1966) and Bluebird and Kavkaz in the pedigree of Kwale are known to carry the gene(GRIS, 2017) . An invaluable trait of this gene is its race non-specificity, making it a major component of durable "slow rusting" resistance additionally, it also acts synergistically with other rust resistance genes (German and Kolmer 1992; Lagudah et al., 2006). In our study this gene was amplified in the first genotype of Kwale (Ka), shown by a 150bp band with the marker csLv34(Lagudah et al., 2006). The first Kwale genotype is the only genotype that was positive for *Lr34*, all the other parental genotypes and bulks amplified a characteristic negative 229bp band.

3.5 Conclusions and Recommendations

Conclusions

In conclusion, the aim of the study was achieved as the two genes in the parents i.e *Sr2* and *SrTmp* were introgressed into the progeny. Superior transgressive segregants that exhibited higher levels of resistance than the parents were also identified.

Recommendations

1. The markers cfd48 observed to have highest PIC in testing the purity of Kwale parental genotypes be further tested and validated to be able to differentiate the different genotypes of Kwale revealed by our study. The markers wmc243 observed to have highest PIC in testing the purity of Robin parental genotypes be further tested and validated to be able to differentiate the different genotypes of Robin revealed by our study.

2. Markers Xgwm533 and xcfd49 can be used effectively to track the presence of *Sr2* and *SrTmp* genes in breeding populations.

CHAPTER FOUR

Quantitative trait loci mapping for adult plant resistance to stem rust in bread wheat cultivar Akuri

Abstract

Resistance is the most economically viable approach to curb the threat of rusts in wheat. The trouncing of *Sr31* and susceptibility of other known resistance genes to the highly virulent *Pgt* race *Ug99* and its lineage of races led to converted efforts to discover and deploy resistance genes/QTLs into new durably resistant varieties. Seedling resistance (major) genes provide protection against rust at all stages of the plant to a particular race of stem rust. Adult plant resistance (minor) genes are however termed "durable" as they provide resistance to a broad spectra or rust races. Akuri is a CIMMYT-developed bread wheat line exhibiting adult plant resistance (APR) in field trials in Kenya despite susceptibility to many races at the seedling stage. This study was conducted to identify genomic regions contributing APR to stem rust in Akuri. One hundred and forty-one RILs and parents of an $F_{2:5}$ Akuri x PBW343 population were evaluated in Njoro for APR to stem rust over three seasons. Composite interval mapping was implemented on Windows QTL Cartographer to detect QTLs at a LOD threshold of 2.5 utilizing 910 high quality SNPs previously typed on the DArT-Seq platform. QTL analyses revealed loci on chromosomes 1B, 2B and 3B consistently contributing to stem rust resistance. These QTL respectively explained ~7, 9, and 8% of the phenotypic variation. A comparison with the recently reported QTL consensus map revealed that the QTL herein discovered are probably novel.

4.1 Introduction

Rust diseases are highly specialized plant pathogens and among the most ancient of plant diseases known to man, dating back to Aristotle's time (384-322 B.C) (Shumann et al., 2000). Wheat stem rust, caused by *Puccinia graminis f. sp. tritici* (Pgt), is the most devastating of the rust diseases, top among them the *Ug99* lineage of stem rust races pose a challenge to global bread baskets resulting in the awakening of breeding programmes worldwide to the need for hastened discovery of effective sources of resistance. The acclaimed versatility of the stem rust pathogen is due to its known genetic plasticity, continuous evolution and adaptation to host environment escaping immune recognition (Sperschneider et. al., 2014).

The 1BL.1RS *Sr31* translocation contributed to global stem rust control for over three decades, but was defeated by TTKSK (*Ug99*) identified in Uganda in 1998 (Pretorius et al., 2000). *Ug99* has continued to exhibit an evolutionary pathway leading to the *Ug99 lineage* of races (Park et. al., 2011), acquiring virulence to a combination of other known rust resistance genes rendering an estimated 85-95% of breeding materials from most countries susceptible to this race (Singh et. al, 2011). Lessons learnt from past notable occurrences by yellow rust pathogen *Puccinia striiformis* indicate the new Pgt races are expected to move to the Middle East, West Africa, and South Asia within a period of approximately 10 years if not sooner (Roelfs and Bushnell, 1985). Odds of these races being introduced into new areas, including North America by means of intentional or accidental human-mediated activities are also likely. Average yield losses on a regional basis under epidemic conditions are commonly 10%, a loss of sufficient magnitude to have disastrous humanitarian consequences on wheat producing countries in the

developing world, as well as substantial secondary impacts on the entire global economy (Cornell University, 2016).

In efforts to mitigate the threat posed by Ug99 and its lineage of races, the late Norman E. Borlaug raised the alarm which led to the conceptualization of the Borlaug Global Rust Initiative; BGRI. BGRI is allied to the Durable Rust Resistance in Wheat (DRRW) Project that aims to reduce steadily the world's vulnerability to stem rust diseases of wheat through an international collaboration unparalleled in scale and scope. Among strategies to combat stem rust, preventive measures in ways of adult plant resistant varieties remain the most effective tool (FAO, 2010). The alternative, chemical control i.e. fungicides, are environmentally detrimental and economically not feasible for resource-poor farmers in developing countries.

Race-specific resistance genes are easily broken down by rust pathogens, as has been evident by the illustrated development of *Ug99* and its lineage of races. Race non-specific adult plant resistance (APR) has in the current past proved to be a practical long-term solution (Sign et. al., 2011). Johnson in 1984 defined the term '*durable*' resistance (APR) as resistance exposed to a broad continuum of the pathogen for an extended time over large vicinity and remained resistant. In this study the term adult plant resistance (APR) is adopted.

APR is quantitatively inherited and is characterized by lower receptivity, longer latent periods, and smaller uredinia and less urediniospore production (Liang et. al., 2006, Dubin et. al., 2009). APR genes confer resistance more often expressed only in adult plants and is characterized by less and slower pathogen growth devoid of a necrotic

response also termed “slow rusting” (Ellis et al., 2014). The only well characterized catalogued APR genes include *Sr2/Yr30*, *Lr34/Yr18*, *Lr46/Yr29* and *Lr68* (Singh et. al 2012). These are non-hypersensitive and function in a pleiotropic manner (Silva et al., 2015).

Breeding for durable, partial APR genes is a dynamic process and proves to be a daunting task through conventional methods, owing to the fact that they are polygenic (Knott 1982). This is further complicated as phenotyping and identification of APR genes is often obscured by presence of qualitative major genes with large phenotypic effects (Sukhwinder et. al., 2013). Development of DNA/RNA molecular techniques has provided powerful tools for characterization of quantitative traits such as the APR genes, and allows for manipulation of genotyping data to evaluations at a molecular level. Identified molecular markers are utilized in marker assisted selection through (MAS), aiding in pyramiding of APR genes through procedures such as limited or repeated backcrossing (Singh et. al., 2011). MAS additionally provides a platform to pyramid both minor (APR) and major genes, previously impractical through conventional breeding (Sukhwinder et. al., 2013). Studies have been done are incessantly ongoing to identify sources of APR and map genomic regions contributing to slow rusting resistance, as well as utilizing MAS to aid in gene pyramiding (Kuchel et al 2007; Bhavani et al 2011; Long et al., 2014).

In line with these efforts to breed for durable adult plant resistance, the aims of these study were (1) to evaluate bread wheat recombinant inbred line (RIL) population derived from the cross of moderately susceptible wheat PBW343 with APR wheat ‘Akuri’ in the

field for adult plant resistance to stem rust race *Ug99*, (2) to genotypically characterize the afore mentioned population with DArT markers and (3) identify genomic regions harboring quantitative trait loci significantly contributing to observed adult plant resistance in the test population.

4.2 Materials and methods

4.2.1 Plant Material

One hundred and forty-eight (148) $F_{2:5}$ RILs of the cross between susceptible PBW343 and resistant PGO/**Croc_1/Ae. Squarrosa/Circus/Borl 95/Oasis** were evaluated in this study. The resistant parent was recently named '***Akuri***' and henceforth the name is adopted for the resistant parent (Ravi, 2104 personal communication). This population was generously provided by Dr. Ravi Singh and Dr. Sridhar Bhavani from CIMMYT, Mexico.

PBW343 is a moderately susceptible spring wheat variety, a descendant and selection (GID2430154) (Sukhwinder *et. al.*, 2013) of the popular CIMMYT variety Attila, and was released in India as a high yielding stem rust resistant variety carrying the gene *Sr31* in the year 1995. As is known this gene was later broken down by the Ug99 lineage of stem rust races. PBW343 is of the pedigree '***Nord Deprez/VG9144//Kalyansona/Bluebird/3/Yaco/4/Veery#5'***, (GRIS, 2017). The resistant parent Akuri has the pedigree ***PGO//CROC_1/Ae. Squarrosa (224)/3/2*BORL95/4/Circus***.

4.2.2 Phenotyping

4.2.2.1 Seedling Resistance

Approximately 10grams of seed each for both parents were sent to a collaborator Dr. Matt Rouse at USDA - Cereal Disease Laboratory, St. Paul Minnesota, USA for seedling resistance screening as described by Rouse et al, 2014. This location was preferred for the seedling resistance tests because of the advanced greenhouse facilities available for seedling tests.

4.2.2.2 Adult Plant Resistance

Evaluation of slow rusting APR in reaction to stem rust was conducted at the Kenya Agricultural Livestock Research Organization (KALRO), Njoro International wheat screening nurseries in the main and off seasons of 2010, 2013 and the main season of 2014. Plots were cultivated as 0.5 m double rows 20cm apart with 0.5 m pathway. Hill plots of stem rust disease spreader plants (a mixture of the highly susceptible wheat cultivars Thatcher, Morocco, and Cacuke) were planted perpendicular to the rows on one side of each plot. To further boost disease infection, several continuous rows of spreader plants were planted around the whole block to facilitate uniform disease infection.

At planting, DAP fertilizer providing Nitrogen and Phosphorus at the rate of 22.5 kg N ha^{-1} and 25.3 kg P ha^{-1} were applied respectively. Buctril MC that contains 225 g L^{-1} Bromoxynil octanoate and 225 g L^{-1} MCPA Ethylhexylester were applied to control post emergence weeds after planting and at tillering stage at the rate of 7 ml L^{-1} of water to control broad-leafed weeds. To control insect pests, Buldock Duo (225 g L^{-1} Beta-

Cyfluthrin) was sprayed at the rate of 10 ml L^{-1} of water. When needed manual weeding was done to control for grass weeds.

To initiate artificial stem rust epidemic, spreader rows and plants were inoculated twice prior to booting and during stem elongation. A solution of fresh urediniospores collected from the KARI-Njoro trap nurseries that were predominantly of the *Sr31 + Sr24* virulent variant TTKSK, of the race *Ug99* (Kimani et, al., unpublished) were used. Urediniospores were suspended into water then injected into1-3 individual spreader plants every one meter (growth stage Z35-37; Zadoks et al, 1974). Spreader plants were also sprayed with a suspension of urediniospores suspended in a light-weight mineral oil Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX) twice during stem elongation.

4.2.3 Stem rust evaluation

Assessment of the seedlings reaction (infection type) to the stem rust pathogen was conducted on 21 day old seedlings based on a 0 to 4 infection type scale described by Stakman et al. (1962).

Wheat test plants reaction to stem rust infection was scored as a % infestation of disease on the plant (total area of stem and leaves covered by the disease) (Peterson et al., 1948,) and host plant response recorded as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) as described by Knott, 1989.

4.2.4 Statistical Analyses

The mean disease severity was calculated for each RIL family. Comparison of means of the different seasons was performed using one-way analysis of variance (ANOVA) with function "*aov*" in R software (R Development Core Team, 2011). In the analysis, phenotype (disease severity of the RILs) was modeled as dependent variable on the dependable factor that is season. Post-hoc analysis of Anova results was performed using Tukey Honestly Significant Difference (Tukey, 1949).

A coefficient of infection (CI) was computed by combining disease severity and host response data into a single value. Thus, severity was multiplied with a constant for host response (Yu et al. 2011) where immune = 0.0, R = 0.2, MR = 0.4, M = 0.6, MS = 0.8 and S = 1.0. The CI was used to plot box plots and frequency distribution histograms of mean disease severity between the seasons.

4.2.5 Genotyping

4.2.5.1 DNA Isolation

DNA was extracted from 2 seeds of each RIL family. The seeds were crushed in a mortar and pestle into a fine powder then transferred into a 1.5 ml centrifuge tube. 600µl of extraction buffer (1.4 NaCl, 100mM Tris HCl, pH 8.0, 20mM EDTA, and pH 8.0, 2% CTAB) was added to the tube and the solution vortexed for 1 minute. 40µl of 20% SDS (Soduimdodecyl sulphate) was then added and the solution incubated for 45 minutes in a water bath at 65ºC. 160µl of 5M KAoC was then added and the solution incubated on ice for 10 minutes. The solution was then centrifuged at 13000 rpm (rounds per minute) for ten minutes and 600µl of the supernatant transferred into a fresh 1.5ml microfuge tube.

An equal volume (600µl) of chilled isopropanol was added and the solution inverted several times to mix then put in a -20ºC freezer for 20 minutes to hasten DNA precipitation. After this it was centrifuged at 13000rpm to allow for pellet formation. The supernatant was discarded leaving the pellet which was washed with 500µl 70% EtOH and centrifuged at 13000rpm for 5 minutes. The pellet was air dried at room temperature for 20 minutes and suspended in 100µl deionised water and kept at 4ºC overnight for the pellet to dissolve then stored at -20ºC.

Quantification and assessment of the quality of isolated DNA was done by comparing DNA samples with known concentrations of uncut, unmethylated lambda (λ) DNA standards resolved on a 0.8% agarose (Sigma, UK) gel in 1x TBE buffer (89.2mM Tris, 89.0mM Boric acid, 1.25mM EDTA pH 8.0). Lambda DNA of different concentrations and the isolated DNA samples were mixed with 2µl of 6X loading dye (ThermoSCIENTIFIC) and resolved on agarose gels at 80 volts for 40 minutes.

50-100 ng DNA aliquots of the RILs and parents were submitted to Diversity Arrays Technology Pty Ltd ABN in Yarralumla Australia for genotyping using DArTseq.

4.2.5.2 Linkage Mapping and QTL Analysis

Markers were assigned to chromosomes based on the recently updated DArT consensus maps (Kilan, 2013, personal communication). Join Map v 4.1 was used to create linkage groups (Van Ooijen, 2006). Linkage groups were separated using the independence LOD score > 3.0. The order of markers within linkage groups was established with the regression mapping algorithm of JoinMap as it gives a less extended map as opposed to maximum likelihood ratio (ML) mapping algorithm. The Kosambi mapping function

was used to calculate the genetic distance between markers in centimorgan (cM) values. The DArT Wheat consensus maps were used as a reference in this study. These maps were recently updated to include the clones that are used for

DArTSeq technique used for identifying silico-DArTs and SNPs (Li et al., 2015). QTL IciMapping software (ICiM) and WinQTL Cartographer softwares were used for QTL mapping using inclusive composite interval mapping (ICIM) and composite interval mapping (CIM) algorithms respectively.

4.3 Results

4.3.1 Phenotyping

4.3.1.1 Seedling Analysis

The parents PBW343 and Akuri were screened with races TTKSK, the original *Ug99* virulent to *Sr31*, and TTKST, a variant with combined virulence to *Sr31* and *Sr24*. Both parents were susceptible to both races as shown in the *Table 4.1*.

Table 4.1; Seedling infection types of parents PBW343 and Akuri screened with race TTKSK and TTKST

Pgt[a] Race	TTKSK[b] Rep1	TTKSK[b] Rep2	TTKST[b] Rep1	TTKST[b] Rep2
Pgt Isolate	04KEN156/04	04KEN156/04	06KEN19V3	06KEN19V3
Date Scored	3/13/15	3/13/15	3/13/15	3/13/15
PBW343	3 lif[c]	3+[d]	3+	3+
Akuri	3+	-[e]	3+	3+

Pgt[a] – Puccina graminis

TTKSK[b] – races of rust used to do the seedling tests

Lif[c] – low infection type

3+[d] – susceptible infection type 3+

(-)[e] – missing data point, plant did not grow

These results confirmed that the observed resistance was indeed inherited in a quantitative manner. i.e adult plant resistance (APR).

4.3.1.2 Adult Plant Resistance Analysis

Adequate disease pressure was observed in all cropping seasons that allowed for appropriate recording of data of response to stem rust (data presented in appendix 6.1). Difference in response of the population to stem rust across seasons was found to be significantly different as depicted by the one-way analysis of variance performed *(Table*

4.2). A box plot of means and variances computed from co-efficient of infection data also revealed that the seasons differed quite significantly *(Figure 4.1)*. Pairwise comparisons of seasons' mean using TukeyHSD *(Table 4.3)* revealed that season 3- season 5; season 5 season 3 and season 5 –season 4 did not differ significantly in their means, while all other pairwise comparisons had a significant difference ($p<0.05$). This difference in seasons could be attributed to different weather conditions being experienced in the different seasons. Seasons with high moisture and humidity tend to favor higher inoculum build up and hence the increase in disease severity.

Table 4.2: Summary Table of One Way Analysis of Variance of Mean Disease Severities to stem rust

	Df	***Sum Sq***	***Mean Sq***	***F value***	***Pr(>F)***
SEASON	**4**	**85034**	**21258**	**98.96**	**<2e-16*** **
Error	**731**	**157032**	**215**		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4.3: TukeyHSD test showing pair wise comparisons of season's means depicting which seasons differ and by how much

	diff[a]	lwr[b]	upr[c]	p value[d]
Season 1-Season 3	20.686667	16.0586	25.3147233	0.0000000*
Season 4-Season 3	-4.502313	-9.1539	0.1492963	0.0632174
Season 5-Season 3	-2.767619	-7.4192	1.8839902	0.4804897
Season 6-Season 3	-10.957089	-15.65	-6.2643014	0.0000000*
Season 4-Season 1	-25.18898	-29.841	-20.53737	0.0000000*
Season 5-Season 1	-23.454286	-28.106	-18.802677	0.0000000*
Season 6-Season 1	-31.643756	-36.337	-26.950968	0.0000000*
Season 5-Season 4	1.734694	-2.9403	6.4097371	0.8486481
Season 6-Season 4	-6.454776	-11.171	-1.7387591	0.0018311*
Season 6-Season 5	-8.18947	-12.905	-3.473453	0.0000243*
Season 6-Season 5	-8.18947	-12.905	-3.473453	0.0000243*

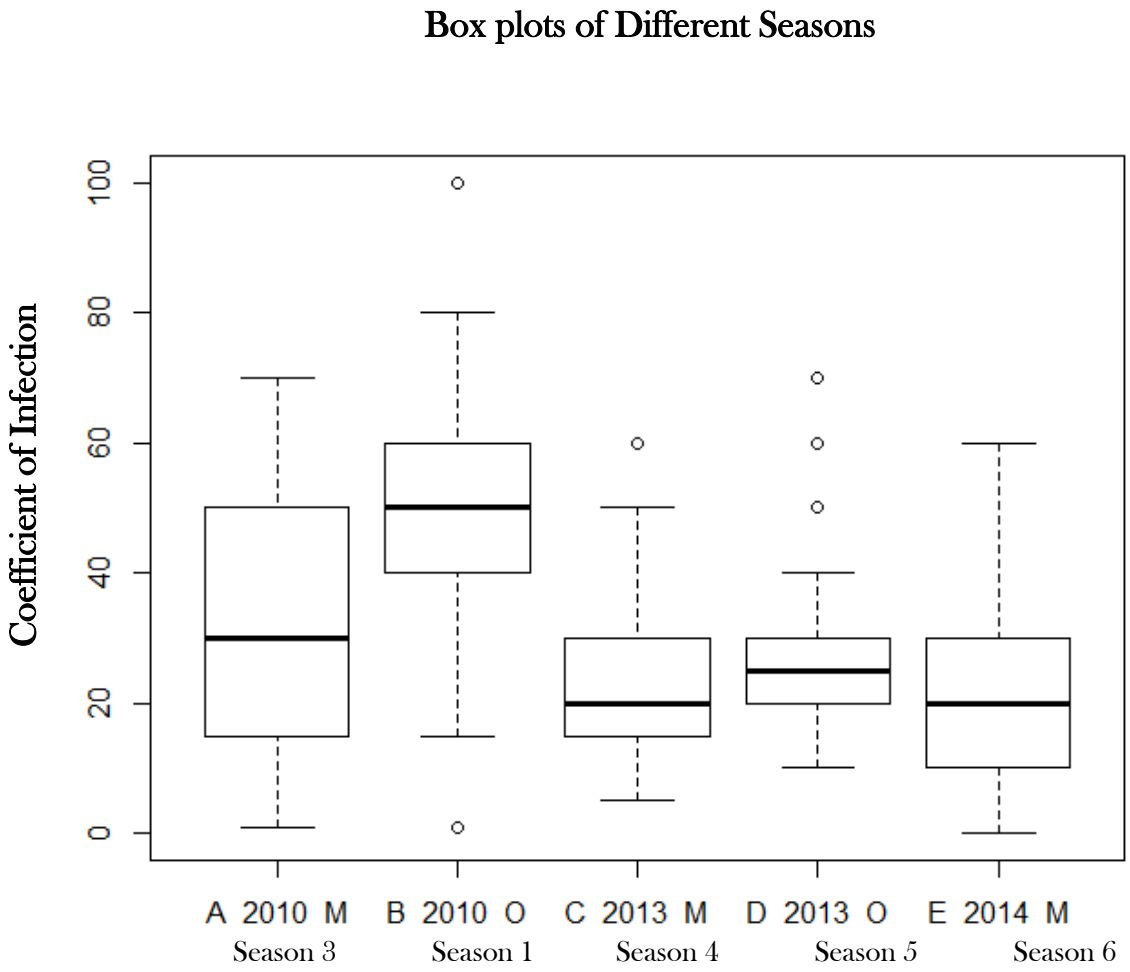
*Significant at α=0.05

[a] difference between means for each pair of groups

[b, c] the lower and upper limit of the 0.05 confidence interval of the difference

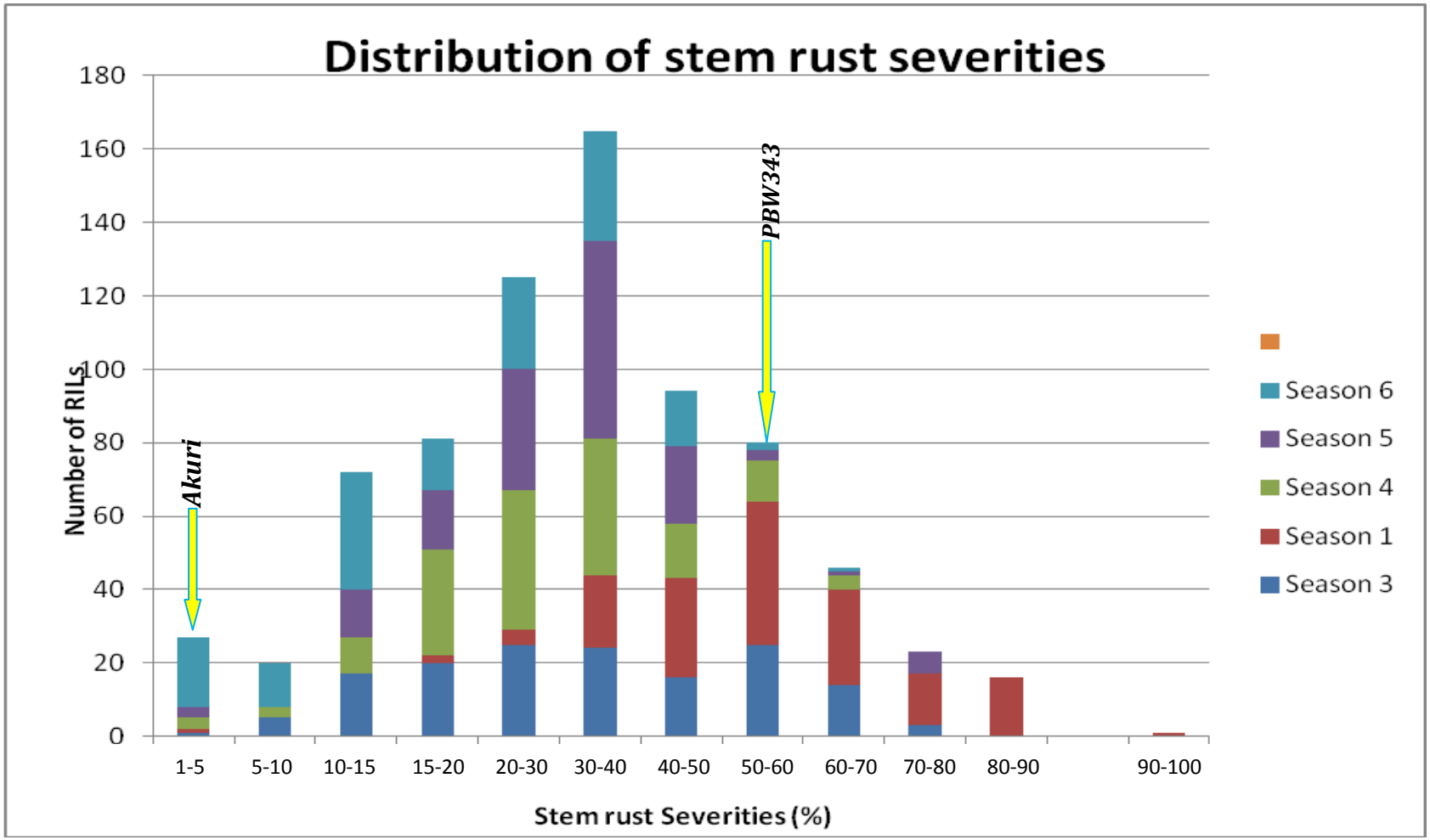
[d] p-value at 0.05 significance level

Season 3 -2010 Main season, Season 1 – 2010 Off season, Season 4 – 2013 Main season, Season 5 – 2013 Off season, Season 6 – 2014 Main season



Season 3 -2010 Main season, *Season 1* – 2010 Off season, *Season 4* – 2013 Main season, *Season 5* – 2013 Off season, *Season 6* – 2014 Main season

Figure 4.1: Box plots illustrating differences in resistance responses to stem rust in different season for the mapping population. Box boundaries represent the upper and lower quantiles with median represented by the line in the middle of the box. Whiskers represent 1.5 times the quantile of the data with outliers shown as dots. The clear differences of the median of data recorded in different seasons indicated how the population differed in response to stem rust in different seasons.



Season 3 -2010 Main season, Season 1 – 2010 Off season, Season 4 – 2013 Main season, Season 5 – 2013 Off season, Season 6 – 2014 Main season

Figure 4.2; Frequency Distribution Graph of Stem Rust Disease Severities in the Different Seasons

Evaluation of the disease severity in the field revealed differences within the mapping population and between the parents. The parents, PBW343 and Akuri, significantly deferred in their observed field responses to stem rust as depicted by their mean disease severities as shown in *Table 4.4.* A plot of disease severities from the cropping seasons revealed a gaussian type of distribution as shown in *Figure 4.2*. Resistance was hypothesized to be quantitatively distributed based on the continuous distribution observed (*Figure 4.2*) and ranged from 1-100 with an average of 32.5% (*Table 4.4*). Mean stem rust severity of 4% for the resistant parent Akuri differed significantly (α 0.05; *p value* = 9.65702E-06) with that of the moderately susceptible parent PBW343 of 51.67% (*Table 4.4*).

Table 4.4; Mean and Range of stem rust severity in PBW343/Akuri RIL mapping population and their parts over 5 cropping seasons in field trials at Njoro, Kenya.

Year	**Stem Rust Severity**			
	Parents		PBW343/Akuri RILs	
	PBW343	Akuri	Mean	Range
Season 1	70	1	46.1	1-100
Season 2	50	1	45.4	1-100
Season 3	50	1	30.5	1-100
Season 4	50	10	25.8	1-100
Season 5	50	10	27.6	1-100
Season 6	40	1	19.69	1-100
Mean	*51.67***	*4***	*32.5*	

**Differed significantly at (p=0.05)

4.3.2 Genotyping

4.3.2.1 Estimation of DNA quality and quantity

The yield of DNA isolated ranged from 50 – 100 µg. A gel picture showing the quality and quantity of DNA as assessed is shown below in *Plate 4.1*. The DNA isolated from the RILs was intact and of high quality for the subsequent genotyping procedures.

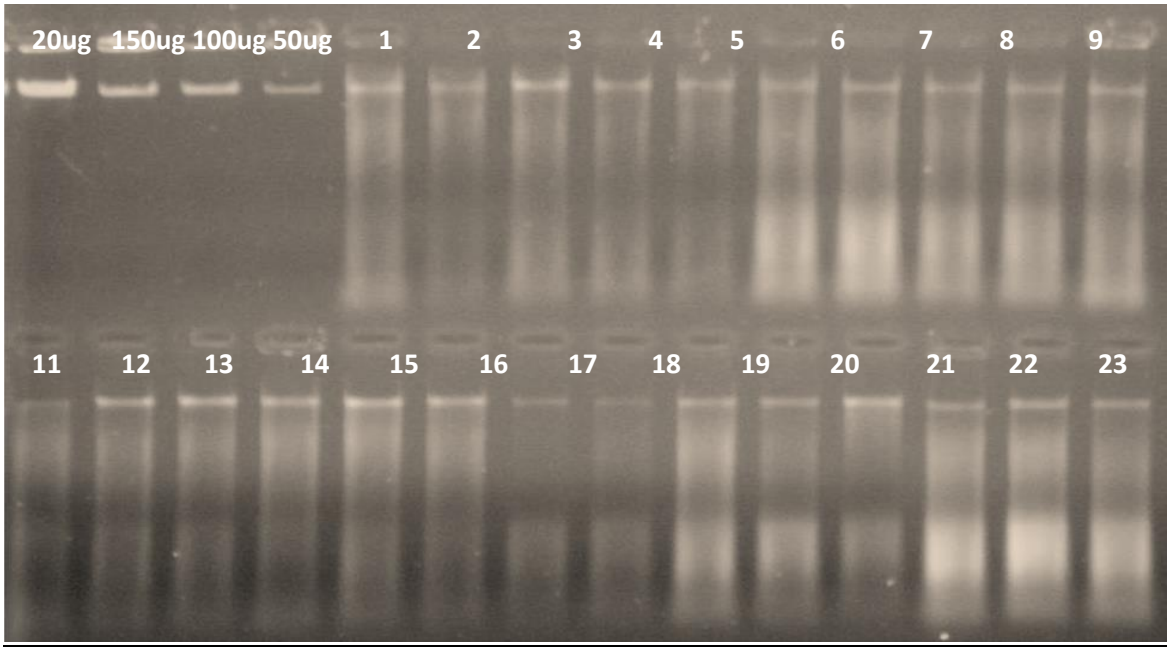


Plate 4.1; Gel electrophoresis image showing Quantification of DNA isolated from RILs 1-24 compared to different concentrations of lambda DNA, resolved on a 0.8% gel at 80 volts for 30 minutes.

4.3.2.2 DArT Genotyping

DArTseq method deploys sequencing of the representations on the Next Generation Sequencing (NGS) platforms. DArTseq generates presence absence markers and SNPs in fragments present in the representation. A total of 7,078 SNPs were found to be polymorphic and genotyped across 141 RILs and the parents. Of these 4,017 were assigned chromosome positions from the recently updated wheat consensus maps (Kilan, 2013, personal communication). These were further filtered using a 0.05 minimum allele frequency (MAF) and <10% missing data points to remain with 1,612 polymorphic markers that were used to construct linkage maps.

4.3.2.3 Linkage Mapping

The genetic map consisted total of 44 linkage groups, with some chromosomes being represented by more than one linkage group. The final map had 910 markers spanning a length 2759.39 cMs that were used to identify genomic regions harboring quantitative trait loci conferring resistance to stem rust in the population under study (PBW343/Akuri). Chromosome 3B had the highest number of markers (143) distributed in 3 linkage groups, while chromosome 7D had the least number of markers (9) in two linkage groups. The average number of markers per chromosome was 50.56 while the average number of linkage groups per chromosome was 2.4. Chromosome 6D had the highest number linkage groups (4). Markers on chromosomes 2D, 4A and 4B did not have sufficient linkage to have linkage groups.

4.3.2.4 Quantitative mapping of APR to stem rust

Ten significant QTL on chromosomes 1B, 2B, 3A, 3B, 4A, 5B, 6B, 6D, 7A and 7B for resistance to stem rust were identified through Composite Interval Mapping with WinQTL Cartographer v2.5_011, with LOD score ranging from 2.5 to 6.06. Naming of the QTLs was done following the stipulated nomenclature for designation of quantitative loci in wheat (McIntosh et. al., 2003).

Three QTL on 1BL, 2BL and 3B and were consistent in more than one season, and were designated as *QSr.cim-1BL, QSr.cim-2BL*, and *QSr.cim-3B*-. These QTL are illustrated in *Figure 4.3.* Among these QTL, *QSr.cim-2BL* at a support interval of 135.4 – 154.7 cM on chromosome 2B was detected in both in season 6 and season 2. QTL *QSr.cim-1BL* was consistent in both season 4 and season 5 with an LOD ranging from 2.8 – 4.1. The QTL on the 3B chromosome designated as *QSr.cim-3B*- was detected in all seasons except the season 1 and 2, with an LOD ranging from 2.8 – 4.1, at a supporting interval of between 53.1 and 74.4 cM. This QTL, *QSr.cim-3B,* was responsible for 6.73 to 14.02 % of the phenotypic variance across seasons.

All other QTL, *QSr.cim-3A, QSr.cim-4AL, QSr.cim-5BL, QSr.cim-6B, QSr.cim-6D, QSr.cim-7AL* and *QSr.cim-7BL* shown in *Table 4,5* were detected only in one season with LODs ranging from 2.6 – 4.1, responsible for 12.95%, 11.7%, 10.5%, 6.17%, 7.42%, 6.87% and 7.9% of the phenotypic variance respectively in the seasons in which they were significant. The total R^2 from these QTLs ranged from 30.71% - 36.17% *(Table 4.5).* Further phenotyping coupled with genotyping is needed for verification. Chromosome regions with sparse markers could have existing undetected QTL

Table 4.5; QTL for adult plant resistance to stem rust in PBW343/Akuri RIL population showing chromosome location, position, peak marker associated with the QTL, LOD, PVE (R^2), estimated additive effect and adjusted total R^2 explained by QTL.

Chromosome	Marker	Position	Peak Marker	LOD Interval (cM)	LOD	Est. Add[a]	R^2	TR^2
Season 4								
1B	2	2.4	979184	0.0 - 3.4	4.5587	5.1549	10.65	39.36
3B	21	52.1	1120943	49.6 - 53.8	3.0122	-4.1647	9.48	39.35
3B	32	60.8	1055305	59.0 - 61.8	3.6868	-3.828	7.97	37.84
3B	43	71.9	996899	70.4 - 72.0	2.7976	-3.3915	6.7	36.57
Season 5								
1B	4	10.7	992991	9.3 - 10.6	2.7746	-3.6622	7.04	31.34
3B	21	53.1	1120943	49.8 - 57.0	4.1256	-4.9938	12.31	33.37
5B	69	121.2	2278566	120.8 - 121.5	3.038	-4.4592	10.49	35.08
Season 6								
2B	59	171.2	1026541	158.8 - 174.4	5.0834	-5.0462	14.49	39.27
3B	4	16.3	1007283	14.9 - 16.4	2.6482	-3.2538	5.89	36.11
3B	31	40.3	1721611	38.0 - 41.3	3.2893	4.563	7.98	30.02
3B	44	45.1	1053549	44.4 - 48.9	6.0625	4.9875	14.02	36.07
Season 1								
4A	21	103.3	1131791	92.2 - 105.4	4.0973	-6.8875	11.73	35.04
4A	3	3.7	1001988	2.6 - 5.5	2.6706	5.019	6.26	32.8
7A	7	39.8	986684	37.8 - 40.5	2.9427	5.2011	6.86	32.73
Season 2								

2B	51	148.6	1047891	135.4 - 154.7	3.8641	4.4698	9.2	32.34
2B	53	165.3	1122961	161.5 - 169.2	2.7062	4.1724	8.05	31.19
6D	2	0.9	1130017	0.4 - 1.6	3.1816	3.9944	7.42	32.37
Season 3								
3A	4	17.4	1075651	15.6 - 18.4	3.1223	4.678	7.49	30.72
3A	6	26.6	1103158	19.8 - 28.9	3.4868	6.0048	12.91	36.14
3B	23	55.8	1065763	55.0 - 57.0	3.5727	-5.1255	8.86	28.91
3B	37	65.4	3026758	64.4 - 74.4	3.5868	-5.4451	8.85	28.9
6B	7	49.1	1017910	48.9 - 50.6	2.6095	4.2558	6.17	30.74
7B	24	41.9	988742	40.7 - 46.1	2.8089	-4.7973	7.9	32.2

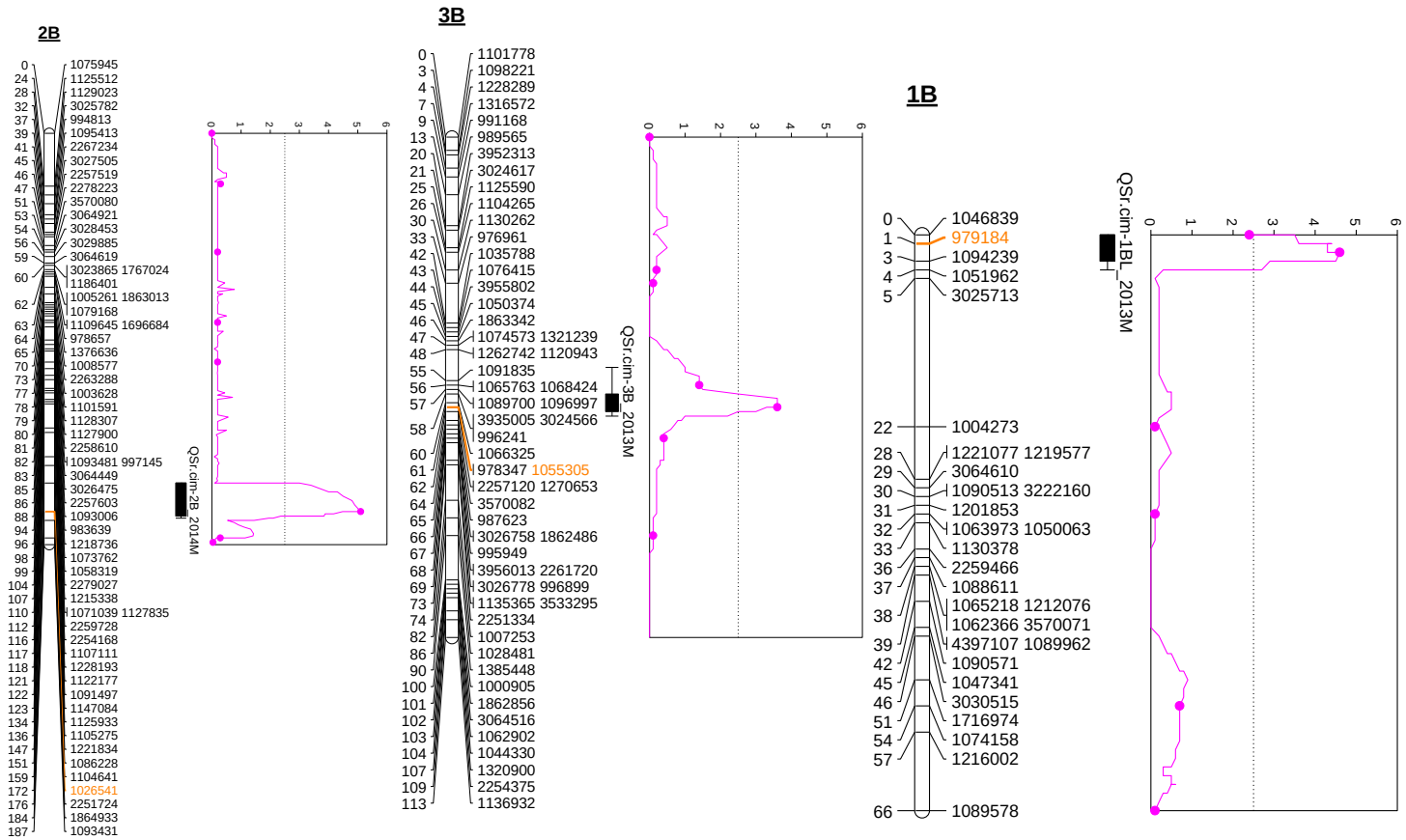


Figure 4.3: Linkage groups showing significant QTL for Stem rust with corresponding LOD contours obtained from CIM. The LOD significance threshold of 2.5 is indicated by a dashed line. In parenthesis after QTL name is the environment of detection. Genetic distances in centimorgans are indicated on the right of each linkage group.

4.4 Discussion

A RIL population developed from the parents PBW343 and Akuri has presented the nature of resistance in our study. High and uniform disease pressure for accurate phenotyping to stem rust is a pre-requisite for QTL mapping of APR. Both parents were observed to be susceptible at seedling stage to the prevalent races of stem rust race *Ug99* used to screen them. This is a clear indication that the resistance observed is most likely minor quantitative i.e. adult plant resistance and not qualitative major gene resistance. The quantitative nature of APR to stem rust was also observed from the continuous variation of the PBW343/Akuri RILs ranging from 1 – 100% observed in the frequency distribution histogram. Significantly higher average stem rust severity of 51.67% was observed for PBW343 as opposed to the low severity average of 4% observed in Akuri. Transgressive segregants observed exhibiting higher resistance or susceptibility than the parents to stem rust was an indication of diverse APR QTL present in both parents.

Mean disease severities of the seasons were revealed to differ significantly ($p<0.05$) as revealed by the different statistical test performed, most probably due to the different weather condition experienced that tend to either favor or suppress the stem rust dispersal and infection

A total of ten quantitative trait loci were detected in our study, three of which were consistent in conferring APR to stem rust namely *QSr.cim-1BL*, *QSr.cim-2BL*, and *QSr.cim-3B.* All the other QTL were inconsistent *i.e.* they were significant only in one season, but potentially could contain loci that have been unexploited for APR to stem rust

(*QSr.cim-3A, QSr.cim-4A, QSr.cim-5B, QSr.cim-6B, QSr.cim-6D, QSr.cim-7B, QSr.cim-7A).*

QSr.cim-1BL located on the long arm of chromosome 1B had significant effect on rust severity explaining 7.04 – 10.65% of the resistance observed in the study population. Previously done studies on Avocet/Pavon, (Njau et al., 2013) PBW43/Kenya Nyangumi and PBW343/Cross Bill (Long et al., 2014) also reported homologous QTLs at the same location on this chromosome. The *1BL.1RS* translocation carrying the *Ug99* ineffective *Sr31* gene, documented to be linked to marker *wPt-8949,* maps to this location (Long et al., 2014). Peak markers *992991* and *979184,* for the *QSr.cim-1BL QTL,* map to the same region as marker *wPt-8949* (Li et al., 2015) in this studies. The pleiotropic gene *Lr46/Yr29* also maps to chromosome 1BL, which could explain the positive additive effect of this QTL in both the 2013 main and off season. Njau et al in 2013 also found a QTL on 1B most likely Lr46; with the peak marker for the QTL wPt-1560 located 10.26cM. The peak marker in the current study for this QTL *979184* was located 17.76cM from wPt-1560.

In studies by Bhavani et al., 2011 and Sukhwinder et al., 2013 a QTL on the 2B chromosome was identified in PBW343/Juchi, and PBW343/Hurivis#1; and PBW343/Muu populations respectively, when they were tested in 2009-2010 cropping seasons at the KALRO-Njoro screening fields. The peak markers for QTL *QSr.cim-2BL,* marker 1026541 co-located with markers Xwpt-92230 and Xwpt-744022 reported by Sukhwinder et al., 2013 in the wheat consensus maps of 2014 (Li et al., 2015). This QTL

was responsible for up to 14.55% of the phenotypic variation in the 2014 main season, at an LOD of 5.1.

QTL *QSr.cim-3B* was detected in all the seasons except in season 1 and 2 at an LOD ranging from 2.6 to 6.1 and was responsible for between 5 to 14% of the phenotypic variation observed in the different seasons. A study done on PBW343/Muu by Sukhwinder et al, 2013 and an elaborate study by Bhavani et al., 2011 on six populations reported DArT marker Xwpt-800213 to co-segregate with a QTL on the 3B chromosome which they implicated to be *Sr2*. Among the peak markers for the QTL *QSr.cim-3B* in this study 1007283 co-locates with the reported DArT marker, suggesting that QTL *QSr.cim-3B* could possibly be *Sr2*. Until recently, the only cataloged APR gene to stem rust was *Sr2,* (McIntosh 1988) located on chromosome 3BS. *Sr2* is arguably the most important stem rust resistance gene having provided almost 50 years and counting of stem rust free green revolution until *Ug99* (Jeffrey et al., 2014). Due to its nature of resistance, it still continues to provide durable broad-spectrum APR to rust including race *Ug99* of stem rust (Speilmeyer et al., 2001). *Sr2* was introduced into the CIMMYT wheat programme in early 1940s through cultivar '*Newthatch*' (Jeffrey et al, 2014). It provides partial resistance only when present in its homozygous, recessive state (Speilmeyer et al., 2003)

All the other QTL were inconsistent *i.e.* they were significant only in one season, but potentially could contain loci that have been unexploited for APR to stem rust (*QSr.cim-3A, QSr.cim-4A, QSr.cim-5B, QSr.cim-6B, QSr.cim-6D, QSr.cim-7B, QSr.cim-7A)*. QTLs on 1B and 5B are from PBW343, while all other QTLs originate from the resistant parent

Akuri. Sukhwinder et al, 2013 reported QTLs on chromosomes 1B and 5B from PBW343 that corresponds with the results of the study.

QTLs are known to be highly influenced by the environment, more so minor effect "partial" APR QTLs (Silva et al., 2015). Different genotypes will interact differently with the environment to enhance or suppress the expression of different resistant QTL. The 3B QTL was quite consistent, but most likely due to the interaction of alleles present in the RILs and the different environmental conditions, this QTL contributed negatively in most of the seasons it was detected, and acted to contribute positively to resistance only in season 6 observed from the additive effect in *Table 4.5.* Interaction of the alleles present in the RILs and the environment resulted in either a positive or negative additive effect from the different QTL detected. Independent multiple interval mapping (MIM) trait analysis of data of each of the traits (i.e. each season was treated as a trait for the purpose of the MIM analysis.) detected several additive epistatic interaction, particularly between the 1B and 3B QTL though not significantly above the 2.5 LOD threshold.

Given the continuous evolution of stem rust race *Ug99*, with an additional variants already being reported and spread into new territory (CIMMYT, 2016), efforts should ensue to validate these QTL in independent mapping populations.

4.5 Conclusions and recommendations

Conclusion

In the study, QTLs were detected in the PBW/Akuri population were seen to significantly confer resistance of a qualitative nature. These will be invaluable in introgression of identified resistance into the breeding programmes.

Recommendations

RILs observed to have consistent resistant phenotypes thorough the seasons, with further testing, should also be incorporated in the breeding programme to help transfer QTLs.

We recommend that a further fine mapping of the study be done to pinpoint exact location of the underlying genes and to find tightly linked markers.

CHAPTER FIVE

5.1 GENERAL DISCUSSION

The reproductive capacity of the rust pathogens, particularly stem rust, coupled with its ability to disperse over long distances in the air and high genetic variation serves as a constant reminder to breeders that new races will keep emerging as is already evident. The need for better understanding of rust dynamics and strategies to cub them cannot be overemphasized, top among them being breeding for host resistant wheat varieties and the maintenance of these varieties' genetic purity.

Conventional breeding methods have been quite successfully used to breed for varieties of different crops in the past, as did Dr. Borlaug, the father of the green revolution who saved millions of people from hunger. The current population growth rate however is increasing fast, an estimated 50 billion people predicted by 2050. Disposable land for agriculture keeps reducing with the increasing need for residential areas for the growing population. Use of modern breeding technologies that combine the phenotypic selection accuracy of conventional breeding and the precision of molecular breeding, is as step in the right direction to reduce the breeding cycle.

The studies described herein are a testament that use of molecular tools in the form of DNA molecular markers is effective in targeting resistance genes conferring resistance to stem rust. Use of validated molecular markers to track genes is demonstrated in the study using SSR markers to track the introgression of genes *Sr2* and S*rTmp* into the progeny of the cross between Robin and Kwale. The subsequent study demonstrates a step in finding this genes responsible for target traits (rust resistance) through QTL mapping studies.

Both this studies demonstrate that combining various molecular techniques and effectively deploying them to crop improvement activities would a long way in efforts to shorten the time of breeding but at the same time increasing the accuracy and efficiency of selection of target traits for better varieties.

REFERENCES

Agropedia (March, 2009) Origin of Wheat. Retrieved from http://agropedia.iitk.ac.in/content/origin-wheat

Anon, (1997) Republic of Kenya development plan; National transformation for the year 2020 Government printers Nairobi sessional paper No. 2

Armour, J. A. L., Alegre, S. A., Miles, S., Williams, L. J., Badge, R. M. (1999). Minisatellites and mutation processes in tandemly repetitive DNA. In: Goldstein, D.B., Schlotterer, C. (eds) Microsatellites: evolution and applications. Oxford university press, Oxford, pp. 24-33Associates Sunderland, MA.

Babu, R., Nair, S. K., Prasanna, B. M., and Gupta, H. S., (2004). Integrating marker-assisted selection in crop breeding – Prospects and challenges Curr. Sci. 87: 607-619.

Bariana, H. S. (2008). Stem rust resistance in wheat - The Australian Experience in: Proceeding of International Conference on Wheat Stem Rust *Ug99*- A Threat to Food Security; (Eds.), GP Singh, K V Prabhu and Anju M Singh, Indian Agricultural Research Institute, New Delhi, India pp 85

Bhardwaj, S. C., Nayar, S. K., Prashar, M., Kumar, J., Menon, M. K., and Sigh, S. B., (1990). A pathotype of *Puccinia graminis* f. sp. *tritici* on *Sr24* in India. Cereal Rusts Powdery Mildews Bull. 18:35-37

Bhattacharya, S. (2017) Deadly new wheat disease threatens Europe's crops. Nature. 2017;542:145–146. doi: 10.1038/nature.2017.21424

Bhavani, S., Singh, R. P., Argillier, O., Huerta-Espino, J., Singh, S., Njau, P., Brun, S., Lacam, S., and Desmouceaux, N. (2011) Mapping durable adult plant stem rust resistance to the race *Ug99* group in six CIMMYT wheats. In McIntosh R (ed) Proceedings of Borlaug global rust initiative, Technical Workshop, Saint Paul, Minnesota, USA, pp 43–53, 13–16 June 2011

Blake, T. K., Kadyrzhanova, D., Shepherd, K. W., Islam, A. K.., Langridge, P. L., McDonald, C. L., Erpelding, J., Larson, S., Blake, N.K., and Talbert, L.E., (1996). STS-PCR markers appropriate for wheat-barley introgression. Theor. Appl. Genet. 82:715-721

Borlaug, N. E., (1954). Mexican wheat production and its role in the epidemiology of stem rust in North America Phytopathology, 44:398-404

Brown, J. K. M., and Hovmoller, M. S. (2002). Aerial dispersal of pathogens on global and continental scale and its impact on plant disease, Science297:537-41

Buchenauer, H., (1982). Chemical and biological control of cereal rust. In K. J. Scott & A. K. Chakravorty, eds. The rust fungi, pp. 247-279. London, Academic Press.

Burdon, J. J., Barrett, L. G., Rebetzke, G., and Thrall, P. H. (2014) Guiding deployment of resistance in cereals using evolutionary principles. Evolutionary Applications ISSN 1752 4571DOI: 10.1007/s00122-006-0406-z.

Burr, B., Burr, F. A., Thompson, K.H., Albertson, M. C., and Stuber, C. W. (1988) Gene mapping with recombinant in-breds in maize Genetics 118: 519-526

CABI (March 2018). *Puccinia graminis* (stem rust of cereals) ; Invasive species compendium Retrieved October 21, 2018 from https://www.cabi.org/isc/datasheet/45797

Cato, S., Gardner, R., Kent, J., and Richardson, T. (2001). A rapid PCR based method for genetically mapping ESTs. Theor. Appl. Genet. 102: 296–306

Cereal Grains: The Structure & Uses of Wheat. (2017). In *ScienceAid*. Retrieved Oct 21, 2018, from https://scienceaid.net/Economic_Botany

CIMMYT (February, 2016). A Global Wheat Rust Monitoring System. Retrieved from https://rusttracker.cimmyt.org/?page_id=22

Collard, B. C. & Mackill, D. J. (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363, 557–572

Collard, Bertrand & Jahufer, Zulfi & Brouwer, J.B. and Pang, Edwin. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica. 142. 169-196. 10.1007/s10681-005-1681-5.

Cornell University (2016) Durable Rust Resistance in Wheat Project.Retrieved from http://www.wheatrust.cornell.edu/ April 2017

Curtis, B., (2002). Wheat in the world; FAO Plant production and protection series; FAO corporate document repository, www.fao.org

Demeke, T., Sasikumar, B., Hucl, P. and Chibbar, R. N. (1997). Random amplified polymorphic DNA (RAPD) in cereal improvement. Maydica 42: 133–142

Dhingani, M. R. Umranja, V. V., Tomar, S. T., Parakhia, V. M., and Golakiya, B. A. (2015) Introduction to QTL mapping in plants. Annals of Plant sciences, 4(04), 1072-1079

Doyle, J. J. and Doyle, J. L. (1990). A rapid total DNA preparation procedure for fresh plant tissue. Focus 12: 13-15.

Dubin, J. H., and Brennan, J. P. (2009) Combating stem and leaf rust of wheat: Historical perspective, impacts, and lessons learned. Intl Food Policy Res Inst-Social Science

DuToit, F., and Walters, M. C. (1984). Damage assessment and economic threshold values for chemical control of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) on winter wheat, pp. 58-62. In M. C Walters (eds): Progress in Russian wheat aphid research in Republic of South Africa. Technical communication 191 Department of Agriculture, Republic of South Afric

Dyck, P. L., Samborski, D. J., Anderson, R. G. (1966) Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana In**:** Canadian Journal of Genetics and Cytology, 1966, 8:665-671.

Ellis, J. G., Lagudah, E. S., Spielmeyer, W., and Dodds, P. N. (2014). The past, present and future of breeding rust resistant wheat. *Frontiers in Plant Science Vol: 5 article 641*.

Eujayl, I., Sledge, M.K.., Wang, L., May, G.D., Chekhovskiy, K.., Zwonitzer, J.C., and Mian, M. A. R. (2004). *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for Medicago spp. Theory of Applied Genetics. 108: 414–422.

Eurofins Bio-Diagnostics (2018) Genetic Purity. Retrieved from https://www.eurofinsus.com/biodiagnostics/our-services/genetic-purity/ October 2018

FAO (2010). Patho-type Tracker Status Summary: Ug99 Lineage Retrieved from http://www.fao.org/agriculture/crops/rust/stem/stem-pathotypetracker/en/

FAO (2010). Wheat Stem Rust – UG99 (RACE TTKSK) Retrieved from http://www.fao.org/agriculture/crops/rust/stem/rust-report/stem-ug99racettksk/en/

FAO Global Wheat Rust Monitoring System Rust report of 2011 accessed from www.fao.org on January 2012

FAOSTAT, (October, 2018). World Wheat Production Figures. Retrieved from http://www.fao.org/faostat/en/#data/QC

Gao, J., Kielsmeier-Cook, J., Bajgain, P., Zhang, X., Chao, S., Rouse M. N., Anderson, J. A. (2015) Development of genotyping by sequencing (GBS)- and array-derived SNP markers for stem rust resistance gene *Sr42*. Mol Breeding 35:207 DOI 10.1007/s11032015-0404-4

German, S. E, Kolmer, J. A. (1992) Effect of gene *Lr34* on the enhancement of resistance to leaf rust of wheat Theor Appl Genet 84:97–105

Ghazvini, H., Hiebert, C.W., Zegeye, T., Liu, S., Dilawari, M., Tsilo, T., Anderson, J. A., Rouse M. N., Jin, Y., and Fetch, T. (2012). Inheritance of resistance to *Ug99* stem rust in wheat cultivar Norin 40 and genetic mapping of *Sr42*. Theor Appl Genet 125(4):817–824. doi:10.1007/s00122-012-1874-y

Government of Kenya (2002) National development plan, Kenya NDP (2003-2007)

Grain and Feed Annual (2018). Kenya's imports of key commodities set to increase. USDA Foreign Agricultural Service GAIN Report; Global Agricultural Information Network

GRIS, Genetic Resources Information System for Wheat and Barley (2017) KENYA-ROBIN. Retrieved from http://www.wheatpedigree.net

Gupta, P. K., Rustgi, S., Sharma, S., Sing, R., Kumar, N., Balyan, H. S. (2003). Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Molecular Genetics and Genomics 270:315-323

Gupta, P. K., Varshney, R. K., Sharma, P. C. and Ramaesh, B. (1999). Molecular markers and their applications in wheat breeding. Plant Breeding 118:369-390

Halentjaris, T., Weber, D., Wright, S., Schaefer, A., Nienhuis, J. (1986). Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118: 356-363

Haley, C. S. and Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity vol.69, pp 315-324

Hare, R. A. and McIntosh, R. A. (1979). Genetic and cytogenetic studies of the durable adult plant resistance in 'Hope and related cultivars to wheat rusts.' Zeitschrift fur Pflanzenzuchtung 83:350-367.

Harushima, Y., Yano, M., Shomura, A., Sato, M., Shimano, T., and Kuboki, Y. (1998). A high- density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148: 479–494.

Hibbett, D. (2012). 21st Century Guidebook to Fungi by Moore, D., Robson, G. D., and Trinci, A. P. J. The Quarterly Review of Biology. 87. 10.1086/668171.

Hiebert, C. W., Kassa, M. T., McCartney, C. A., You, F. M., Rouse, M. N., Fobert, P., Fetch, T. G. (2016) Genetics and mapping of seedling resistance to Ug99 stem rust in winter wheat cultivar Triumph 64 and differentiation of SrTmp, SrCad, and Sr42 Theor Appl Genet. 2016 Nov;129 (11):2171-2177

Hiebert, C. W., Fetch, T. G., Zegeye, T., Thomas, J. B., Somers, D. J., Humphreys, D. G., McCallum, B. D., Cloutier, S., Singh, D., Knott, D. R. (2011). Genetics and mapping of seedling resistance to *Ug99* stem rust in Canadian wheat cultivars 'Peace' and 'AC Cadillac'. Theor Appl Genet 122(1):143–149. doi:10. 1007/s00122-010-1430-6

Huihui, L., Guoyou, Y., and Jiankang, W. (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361-374

Jiang, G. L. (2013) Molecular Markers and Marker-Assisted Breeding in Plants, Plant Breeding from Laboratories to Fields ed. Andersen S. B. DOI: 10.5772/52583 InTech (2013)

Jin, Y., Pretorius, Z., and Singh R. P. (2007). New virulence within the race TTKS (*Ug99*) of the stem rust pathogen and effective resistance genes Phytopathology 97, S173

Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M., Njau, P., Fetch, T., Pretorius, Z.A., Yahyaoui, A. (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 91:1096-1099.

Johnson, R. (1984). A critical analysis of durable resistance. Annu. Rev. Phytopathol. 22, 309–330. doi: 10.1146/annurev.py.22.090184.001521.

Kao, C. H., Zeng, Z. and Teasdale, R. D. (1999). Multiple interval mapping quantitative trait loci. Genetics volume 152 pp 1203-1216

Kenya Consumer Trends (Friday 26 August 2011) Business Daily. Retrieved October 21 2018 from http://businessdailyafrica.com/blob/view/-/1225404/data/289916/-/15mqupez/-/edge-26082011.pdf

Kenya Production potential; Kenya-FAO Wheat database. Retrieved from www.fao.org on 23rd January, 2012

Keurentjes, J. J. B., Willems, G, Van Eeuwijk, F., Magnus Nordborg, M., and Koornneef M. (2011) A comparison of population types used for QTL mapping in Arabidopsis thaliana. Plant Genetic Resources: Characterization and Utilization (2011) 9(2); 185–188 doi:10.1017/S1479262111000086

King, R. C., and Stansfield, W.D. (1990) A dictionary of genetics 4th edition, Oxford University Press, New York-Oxford, page 188

Kirk, P.M., Cannon, P.F., David, J.V. and Stalpers, J.A. (2001). Ainsworth and Bisby's dictionary of the fungi, 9th Edition page 569, 610, & 624. Wallingford, UK: CAB International.

Knott, D. R. (1982) Multigenic inheritance of stem rust resistance in wheat. Crop Sci 22:393–399

Knott, D. R. (1989) The origin and evolution of wheat In: The wheat rusts – breeding for resistance. Springer-Verlag, Berlin, monographs Theor. Appl. Genet., pp 1-6

Knott, D.R. (1988). Using polygenic resistance to breed for stem rust resistance in wheat In: N.W. Simmonds & S. Rajaram, (eds.) Breeding strategies for resistance to the rusts of wheat, Mexico, DF, CIMMYT page 39-47.

Kojima,T., Nagaoka, T., Noda, N., and Ogihara, Y. (1998). Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theory of Applied Genetics 96: 37–45

Kuchel H, Fox R, Reinheimer J, Mosionek L, Willey N, Bariana H, Jefferies S (2007) The successful application of a marker assisted wheat breeding strategy. Mol Breed 20(4):295–308

Kurt, J. L., and Les, J. S. (2005) Stem rust of small grains and grasses caused by *Puccinia graminis*, Molecular plant pathology Volume 6(2) 99-111

Lagudah, E. S., McFadden, H., Singh, R. P., Huerta-Espino, J., Bariana, H. S., Spielmeyer, W. (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat**. In:** TAG Theoretical and Applied Genetics, 2006, 114:21-30.

Lance, G., and Garren, B. (2002) Origin, History, and Uses of Oats (*Avena sativa*) and Wheat (*Triticum aestivum*):www.agron.iastate.edu

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

Leonard, W. H., and Martin, J. H. (1963) Cereal crops New York, NY, USA, MacMillan Publishing.

Lewis, C. M., Persoons, A., Bebber, D. P., Kigathi, R. N., Maintz, J., Findlay, K., Saunders, D. G. O. (2018). Potential for re-emergence of wheat stem rust in the United Kingdom. *Communications Biology*, *1*, 13 http://doi.org/10.1038/s42003-018-0013-y

Li, H., Vikram, P., Singh, R. P., Kilian, A., Carling, J., Song, J., Burgueno-Ferreira, J. A., Bhavani, S., Huerta-Espino, J., Payne, T., Sehgal, D., Wenzl, P., and Singh, S. (2015) A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. BMC Genomics 16:216 DOI 10.1186/s12864-015-1424-5

Li, H., Ye, G. Y., and Wang, J. (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:1-14

Liang, S. S., K. Suenaga, Z.H. He, Z. L. Wang, H. Y. Liu, D. S. Wang, R. P. Singh, P. Sourdille, and X. C. Xia. 2006. Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96 (7): 784-789.

Liu, Z., Bowden, L. R., and Bai, G. (2013) Molecular Markers for Leaf Rust Resistance Gene *Lr42* in Wheat Crop Sci. 53:1566–1570

Long, X. Y., Hugues, B., Rouse, M. N., Singh, S., Singh, P. R., Bhavani, S., Huerta-Espino, J., Sorrells, M. (2014) A consensus map for Ug99 stem rust resistant loci in wheat. Theor Appl Genet 127(7):1561-81

Lowe, I., Cantu, D., and Dubcovsky J. (2010) Durable resistance to wheat rusts: Integrating classical systems and biology approaches. BGRI Technical workshop 30-31 May 2010, St. Petersburg, Russia

Luig, N. H. (1985) Epidemiology in Australia and New Zealand In: Roelfs AP, Bushnell WR (Eds), Cereal Rusts, Vol. II: Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando, pp. 301–328

Lynch, M., and Walsh, B. (1998) Genetics and analysis of quantitative traits Sinauer

Mackay, T. F. C. (2001) The genetic architecture of quantitative traits. Annals of Revolutionary Genetics 35:303-339

McDonald, J. H. (2014). Handbook of Biological Statistics (3rd ed.) Sparky House Publishing Baltimore, Maryland pages 45-52 in the printed version.

McFadden, E. S. (1930). A successful transfer of emmer characters to *Vulgare* wheat J. Am. Soc. Agron. 22, 1020–1034.

McIntosh, R. A. (1988) The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, pp 1–9

McIntosh, R. A. (1992). Genetics of wheat and wheat rusts since Farrer: Farrer memorial oration 1976. J. Australian Institute of Agricultural Science 42:203-216

McIntosh, R. A., Hart, G. E., Devos, K. M., Gale, M. D., Rogers, W. J., Dubcovsky, J., and Morris, C. F. (2003) Catalogue of gene symbols for wheat. GrainGenes. http://wheat.pw.usda.gov/wgc/2003 (March 28, 2005)

McIntosh, R. A., Wellings, C. R., and Park, R. F. (1995). Wheat Rusts: An Atlas of Resistance Genes. Melbourne: CSIRO Publishing

Mehmet, A. and Nuh, B. (2012). Stem rust (Ug99) seen as a threat globally. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 25. 23-28.

Mishra, A. N., Kaushal, K., Yadav, S. R., Shirsekar, G. S. and Pandey, H. N. (2005). The linkage between the stem rust resistance gene *Sr2* and pseudo-black chaff in wheat can be broken. Plant Breeding, 124: 520–522. doi:10.1111/j.1439-0523.2005. 01136.x

Muasya, R. M., and Mwakha. (1996). Initial wheat response to low and high rates of nitrogen and phosphorus fortified compost in Kenya farrosol. Focus on Agricultural research for sustainable development in changing economic environment, proceedings of the 5th KARI scientific conference in Nairobi.

Nagarajan, S. and Joshi, L. M. (1985). Epidemiology in the Indian Subcontinent In "The cereals rusts, Volume II; Diseases, Distribution, Epidemiology and Control". (A. P. Roelfs and W. R. Bushnell, eds)

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals *Genetics 89: 583–590*

Nuttonson, M.Y. (1995) Wheat climatic relationships and the use of phenology in ascertaining the thermal and photo thermal requirements of wheat Washington, DC, American Institute of Crop Ecology.

Olivera, F. P. D., Newcomb, M., Flath, K., Sommefeldt-Impe, N., Szabo, L. J., Carter, M., Luster, D. G., and Jin, Y. (2017) Characterization of *Puccinia graminis* f. sp. *tritici* isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. *Plant Pathol.*10.1111/ppa.12674 (2017)

Olson, E. L., Brown-Guedira, G., Marshall D. S., Jin, Y., Mergoum, M., Lowe, I., and Dubcovsky, J. (2010) Genotyping of U.S. Wheat Germplasm for Presence of Stem Rust Resistance Genes *Sr24*, *Sr36* and *Sr1RSAmigo* Crop Sci. 50:668–675

Paran, I. and Michelmore, R.W. (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theory of Applied Genetics 85:985-993.

Park, R., T. Fetch, D. Hodson, Y. Jin, K. Nazari, M. Prashar, and Z. Pretorius. 2011. International surveillance of wheat rust pathogens: *Progress and challenges Euphytica 179 (1): 109-117.*

Park, S. D. E. (2001) Trypano-tolerance in West African Cattle and the Population Genetic Effects of Selection. [Ph.D. thesis] University of Dublin

Parlevliet, J. E. (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124: 147-156

Percival, J., (1921). The wheat plant; A monograph. New York, NY, USA E. P Dutton & Company.

Peterson, R. F., Campbell, A. B., and Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Res

Plant and Soil Science **e**Library (2018) Quantitative Trait Analysis (QTL) Analysis 2. Retrieved October 2018 from http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1067442598&topicorder=7

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis, Molecular Breeding 2: 225-238

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., and Payne, T. S. (2000) Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f sp tritici in Uganda. Plant Dis 84:203

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., and Payne, T. S. (2000). Detection of Virulence to Wheat Stem Rust Resistance Gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda Plant Diseases February 2000, Volume 84, Number 2 Page 203

Priyamvada, M. S., and Tiwari, R. (2011). Durable resistance in wheat. Journal of genetics and molecular biology Volume 3(8), pp 108-114

Queller, D. C., Strassman, J. E., and Hughes, C. R. (1993). Microsatellites and kinship. Trends in Ecology and Evolution 8: 285-288

Reamon-Buttner, S. M., and Jung, C. (2000) AFLP-derived STS markers for the identification of sex in *Asparagus officinalis* L. Theory of Applied Genetics 100:432–438

Robinson, R.A. (1973). Horizontal resistance. Revolutionary Plant Pathology 52: 483-501.

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

Roelfs, A. P. and Bushnell, W. R. (1985*)* The Cereal Rusts Volume II: Diseases, Distribution, Epidemiology, and Control. ACADEMIC PRESS, INC**.** Orlando, Florida 32887

Roelfs, A. P., Singh, R. P., and Saari, E. E. (1992) Rust diseases of wheat: Concepts and methods of management. Mexico, DF: CIMMYT.

Rungis, D., Hamberger, B., Berube, Y., Wilkin, J., Bohlmann, J, and Ritland, K. (2005) Efficient genetic mapping of single nucleotide polymorphisms based upon DNA mismatch digestion. Molecular Breeding 16: 261-270

Saal, B., and Wricke, G. (1999) Development of simple sequence repeat markers in rye *Secale cereale* L. Genome 42, 964—972.

Saari, E. E., and Prescott, J. M. (1985) World distribution in relation to economic losses. In 'The cereal Rusts, Vol. II; Diseases, Distribution, Epidemiology, and control (A. P. Roelfs and W. R. Bunshell, eds.), pp. 259-298. Academic Press, Orlando

Santra M., Wang H., Seifert S., Haley S. (2017) Doubled Haploid Laboratory Protocol for Wheat Using Wheat–Maize Wide Hybridization. In: Bhalla P., Singh M. (eds) Wheat Biotechnology. Methods in Molecular Biology, vol 1679. Humana Press, New York, NY

Schumann, G.L. and K.J. Leonard. 2000. Stem rust of wheat (black rust). The Plant Health Instructor. DOI: 10.1094/PHI-I-2000-0721-01(updated in 2011)

Semagn, K., Bjornstad, A., Xu, Y. (2010) The genetic dissection of quantitative traits in crops. Electronic Journal of Biotechnology Volume 13 issue 5

Semagn, K., Bjorstad, A., and Ndjiondjop, M. N. (2006) An overview of molecular marker methods for plants. Africa journal of biotechnology Volume 5:2540-2568

Septiningsih, E. M., Trijatmiko, K. R., Moeljopawiro, S. and Mccouch, S. R. (2003) Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the Oryza sativa variety IR64 and the wild relative O. rufipogon. Theoretical and Applied Genetics. 2003;107(8):1419–32. pmid:14513215

Shank, R. (1994). Wheat stem rust and drought effects on Bale Agricultural production and future prospects. Report on February 17-28 assessment. In "United Nations Emergencies Unit for Ethiopia." http://www.africa.upenn.edu/eue_web/Bale_mar.txt Retrieved on 15th January,2012

Silva, P., Calvo-Salazar, V., Condo,´n F., Quincke, M., Pritsch, C., Gutie´rrez, L., Castro, A., Herrera-Foessel, S., Von Zitzewitz, J. and, Germa´n, S. (2105) Effects and interactions of genes Lr34, Lr68 and Sr2 on wheat leaf rust adult plant resistance in Uruguay. Euphytica (2015) 204:599–608

Singh, B.D., Singh A.K. (2015) Mapping Populations. In: Marker-Assisted Plant Breeding: Principles and Practices. Springer, New Delhi

Singh, R. P. (2012). Pros and cons of utilizing major, race-specific resistance genes versus partial resistance in breeding rust resistant wheat. Paper presented at 2012 BGRI Technical Workshop, September1-4. Beijing, China.

Singh, R. P., D. P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, S. Herrera-Foessel, P. K. Singh, S. Singh, and V. Govindan. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annual Review of Phytopathology 49: 465-81.

Singh, R. P., Hodson, D. P., Huerta-Espinno, J., Yue, J. Njau, P., Wanyera, R., Herrera-Foessel, S. A. and Ward, R. W. (2008) Will stem rust destroy the world's wheat crop? Advances in agronomy Volume 98 page 272-298

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M. G., Wanyera, R., Njau, P., Ward, R. W. (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in agriculture, veterinary science, nutrition and natural resources 1:1-13

Singh, R. P., Hodson, D. P., Jin, Y., Lagudah, E. S., Ayliffe, M. A., Bhavani, S., Rouse, M. N., Pretorius, Z. A., Szabo, L. J., Huerta-Espino, J., Basnet, B. R., Lan, C., and Hovmøller, M. S. (2015) Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic Control. July 2015, Volume 105, Number 7 Pages 872-884 http://dx.doi.org/10.1094/PHYTO-01-15- 0030-FI

Singh, R. P., Huerta-Espino, J., Bhavani, S., Herrera-Foessel S. A., Singh, D., P. K. Singh, P. K., Velu, G., Mason, E. R., Jin, Y., Njau, P., and Crossa, J. (2011) Race non-specific resistance to rust diseases in CIMMYT spring wheats. *Euphytica 179:175–186*

Singh, R. P., Huerta-Espino, J., Rajaram, S., (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta phytopathology Entomology Hung 35:133-139

Singh, R. P., Huerta-Espino, J., Roelfs, A. P. (2002). The wheat rusts. In "Bread wheat; improvement and production" FAO plant production and protection series No. 30

Singh, R. P., William, H. M., Huerto-Espino, J., and Rosewarne, G. (2004). Wheat rust in Asia: Meeting the challenge with old and new technologies. In "New directions for a diverse planet: Proceedings of the 4th international crop science congress," September 26-October 1 2004. Brisbane, Australia.

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P.K., Singh, K., and Govindan, V. (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Ann. Review of Phytopathology 49:465-81

Speilmeyer, W., McIntosh, R. A., Kolmer, J., Lagudah, E. S. (2005) Powdery mildew resistance and Lr34/Yr18 genes for durable resistance to leaf and stripe rust co-segregate at a locus on the short arm of chromosome 7D of wheat. Theoretical and Applied Genetics 111: 731-735

Sperschneider, J., Ying, H., Dodds, P. N., Gardiner, D. M., Upadhyaya, N., Singh, K. B, Manners, J. M. and Taylor, J. M. (2015) Diversifying selection in the wheat stem rust fungus acts predominantly on pathogen-associated gene families and reveals candidate effectors. Frontiers in Plant Science Vol. 5 article 372

Spielmeyer, W., Sharp, P. J., Lagudah, E. S. (2003) Identification of markers linked to resistance gene *Sr2* in wheat (*Triticum aestivum L.)* Crop Science 43:333-336 Wanyera, R., Kinyua, M. G., Jin, Y., and Singh, R. P., (2006) The spread of stem rust caused by Puccinia graminis f. sp. tritici, with virulence on Sr31 in wheat in Eastern Africa. Plant Diseases 90:113

Spielmeyer, W., P. J. Sharp, P. J., and Lagudah, E. S. (2001) Identification and Validation of Markers Linked to Broad-Spectrum Stem Rust Resistance Gene Sr2 in Wheat (Triticum aestivum L.) Crop Science, 2003, 43:333–336

Steele, K. A., Humphreys, E., Wellings, C. R., and Dickson, M. J. (2001). Support for a stepwise mutation model for pathogen evolution in Australian *Puccinia striiformis f. sp. tritici* by use of molecular markers. Plant Pathology 50:174-180

Sukhwinder Singh, Ravi P. Singh, Sridhar Bhavani, Julio Huerta-Espino, Lopez-Vera Eric Eugenio. (2013) QTL mapping of slow-rusting, adult plant resistance to race Ug99 of stem rust fungus in PBW343/Muu RIL population. Theory of Applied Genetics 126:1367-1375

Tanksley, S.D., Ganal, M.W., and Martin, G.B. (1995). Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends in Genetics 11: 63-68.Technical Workshop, Saint Paul, Minnesota, USA, pp 43–53, 13– 16 June 2011

The Natural History of Wheat (2003) Encyclopedia of Food and Culture. Retrieved October 21, 2018 from Encyclopedia.com: http://www.encyclopedia.com/food/encyclopedias-almanacs-transcripts-and-maps/natural-history-wheat

Tukey, John (1949). "Comparing Individual Means in the Analysis of Variance". Biometrics. 5 (2): 99–114. JSTOR 3001913

Van Ooijen, J. W., 2006. JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B. V., Wageningen, Netherlands.

Vanderplank, J.E. (1963). Plant Diseases: Epidemics and Control. New York: Academic, p. 349.

VanZandvoort, P., vanEck, H. J., Folkertsma, R. T., Hutten. R. C. B., Draaistra, J., Gommers, F. J., Jacobsen, E., Helder, J., and Bakker, J. (1997). Use of allele specificity of co-migrating AFLP markers to align genetic maps from different potato genotypes. Mol. General Genet. 255:438-447.

Vignal, A., Milana, D., Sancristobala, M., and Eggenb, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics Genet. Sel. Evol. 34:275-305.

Wanyera, R., Macharia, J. K., Kilonzo, S. M., and Kamundia, J. W. (2009) Foliar fungicides to control wheat stem rust race TTKS (Ug99), in Kenya. Plant Dis. 93:929-932

Warwick Crop Centre (September, 2016) What are Mapping populations? Retrieved on 25th October, 2016

Wellings, C. R., Singh, R. P., Yahyaoui, A. H., Nazari, K., and McIntosh, R. A (2009) The development and application of near-isogenic lines for monitoring cereal rust pathogens BGRI In: Proceedings of the Borlaug Global Rust Technical Workshop 2009

Weng, Y., Azhaguvel, P., Devkota, R.N., and Rudd, J.C. (2007). PCR-based markers for detection of different sources of 1AL.1RS and 1BL.1RS wheat-rye translocations in wheat background. Plant Breeding 126:482-486.

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A., and Kilian, A. (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proceedings of the National Academy of Sciences Jun 2004, 101 (26) 9915-9920; DOI:10.1073/pnas.0401076101

Wheat & Small Grains (2012) Stem rust and Barberry in the pacific north west. Retrieved August,2016 from http://smallgrains.wsu.edu/disease-resources/foliar-fungal-diseases/stem-rust/

William M, Singh RP, Huerta-Espino J, Ortiz Islas S, Hoisington D (2003) Molecular Marker Mapping of Leaf Rust Resistance Gene Lr46 and Its Association with Stripe Rust Resistance Gene Yr29 in Wheat. In: Phytopathology, 2003, 93(2):153-159.

World Agriculture (2018) Maintaining Genetic Purity of Varieties in Seed Production. Retrieved from https://www.agrotechnomarket.com/2011/08/maintaining-genetic-purity-of-varieties.html , October 2018

World Bank., (1989) Kenya Agricultural Growth and Strategy Options. Unpublished Sector Report, Nairobi, Kenya: The World Bank

Yang, W., de Oliveira, A. C., Goodwin, I., Schertz, K., and Bennetzen, J. L. (1996). Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Sci. 36: 1669–1676.

APPENDICES

APPENDIX I: Disease severity and infection type responses to field stem rust evaluation of the Robin/Kwale population at the F_2 and the F_3 generations. The Co-efficient of infection for the data recorded at the F_2 generation is also shown

$F_{2:3}$ ROBIN/KWALE Families							
	***F_2_TDS*[a]**	***F_2_TDS*[a]**	***F_3_TDS*[c]**		***F_2_TDS*[a]**	***F_2_TDS*[a]**	***F_3_TDS*[c]**
F_3 field No.	**13/05/13**	**CI[b]**	***31/10/2013***	**F_3 field No.**	**13/05/13**	**CI[b]**	***31/10/2013***
1	5MS	4	20MSS	**161**	10MR	4	10RMR 5M
2	15MR	6	25RMR	**162**	15MR	6	20RMR 5M
3	5MR	2	20RMR	**163**	20MR	8	15MR 15MS MR
4	5MS	4	15MSMR	**164**	15MSS	12	20M
5	10MR	4	10RMR TMS	**165**	20M	12	15M
6	15MR	6	30MSS	**166**	5MR	2	5RMR 15MSMR
7	20MR	8	20RMR	**167**	5MR	2	5RMR
8	10MR	4	20MR;30M	**168**	15MS	12	20M
9	5MR	2	20M	**169**	5MR	2	10M
10	15MR	6	20MRTMS	**170**	5MR	2	10RMR TMS
11	50MSS	40	15MSSMR	**171**	TR RMR	0.08	15M
12	10MR	4	10M	**172**	5MR	2	10RMR 10M
13	5MR	2	5RMR;10MSMR	**173**	5MR	2	10RMR
14	40MSS	32	-	**174**	15MR	6	15RMR 5M
15	5MR	2	15RMRTMS;10M	**175**	15MR	6	15RMR TMS
16	20MR	8	-	**176**	10MSS	8	15MS MR
17	5MR	2	TRMR;5M;5MS	**177**	5MR	2	5RMR 15MSMR
18	5MR	2	10RMR;5MS	**178**	10MR	4	15RMR
19	15MR	6	20MSMR	**179**	10MR	4	10RMR 10MSS
20	30MR	12	15MR	**180**	20MSS	16	20M
21	30MSS	24	20MSS	**181**	10M	6	10M
22	30MSS	24	-	**182**	30MSS	24	20MSS MR
23	10MR	4	40M	**183**	5MR	2	10RMR

24	5MR	2	10RMR	**184**	20MSS	16	20MSS MR
25	5MR	2	15RMR	**185**	30MSS	24	15M
26	10MR	4	10RMR	**186**	10MR	4	10RMR
27	TR R	0.2	5RMR	**187**	15M	9	15RMR TMS
28	5MR	2	15M	**188**	TR MR	0.4	15M
29	10MR	4	10M	**189**	10MR	4	15RMR 5MS
30	5MR	2	20M	**190**	10MR	4	15RMR
31	15MR	6	10RMR	**191**	10MR	4	15RMR TMS
32	15MR	6	10RMR;10M	**192**	10MR	4	15RMR TMS
33	5MR	2	15RMR	**193**	5RMR	0.4	10RMR
34	5MR	2	-	**194**	15MR	6	30MR TMS
35	TR MR	0.4	10RMRTMS	**195**	15MR	6	20M
36	5MR	2	10RMR	**196**	5MR	2	10RMR
37	20M	12	30M	**197**	15MR	6	10RMR TMS
38	TR MR	0.4	5R	**198**	10MR	4	15RMR
39	15MR	6	15RMR	**199**	10MR	4	15RMR 20MS MR
40	5MR	2	10M	**200**	5RMR	0.4	10RMR
41	15MR	6	15RMR	**201**	30MSS	24	15MSS MR
42	10MR	4	-	**202**	5RMR	0.4	15RMR
43	25MSS	20	20MSS	**203**	5MR	2	15RMR
44	5MR	2	30MSS	**204**	5M	3	
45	15MS	12	15MSS	**205**	5MR	2	10RMR
46	15MR	6	20MR0(M)	**206**	10M	6	10RMR 15MS MR
47	10MR	4	-	**207**	TR MR	0.4	10RMR
48	20M	12	20MR	**208**	15MSS	12	10RMR 15M
49	20MR	8	10M	**209**	5MR	2	15M
50	5MR	2	10RMR	**210**	5MS	4	10M
51	5MR	2		**211**	15MSS	12	15M
52	5MR	2	15M	**212**	10MR	4	15RMR TMS
53	20MR	8	25MS 15M	**213**	5MR	2	10RMR
54	10MR	4	10RMR	**214**	10MSS	8	30MS MR
55	15MR	6	10RMR	**215**	5MR	2	10RMR

56	5M	3	20MSS	**216**	5MR	2	
57	15MR	6	15M	**217**	10MR	4	15M .
58	5MR	2	10RMR 10MSMR	**218**	15MR	6	15MR TMS
59	20MSS	16	15MSS	**219**	5RMR	0.4	10RMR TMS
60	5MR	2	15RMR	**220**	10MR	4	15RMR TMS
61	15MSS	12	15MS 10MR	**221**	10MSS	8	30MSS
62	5MR	2		**222**	10MSS	8	20M
63	TR R	0.2	5M	**223**	5MR	2	10RMR 15M
64	10MR	4	15RMR	**224**	5MR	2	10RMR
65	30MSS	24	30MSS	**225**	10M	6	15M
66	10MR	4	15RMR 15M	**226**	5MR	2	15M
67	40MSS	32	30MSS 10M	**227**	0	0	5MR MS
68	5MR	2	10RMR	**228**	5MR	2	
69	10MR	4	20RMR	**229**	15MR	6	
70	10MR	4	15M	**230**	10MR	4	
71	10MR	4	15RMR	**231**	10MS	8	20M
72	TR RMR	0.08	5RMR	**232**	10MR	4	15RMR 10M
73	15M	9	15RMR	**233**	5MR	2	
74	5MR	2	10RMR	**234**	5MR	2	10RMR TMS
75	15MR	6	5R	**235**	5MR	2	20M
76	15MR	6	5RMR	**236**	15MR	6	15RMR TMS
77	10MR	4		**237**	5MR	2	15RMR
78	5MR	2	20RMR	**238**	R	0.2	15RMR
79	5MR	2		**239**	10MS	8	30MSS
80	10MR	4		**240**	5M	3	
81	5MR	2	15RMR	**241**	5RMR	0.4	10RMR TMS
82	20MSS	16	30M	**242**	10MR	4	
83	15MSS	12	5RMR	**243**	10MR	4	15RMR TMS
84	20M	12	20RMR .	**244**	5RMR	0.4	10RMR
85	5MR	2	10RMR	**245**	5MR	2	20M
86	5MR	2		**246**	5RMR	0.4	10RMR
87	5RMR	0.4	10RMR	**247**	5MR	2	15RMR TMS
88	10MR	4	15RMR TMS	**248**	5MR	2	10RMR TMS
89	10MSS	8	20MSS	**249**	5MR	2	15M

90	5MR	2	15M	**250**	15MR	6	10RMR 10MSS
91	5MR	2	15M	**251**	5MR	2	15RMR
92	10MSS	8	15M	**252**	TR RMR	0.08	10RMR
93	5MR	2	15RMR	**253**	5MR	2	
94	10MR	4	20M	**254**	15MSS	12	
95	30MSS	24	30M	**255**	5MR	2	10RMR TMS
96	10M	6	10M	**256**	10MR	4	15M
97	15M	9	15M	**257**	10MR	4	15RMR
98	10M	6	10RMR TMS	**258**	5MR	2	10M
99	5MR	2	20M	**259**	10MS	8	10M
100	5MR	2	5RMR	**260**	10MR	4	20M
101	40MSS	32	15M	**261**	5MR	2	15RMR 20MS
102	15MR	6	10RMR	**262**	TR MR	0.4	10M
103	10MR	4	10RMR	**263**	5MR	2	10RMR
104	10MR	4	10RMR	**264**	5M	3	15RMR
105	10MR	4	10RMR 5MS	**265**	5RMR	0.4	10M 10RMR
106	15MSS	12	20M	**266**	TR MR	0.4	5RMR
107	5MR	2	10M	**267**	10MR	4	15RMR
108	40MSS	32	20MSS 20RMR	**268**	15M	9	15M
109	15MR	6	20M	**269**	TR RMR	0.08	15RMR
110	25MSS	20	15M	**270**	10MR	4	10RMR 15M
111	10MR	4	10RMR TMS	**271**	10MR	4	10RMR 5MS
112	10MR	4	15RMR TMS	**272**	15MR	6	20MSS
113	5MR	2	10RMR	**273**	15M	9	15M 15MSS
114	10MR	4	15RMR 5MS	**274**	30MSS	24	20MSS
115	40MSS	32	20M	**275**	5MR	2	10RMR 5M
116	10MR	4	15RMR TMS	**276**	15M	9	10RMR TMS
117	20MS	16	20M	**277**	5MR	2	15RMR 5MS
118	30MSS	24	15MSS	**278**	5MR	2	10RMR
119	15MSS	12	15RMR	**279**	10MR	4	15M
120	15MR	6	15RMR TMS	**280**	5MR	2	10RMR
121	20MS	16	15M	**281**	15MR	6	15RMR TMS
122	10MR	4	20MS MR	**282**	5MR	2	15RMR TMS
123	30MSS	24	15MS MR	**283**	10MR	4	15RMR

124	40MSS	32	30MSS	**284**	20MR	8	15MR TMS
125	5MR	2	10RMS TMS	**285**	5MR	2	10M
126	10MR	4	15RMR 5MS	**286**	15MSS	12	30MSS
127	10MR	4	15RMR TMS	**287**	20MSS	16	30M
128	10MR	4	15MR MS	**288**	10MS	8	15M
129	30MSS	24	20MSSMR	**289**	40MSS	32	40MSS
130	40MSS	32	20M	**290**	TR RMR	0.08	10RMR
131	TR RMR	0.08	10RMR TMS	**291**	30MR	12	30MR
132	15MR	6	20RMR	**292**	10MR	4	20MR
133	10MR	4	15RMR TMS	**293**	5MR	2	15RMR 10M
134	10M	6	15RMR	**294**	15MSS	12	20M
135	10MR	4	10RMR	**295**	5MR	2	15RMR
136	10MR	4	10RMR 1PLT 10M	**296**	20MSS	16	20MSS 5M
137	10M	6	15RMR	**297**	15M	9	15RMR 15M
138	5MR	2	10RMR	**298**	5MR	2	10RMR 10M
139	15MS	12	10M	**299**	10MR	4	20RMR
140	R	0.2		**300**	5RMR	0.4	15RMR 5M
141	10MR	4	15RMR TMS	**301**	10MR	4	20RMR
142	5MR	2	10RMR	**302**	5MR	2	15RMR TMS
143	10M	6	15MR MS	**303**	10M	6	15M
144	20MR	8	15RMR TMS	**304**	5MS	4	15MS MR
145	10M	6	20M	**305**	15MR	6	20RMR
146	20MR	8	15RMR	**306**	5RMR	0.4	15RMR 20MSS
147	5MR	2	20M	**307**	5RMR	0.4	
148	5MR	2	5RMR	**308**	15M	9	20M
149	5RMR	0.4	5RMR	**309**	TR MR	0.4	15RMR 5MS
150	15M	9	15MR MS	**310**	20MR	8	10MSS 15M
151	50MSS	40	20M	**311**	5RMR	0.4	10RMR
152	10MR	4	5RMR	**312**	20MSS	16	20MSS
153	10M	6	20M 15RMR	**313**	10MR	4	15RMR TMS
154	20MR	8	10RMR	**314**	10MR	4	20M
155	20MR	8	10RMR 10M	**315**	10MR	4	15RMR TMS
156	10MR	4	15RMR				

157	10MR	4	15M
158	10M	6	10RMR 5MSS
159	10MR	4	5RMR
160	30MSS	24	20M

F_2_TDS[a] –**T**erminal Stem Rust **D**isease **S**everity at F_2

CI[b] – Co-efficient of infection

F_3_TDS[c] - **T**erminal Stem Rust **D**isease **S**everity at F_2

(Disease infection types reported; **R** – resistant; **RMR** – resistant to moderately resistant; **MR** - moderately resistant; **MS** - moderately susceptible; **MSS** – moderately susceptible to susceptible; **S** - susceptible as described by Knott, 1989)

APPENDIX II: Terminal Disease Severities of 150 RILs of PBW343/Akuri

	Season 1	*Season 2*	*Season 3*	*Season 4*	*Season 5*	*Season 6*
Date Scored	**13/4/2010**	**13/4/2010**	**12/10/2010**	**29/7/2013**	**2/1/2014**	**3/10/2014**
1	70MSS	50MSS	50MSS	50MSS	50MSS	40MSS
2	1MS	1MS	1MS	10RMR	10RMR;TMS	TR
3	80S	60S	20S	30MSS	100S	40MSS
4	40M	40M	30M	20MSS	-	-
5	80S	70S	60S	40MSS	70MSS	60S
6	50MSS	60MSS	18MSS	20MSS	40M	40MSS
7	30MSS	70MSS	50MSS	40MSS	40MSS	30S
8	15MSS	50MSS	10MSS	30MSS	30M	20MSS
9	70MSS	70MSS	55MSS	60MSS	40MSS	50S
10	20M	40M	50M	50MSS	40M	30MSS
11	20M	30M	40M	50M	30MRMS	10MSS
12	20M	50M	30M	30MSS	15M	20MSS
13	60MSS	60MSS	30MSS	30M	30M	20MS
14	60MSS	60MSS	60MSS	60MSS	40MSS	30MSS
15	40MSS	50MSS	55MSS	60MSS	30M	40MSS
16	30M	40M	10M	20MR	30MRTMS	5MS
17	20M	30M	20M	40MSS	15M	10MSS
18	20MSS	70MSS	20MSS	50M	30M	20MSS
19	30M	30M	15M	15M	40MSS	5MS
20	15M	15M	10M	20M	20M	10S
21	60S	40S	50S	40MSS	25M	20MSS
22	60S	50S	50S	50MSS	60MSS	30MSS
23	80S	50S	50S	40M	40M	30MSS
24	40M	40M	30M	40M	30MSS	20MSS
25	60MSS	60MSS	40MSS	50MSS	30M	40S
26	70S	50S	15S	20MSS	25M	15MSS
27	50S	40S	30S	30M	20M	5MSS
28	30M	30M	8M	20MR	15M	5MS
29	60M	50M	50M	30M	30M	10MSS

30	80S	60S	20S	30M	25M	15M
31	80S	60S	30S	15M	30M	TR
32	40MSS	50MSS	20MSS	20M	15M	5MS
33	60MSS	60MSS	20MSS	10MR	30M	20S
34	70MSS	50MSS	20MSS	15M	40M	10MSS
35	50MS	30MS	10MS	-	30M	10MS
36	70S	40S	50S	-	30M	10MS
37	60S	40S	40S	50MSS	30MSS	20MSS
38	50MSS	50MSS	50MSS	50M	40M	15M
39	50MSS	40MSS	30,15MSS	20MSS	40MS	30MSS
40	70M	30M	20,50M	15M	15M	-
41	70S	40S	55S	40M	30M/MS	30S
42	50M	40M	15M	40M	20M	10MS
43	80S	70S	30S	30M	70MSS	20MSS
44	60M	60M	20M	15MS	30M	15S
45	30MSS	30MSS	30MSS	20M	-	-
46	60M	30M	20M	60MSS	30M	30MSS
47	30M	15M	5M	20MR	-	-
48	50MSS	40MSS	30MSS	30M	20M	10MSS
49	30M	40M	20M	25MSS	30MSS	20M
50	30M	30M	40M	30MSS	30MSS	20MSS
51	20M	20M	5M	15RMR	15M	10S
52	30M	30M	20M	15M	30M	5MSS
53	60MSS	80MSS	50MSS	30M	30M	20MSS
54	60MSS	80MSS	55MSS	20M	20M	30MSS
55	20M	40M	15M	20MR	10M	10MS
56	60MSS	60MSS	25MSS	15MR,20M	30MSS	30S
57	40M	50M	30M	30MSS	15M	10MSS
58	60M	40M	15M	30M	40M	-
59	40M	50M	50M	20M	30M	15MS
60	30MSS	50MSS	20MSS	20MR	20M	15MSS
61	20M	30M	10M	20M	20M	5MS
62	20M	50M	25M	30MR	40M	10MS
63	20M	40M	15M	10RMR	20M	10MSS

64	30M	40M	15M	10MR	15M	10MSS
65	30MSS	40MSS	15MSS	20MR	30M	10MSS
66	20MSS	40MSS	15MSS	20RMR	30M	15S
67	30M	30M	15M	15RMR	40M	40S
68	20M	30M	20M	15MR	20MSS	TS
69	70MSS	50MSS	40MSS	15MR	30M	10MSS
70	60 60	30 60	30 60	20MR	30M	10MSS
71	60M	30M	50M	30MSS	20M	20M
72	20M	20M	15M	30M	25MSS	10MSS
73	60M	40M	40M	30MSS	30MSS	30MSS
74	40M	50M	20M	50M	20MR	30MSS
75	60MSS	80MSS	40MSS	30MSS	40M	40S
76	305M	305M	205M	30MSS	30M	20MSS
77	20M	20M	8M	20MR	15M	5MS
78	30MSS	50MSS	40MSS	50M	70MSS	30MSS
79	30M	50M	15M	20MR	15M	10S
80	40MSS	50MSS	10MSS	20M	20M(MR)	30MSS
81	40M	30M	15M	20MSS	70MSS	30S
82	30MS	50MS	10MS	15RMR	15MR	10M
83	80S	60S	45S	40MSS	25M	40S
84	80S	40S	18S	15MR	30M	20M
85	40M	30M	5M	5M	10M	TS
86	80S	50S	10S	15MR	20M	20MSS
87	60M	50M	20M	20M	50MSS	40S
88	80S	80S	50S	40M	40MSS	50S
89	40MSS	50MSS	10MSS	20MR	20M	10MSS
90	60MSS	50MSS	10MSS	2M	30M	20MSS
91	100S	60S	55S	40MSS	40M	30MSS
92	50M	40M	55M	20M	30MS	30MSS
93	405M	405M	405M	20MR	20M	20MSS
94	70S	80S	55S	30M	25MSS	30MSS
95	70S	60S	55S	-	25M	30MSS
96	30M	50M	15M	15RMR	10M	-
97	50M	50M	30M	15RMR	25MSS	15MSS

98	50M	50M	50M	30MSS	50MSS	40S
99	40MSS	60MSS	30MSS	15M	30M	30MSS
100	30MSS	50MSS	20MSS	20M	20M	30M
101	30MSS	40MSS	30MSS	15RMR	20M	20MSS
102	70S	30S	40S	20MR	20M	30MSS
103	60S	70S	50S	30MSS	30MSS	40S
104	20M	40M	10M	10MR	15M	5MSS
105	70S	60S	40S	30MR	40M	30S
106	15M	15M	30M	30M	20M	10MSS
107	70S	60S	60S	50M	20M	30MSS
108	50M	40M	55M	20M	20M	10MSS
109	30M	40M	30M	30M	20M	5S
110	40M	30M	40M	15RMR	25M	40S
111	40M	50M	30M	10RMR	20M	15S
112	50MSS	80MSS	50MSS	30M	30M	40S
113	30MSS	50MSS	30MSS	30M	30M	20MSS
114	20M	30M	15M	30MSS	10M	TR
115	70S	60S	70S	40M	70MSS	30M
116	20M	50M	20M	15MR	25M	5MS
117	50MSS	50MSS	55MSS	10RMR	20M	10MSS
118	40M	40M	45M	30MSS	30MSS	15MS
119	30M	20M	40M	40MSS	10M	-
120	60S	60S	50S	30MR	30MSS	30MSS
121	50M	40M	40M	30MSS	25M	-
122	50M	30M	50M	40M	40MSS	20M
123	50M	40M	15M	15MR	40M	15S
124	40M	40M	20M	15M	25M	15MSS
125	60M	50M	30M	15MR	30M	20S
126	50MSS	50MSS	30MSS	20M	20M	10MS
127	20M	20M	10M	15MR	10MRTMS	0
128	20M	30M	10M	10RMR	10M	5MS
129	40M	50M	55M	15M	40M	10MS
130	40M	40M	15M	20M	20MR	TS
131	40M	40M	10M	15M	30M(MS)	10MS

132	50M	40M	50M	20MR	20M	10M
133	40M	30M	15M	20M	15MR	TR
134	30M	20M	5M	5MR	15M	10MSS
135	30M	30M	30M	30MSS	20MSS	15MSS
136	50MSS	40MSS	40MSS	30M	10MR	10MS
137	50MSS	50MSS	10MSS	10MR	10MR TMS	TS
138	15MS	40MS	5MS	15RMR	10M	TS
139	40MSS	60MSS	65MSS	30MSS	70MSS	40S
140	60MSS	50MSS	50MSS	30M	30M	30MSS
141	60MSS	50MSS	20MSS	30MSS	20M	40S
142	30M	30M	20M	20M	15M	15MSS
143	30MS	60MS	30MS	10MR	20MS	10MS
144	60MSS	50MSS	45MSS	20MR	20M	30MSS
145	30M	50M	50M	20MR	20M	20MSS
146	30M	30M	20M	20M	30MSS	20MSS
147	30M	40M	15M	15MR	15RMR	20M
148	70S	80S	50S	15M	40MSS	-
149	40M	40M	40M	20MR	20M	30MS
150	70S	50S	20S	40M	10RMR	30MSS

(Disease infection types reported; **R** – resistant; **RMR** – resistant to moderately resistant; **MR** - moderately resistant; **MS** - moderately susceptible; **MSS** – moderately susceptible to susceptible; **S** - susceptible as described by Knott, 1989)