

**IDENTIFICATION OF NOVEL SOURCES OF RESISTANCE GENES TO *UG99*  
PREDOMINANT VARIANTS OF STEM RUST IN KENYAN AND INTRODUCED  
WHEAT GERMPLASM AND THEIR INHERITANCE**

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**A Thesis submitted to Graduate School in partial fulfilment for the requirements of the  
Master of Science Degree in Agronomy (Plant Breeding) of Egerton University**

**EGERTON UNIVERSITY**

**AUGUST, 2015.**

## DECLARATION AND RECOMMENDATION

### DECLARATION

I declare that this is my original work and has not been presented or submitted by anybody in this or any other institution for a degree award.

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## **DEDICATION**

This work is dedicated to the Almighty God, my parents Nicholas and Esther, my siblings Sellah, Titus, Rebbly and Kelvin.

## ABSTRACT

Stem rust (*Puccinia graminis* f.sp. *tritici-Pgt*) disease is currently one of the major biotic constraints in wheat (*Triticum aestivum*) production worldwide. Access to diverse sources of genetic resistance is important in building a broad base resistance to stem rust in our commercial wheat varieties. The objectives of this study were (i) to screen wheat genotypes from diverse origins for both seedling (SPR) and adult plant resistance (APR) to the predominant *Ug99* race and its variants in Kenya, and (ii) to determine the mode and number of genes conferring resistance to *TTKST* race in the identified resistant lines. Screening for seedling plant resistance (SPR) was done under controlled greenhouse conditions while screening for adult plant resistance (APR) was done in the field at KALRO-Njoro, Kenya during 2012 and 2013 wheat growing seasons. Selection for APR was based on area under disease progress curve (AUDPC, <300) and coefficient of infection (CI, <20) values. Under field testing, there were variations in the disease severities and responses within and between seasons. AUDPC values ranged from 0 to 1,285; CI values from 0 to 100 and final disease severities (FDS) from 0 to 100S. This study identified potential sources of adult plant (119 i.e 35%) and seedling (125 i.e 37% -*TTKSK*, 137 i.e 40% -*TTKST*) resistance against stem rust *Ug99* races. Genotypes *KSL18*, *PCB52*, *PCB62*, *PCB76*, *Bounty*, *Lenana*, *K6290 Bulk*, *Kenya Swara*, and *Kenya Nyati* have both resistance genes. In the second experiment, the resistant wheat genotypes *KSL18*, *PCB52*, *PCB62* and *PCB76* were crossed with known susceptible cultivars *Kwale* and *Duma*. The resulting hybrids and F<sub>2</sub> populations alongside the parents were then tested in the greenhouse for response to the stem rust race *TTKST*. The selected wheat lines exhibited infection types ‘;’ to ‘2’ depicting resistance while *Kwale* and *Duma* depicted infection type ‘3+’ to *TTKST*. Evaluation of F<sub>2</sub> populations that derived from *Kwale* × *PCB52* indicated that the resistance is conferred by a single dominant gene (3R:1S ratio). However, all other F<sub>2</sub> populations showed that resistance was conferred by two genes complementing each other (duplicate recessive epistasis) thus the ratios 9R: 7S. These identified resistant genotypes could be evaluated for other qualities and passed as potential varieties or used as sources of valuable resistance to achieve durable resistance against stem rust races.

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## LIST OF ABBREVIATIONS

APR- Adult plant resistance  
AUDPC- Area under progress curve  
Avr- Avirulence  
BGRI- Borlaug Global Rust Initiative  
CAN- Calcium ammonium nitrate  
CIMMYT- International Center for Wheat and Maize Improvement  
CI- Coefficient of infection  
Cm- Centimeter  
FAO- Food and agriculture organization  
FDS- Final disease severity  
F<sub>1</sub>- First filial generation  
F<sub>2</sub>- Second filial generation  
GS- Growth stage  
KALRO- Kenya agricultural and livestock research organization  
KSL- Kenyan selected lines  
Lr- Leaf rust  
MR- Moderately resistant  
MS- Moderately susceptible  
MSS- Moderately susceptible to susceptible  
PBC- Pseudo black chaff  
PCBWR- Parcela chica (small plots) bread wheat rainfed lines  
Pgt- *Puccinia graminis* f.sp. *tritici*  
PM- Powdery mildew  
QTL- Quantitative trait loci  
R- Resistant  
RH- Relative humidity  
SAS- Statistical analysis software  
SPR- Seedling plant resistance  
Sr- Stem rust  
TTKSK- Stem rust race of *Ug99* lineage that is virulent to *Sr31* gene  
TTKST- Stem rust race of *Ug99* lineage that is virulent to *Sr24* gene

Ug99- Stem rust race identified in Uganda in 1999

$\chi^2$  - Chi square

Yr- Yellow rust

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background information

Wheat (*Triticum aestivum*) is an annual, self-pollinated cereal crop of the tribe Triticeae, family Poaceae (Symko, 1999; Sharma, 2012). It is one of the three (the others being rice-*Oryza sativa* and maize-*Zea mays*) major cereals grown providing food to 95% of world population (Mc Kevith, 2004; Gustafson *et al.*, 2009). Worldwide, it is grown in about 225 million hectares annually in areas between 60 °N and 44 °S of the equator at elevations ranging from 3000m above sea level (Singh *et al.*, 2008; <http://www.wheatinitiative.org>). Wheat is grown by approximately 120 countries with the top three being China, India and USA. These produce approximately 138.0, 121.4 and 92.3 million tonnes, respectively (FAO, 2013). Global wheat production in 2013 was 690 million tonnes, an increase of 28 million tonnes from 2012 (FAO, 2013). In 2014, the production was 2542 million tonnes and this need to be increased by 60% in 2050 to meet the demand of a worldwide growing population predicted to be 9.3 billion with changing diet (<http://www.wheatinitiative.org>, FAO 2014). The estimated number of food insecure people in 2013 was 707 million, an increase of 3 million from 2012, 2014 (805 million) an increase of 98 million and by 2023, the number of food insecure people is estimated to increase to 868 million. Sub-Saharan Africa is predicted to remain the most food-insecure region in the world (Meade and Stacy, 2013; FAO, 2014).

Wheat can be described based either on the growing season (winter/spring), the grain colour (red, white and amber), protein content (between the soft and hard bread wheat) or quality of gluten (Sharma, 2012). Wheat grains contain carbohydrates (60 to 80%), protein (8 to 15%), fats (1.5 to 2.0%) and vitamin B<sub>12</sub> hence is the most nutritious food crop among the cereals (Feldman *et al.*, 1995). Globally, more than 20% of the caloric value is provided by wheat (Gupta *et al.*, 2008; Junhua *et al.*, 2011). Gluten and gliadin proteins in wheat make it suitable for making bread, pasta, pastry and cakes. In addition, beer is produced from wheat in Germany (Bavaria state) and Belgium by mixing malt and crushed wheat grains. In Kenya, growing of wheat and research has been undertaken since the early 1900s (Martens, 1975; Mailu, 1997). Wheat production in Kenya is done in three main conditions i.e high rainfall areas like Mau Narok and Timau, Low rainfall areas like lower Narok and Laikipia and acid soil areas like Uasin Gishu and Trans Nzoia. However, it is only bread wheat type that is cultivated in Kenya in an estimated area of 150,000 hectares in altitudes between 1,500 m and 3,000 m above sea level. Due to high costs during production by use of fungicides as a

control method, 80% of small scale farmers produce only 20% of wheat and a greater percentage of wheat i.e 80% is produced by 20% of large scale farmers because they can afford controlling stem rust disease using fungicides.

Low grain yields are attributed to both biotic and abiotic constraints, rust diseases causing yield losses of up to 100% on the crop (Saari and Prescott, 1985; Etienne *et al.*, 2007). Wheat diseases in general are a significant factor in low wheat yields worldwide and rust diseases in particular have been a major problem in wheat cultivation since biblical times (Kislev, 1982). Biblically, cereal rusts epidemics have been referred to as punishment to the Israelites for their sins (Leonard and Szabo, 2005). Pompilus around 700 B.C established a Roman festival called Robigalia that was to protect their cereal crops through prayers and offering of animals as sacrifices to the gods of rusts hoping that rust problem would be solved out but they did not know that their rust epidemics were due to the prevailing favorable weather (warm and wet) conditions (Schumann and Leonard, 2000; Leonard and Szabo, 2005). Globally, there are more than 5,000 rust species attacking different types of crops. However, only stem (black) rust (*Puccinia graminis*), yellow (stripe) rust (*Puccinia striiformis*) and leaf (brown) rust (*Puccinia triticina*) are of economic importance in wheat (Saari and Prescott, 1985; Chen, 2005; Singh *et al.*, 2008).

Rust fungi are specific obligate parasites that interact with wheat in a gene-for-gene relationship (Flor, 1971). Of the three rust types, stem rust caused by fungus *Puccinia graminis* Pers. f. sp. *Tritici* Erik's and E. Henn is perhaps the most destructive in many countries growing wheat because it leads to shriveled kernels or total loss of crop in susceptible cultivars (Sing *et al.*, 2008). Stem rust is a major problem all over Africa and has been studied in Kenya since 1927 (Green *et al.*, 1969). For instance in 1958, there was an outbreak of stem rust which resulted in severe damage to most wheat varieties cultivated then with variety 354 severely infected (Mailu, 1997). However, when breeding work for rust resistance commenced in Kenya in the late 1960s, its damage reduced (Mailu, 1997). The stem rust race *Ug99* and its variants that emerged in 1998 have led to the boom and bust cycles rendering most wheat varieties susceptible causing up to 100% yield losses on susceptible cultivars in Kenya and other worldwide wheat growing regions (Roelfs *et al.*, 1992; Park *et al.*, 2007; Singh *et al.*, 2008). In Ethiopia, there was a stem rust epidemic during 1993 and 1994 that nearly decimated its popular variety Enkoy bearing the *Sr36* gene and the result was famine for up to 300,000 people due to food shortage (UNDP, 1994; Temesgen *et al.*, 1995). The *Ug99* race has broad virulence to the *Sr31* gene(s) and was first

detected in Uganda in 1998 (Pretorius *et al.*, 2000). It migrated into Kenya in 2001 and by 2003 stem rust severity was on the rise (Njau *et al.*, 2010).

This race continues to affect wheat production and food security globally because most cultivated varieties are CIMMYT-derivatives whose resistance is anchored on the *Sr31* gene. Thus this race and its variants continue threatening the livelihoods of hundreds of wheat farmers in Kenya as controlling it currently is mainly by use of chemicals leading to increased production costs. Adult resistances to stem rust involve genes *Sr2* on chromosome 3BS and a recently found *Sr56* on chromosome 5B of cultivar Arina. Additionally, *Sr57* and *Sr55* genes are recent findings. *Sr57* has a pleiotropic effect with *Lr34* and *Yr18* genes whereas *Sr55* gene has a pleiotropic effect with *Lr67* and *Yr46* genes, all these genes conferring resistances to stem rust (Singh *et al.*, 2008; Bhavani *et al.*, 2011). In breeding for resistance to this rust, the emphasis is on durable resistance which can be achieved by combining 2-3 race specific (seedling resistance genes) or 3-4 race-nonspecific (adult plant resistance genes) (Chen, 2005). This study aims at identifying new sources of resistance (both seedling and adult) to stem rust race *Ug99* and its variants in introduced lines and Kenyan genotypes and determining the mode of inheritance of this resistance.

## 1.2 Statement of the problem

Stem rust disease remains a major challenge to wheat production globally due to the rapid mutation of the pathogens to more virulent forms which overcomes any resistance in the cultivated varieties. East Africa is a known hot spot region for the evolution and survival of new rust races because of the favorable environmental conditions. Since its confirmation in Kenya in 2001, the race *Ug99* and its variants have overwhelmed the Kenyan cultivars leading to the low production of 300,000 tonnes of wheat, far short of the national consumption of approximately 900,000 tonnes annually. The first detected *Ug99* (*TTKSK*) variant overcame the stem rust resistance gene *Sr31* which has been effective and durable for many years worldwide, *Ug99* variant *TTKST* overcame *Sr24* gene and *TTTSK Ug99* variant overcame *Sr36* gene. Emergence of new races and their virulence to most wheat cultivars is now a major problem all over Africa, the middle East, Asia, Australia, New Zealand, Europe, North and South America. Recently, a new race designated *TKTTF* which is not of *Ug99* lineage has been confirmed in Ethiopia and has broken down the resistance in Digalu variety known of carrying *SrTmp* gene. However, vigilance has been emphasized in Kenya due to the



noted susceptibility in popular *Kenya Robin* variety whose resistance is provided by the same gene.

### **1.3 Broad objective**

Contribute to the national food security and improved livelihoods of the Kenyan wheat farmers through deployment of disease resistant cultivars.

#### **1.3.1 Specific objectives**

- i. To screen local and introduced wheat genotypes for seedling and adult plant resistance to *Ug99* predominant variants in Kenya.
- ii. To determine the mode of gene action and number of genes conferring resistance to *TTKST* race in identified resistant wheat lines.

#### **1.4 Null hypothesis**

- i. There are no wheat genotypes with seedling and adult plant resistances
- ii. Both the nature and number of resistance genes are unknown.

#### **1.5 Justification**

The destructive nature of stem rust race *Ug99*, designated as *TTKSK* using North American nomenclature and first identified in Uganda in 1999 (hence the term *Ug99*) and its variants (*TTKST*, *TTTSK*) on Kenyan commercial wheat varieties is a result of the ephemeral resistances in them. The alarm on the new *TKTTF* race that has been recently discovered in Ethiopia requires more vigilance by plant breeders and pathologists in Kenya. All these races have been considered the greatest threat to wheat production in East Africa due to either lack of durable resistances in wheat or the changing forms of stem rust pathogens. Though the declines in losses to the disease have been reduced by use of fungicides, this is unaffordable by most farmers and also leads to health problems to the users as well as polluting the environment. Therefore, enhancing the resistance in the adapted susceptible cultivars by combining the effective adult plant resistance (APR) and seedling plant resistance (SPR) gene(s) is the most promising approach. Carrying out inheritance studies on the selected resistant wheat lines against predominant *TTKST* race was also very important in order to determine the kind of the gene action and the numbers of genes conditioning resistance in them. The use of these newly identified genotypes with valuable resistance will contribute to

saving Kenyan commercial wheat varieties from the dangers of the destructive mutating races of stem rust disease. Otherwise, this will lead to diminished yields, low incomes to farmers and a reduction in wheat-derived food and feed products.

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## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Origin and genetics of wheat

Wheat (*Triticum aestivum*) belongs to the grass family *Poaceae* (*Gramineae*) and tribe *Triticeae* which contains more than 15 genera and 300 species (Symko, 1999). About 95% of all the wheat grown worldwide is hexaploid wheat (*Triticum aestivum*) ( $2n = 6x = 42$ ; AABBDD) and the rest is tetraploid durum (*Triticum durum*) ( $2n = 4x = 28$ ; AABB). Bread wheat is an allohexaploid with three sub genomes A, B and D, each having 7 chromosomes resulting in  $2n = 3x = 21$  (Gupta *et al.*, 2008). The sub genome A was derived from *Triticum urartu* ( $2n = 14$ ) while sub genome D was obtained from *T. tauschii*. *Aegilops Speltoides* is closely related to the donor of sub genome B of hexaploid wheat hence it is thought to be the donor of the sub genome (Yen *et al.*, 1996; Gupta *et al.*, 2008). The first event in the origin of hexaploid wheat involved hybridization of *Triticum urartu* ( $2n = 2x = 14$ , AA) and an unconfirmed species (BB genome) related to *Aegilops speltoides* ( $2n = 2x = 14$ , SS), which led to cultivated allotetraploid emmer wheat (*T. turgidum* ssp. *dicoccum*,  $2n = 4x = 28$ , AA BB) (Dvorak *et al.*, 1992). In the second event, which occurred about 10,000 years ago, an ancestor of the diploid *Aegilops tauschii* (DD genome) was hybridized with the allotetraploid to form a hexaploid wheat ( $2n = 6x = 42$ ) (Feldman *et al.*, 1995).

The first cultivation of wheat occurred about 10,000 years ago as part of the neolithic revolution whereby there was a change from hunting to agriculture though its domestication took place 15,000 years ago in the Fertile Crescent, marking the start of modern civilization (Harlan, 1992; Symko, 1999; Gupta *et al.*, 2008). The cultivated types by then were the diploid Einkorn (*Triticum boeoticum*) with genomes AA, BB or DD,  $2n = 14$  and tetraploid Emmer (*Triticum dicoccoides*) with genomes AABB  $2n = 28$  (Nesbitt, 2001; Salamini *et al.*, 2002). The hybridization of the wild diploid wheat (*T. uratu*,  $2n = 2x = 14$ , genome AA) with the goat grass (*Aegilops speltoides*,  $2n = 2x = 14$ , genome BB) 500,000 years earlier led to the creation of Wild Emmer wheat (*T. dicoccoides*,  $2n = 4x = 28$  with genome AABB) (Dvorak and Akhunov, 2005). Einkorn and Emmer were further developed through selection for such traits as high yield. The bread wheat (*Triticum aestivum*) was subsequently developed by hybridization of cultivated emmer with the unrelated wild grass (*Triticum tauschii*). The countries that still grow emmer, einkorn and spelt wheat are Spain, Turkey, Balkans, Indian sub-continent and Europe (Fossati and Ingold, 2001).

## 2.2 Stem rust *Puccinia graminis* f. sp. *tritici* race *Ug99* and its lineage

Race *Ug99* (*TTKSK*) was first reported and identified in Uganda in 1999 and confirmed in Kenya (2001), Ethiopia (2003), Sudan and Yemen (2006), Iran (2007) and Tanzania (2009) (Singh *et al.*, 2008). In 2004, this race severely affected most wheat genotypes developed by International Center for Wheat and Maize Improvement (CIMMYT) in Kenya. This race has virulence to *Sr31* gene located on translocation 1BL.1RS from rye (*Secale cereale*) (Pretorius *et al.*, 2000; Wanyera *et al.*, 2006). Race *TTKSK* is virulent to: *Sr38* gene introduced into wheat from *Triticum ventricosum* (Jin *et al.*, 2007); *Sr34* introduced into wheat from *Triticum comosum*; *Sr21* from *Triticum monococcum*; *Sr9d*, *Sr9e*, *Sr9g*, *Sr11*, *Sr12* and *Sr17* introduced from *Triticum turgidum*; *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9f*, *Sr10*, *Sr15*, *Sr16*, *Sr18*, *Sr19*, *Sr20*, *Sr23*, *Sr30* and *Sr41* introduced from *Triticum aestivum* (Singh *et al.*, 2006). This virulence of *Ug99* increases susceptibility of wheat varieties worldwide. Different races vary in their virulence, aggressiveness and overall fitness to survive in the environment hence the variability in epidemics.

There are eight additional races/variants that have been identified in the *Ug99* lineage i.e. *TTKSF*, *TTKST*, *TTTSK*, *PTKSK*, *PTKST*, *TTKTT*, *TTKTK* and *TTHSK* (Singh *et al.*, 2006; Rust tracker.org, 2015). They all have an identical DNA fingerprint to *Ug99* but shows different virulence patterns (Szabo, 2007; Jin *et al.*, 2008). Race *TTKST* was identified in Kenya in 2006 and caused a major wheat loss in 2007 infecting wheat carrying *Sr31* and *Sr24* genes (Jin *et al.*, 2008). It was found to be predominant in the North Rift region (Kibe *et al.*, 2012). The race was identified in Tanzania in 2009 and Uganda in 2012. However, race *TTTSK* was identified in Kenya in 2007, Tanzania (2009), Ethiopia (2010) and Uganda (2010) infecting wheat carrying *Sr31* and *Sr36* genes causing yield losses of up to 30% (Jin *et al.*, 2008; 2009). On the other hand, race *TTKSP* has virulence to *Sr24* and was identified in South Africa in 2007 but has not been reported in Kenya. The race *PTKSK* was identified in Uganda in 1998, Ethiopia (2007) and Kenya (2009) and broke down resistance conferred by gene *Sr31*. The race *PTKST* was identified in Ethiopia in 2007 and in Kenya in 2008. This race is virulent to genes *Sr31* and *Sr24* (Pretorius *et al.*, 2010). It still threatens wheat farmers in North Rift region where it was identified (Kibe *et al.*, 2012). The variant *TTKSF* has not been reported in Kenya but was identified in Uganda in 1999 and South Africa in 2000 (Rust tracker.org, 2013). In a global wheat rust monitoring system updated in 2014, *TTKSK* and/or *TTKST* are predominant in Tanzania, Kenya, Uganda, Ethiopia, Sudan and Yemen while



*TTKSF* is predominant in South Africa and Zimbabwe, races *TTKST* and/or *PTKST* are coming up in Tanzania, Zimbabwe, Kenya, Uganda and Ethiopia (Rust tracker.org, 2014).

### **2.3 Favorable conditions, symptoms and epidemiology of stem rust**

Stem rust development is favored by heavy dews, high humidity and warm temperatures (Todorovska *et al.*, 2009; Murray *et al.*, 2010). The minimum temperature for the germination of the urediniospore is 2 °C, optimum (15-24 °C) and maximum (30 °C), whereas minimum temperature for sporulation is 5 °C, optimum (30 °C) and maximum (40 °C) (Hogg *et al.*, 1969; Roelfs *et al.*, 1992). Germination of urediniospores begins 1-3 hours after contact with moisture over a range of temperatures and 6-8 hours of dew period or moisture is required for the process of infection to be completed (Sharma, 2012). Stem rust is identified by conspicuous brownish-red blister-like pustules that are oval or spindle-shaped, mostly appearing on stems, leaf sheaths, awns and glumes about 7-10 days after infection (Singh *et al.*, 2006; Singh *et al.*, 2008; Murray *et al.*, 2010). The size of the pustule is determined by the degree of host resistance, age of the tissue, virulence of the pathogen and environmental conditions (Murray *et al.*, 2010). Stem rust is a long distance adapted pathogen that is spread through wind and deposited by rain (Nagarajan and Singh, 1990; Singh *et al.*, 2006).

Neighbouring plants can be infected by rain splash unlike spores blown by wind which can travel hundreds or thousands of miles initiating germination (Roelfs *et al.*, 1992; Murray *et al.*, 2010). In addition to natural dispersal mechanisms, accidental human transmission (infected clothing or plant material) can spread the disease (Singh *et al.*, 2006). Urediniospores are released from young pustules and later in the growing season, the uredinia convert to telia by producing dark-colored teliospores (Leornard and Szabo, 2005; Singh *et al.*, 2008). It is the most devastating of the rusts causing 50 to 100% losses particularly in susceptible cultivars (Roelfs *et al.*, 1992; Leornard, 2001). Bread wheat, durum wheat (*Triticum turgidum*), barley (*Hordeum vulgare*) and triticale (*X. Triticosecale*) are the primary hosts of *Puccinia graminis* f.sp. *tritici* and *Berberis vulgaris* is the main alternate host. Severe stem rust infection leads to shrivelled grains through absorption of plant's nutrients and interference of plant's vascular tissues.

## 2.4 Host pathogen interactions

Understanding of host-pathogen interaction (in this case wheat - *puccinia graminis* f.sp. *tritici*) helps in deciding on the approach used in developing a resistant cultivar. In the 1940s, using flax (*Linum usitatissimum*) and its fungal rust pathogen *Melampsora lini*, Herold Henry Flor developed the “gene-for-gene” model by studying resistance of the plant and virulence of the pathogen. This model stated that the plant contains single dominant resistance genes (R genes) that recognize the complementary avirulence genes (*Avr* genes) of the pathogens (Thakur, 2007). A loss or alteration of either the plant resistance (*R*) gene or the pathogen avirulence (*Avr*) gene leads to disease (compatibility) (Priyamvada *et al.*, 2011). Once the pathogen enters into the host plant, it produces effector molecules lowering the plant’s defense mechanism and eventually colonizing the plant. With presence of the resistance (*R*) genes in the host plant, their product recognizes the presence of the effector molecules hence triggering a defense mechanism resulting into death of cells surrounding the infected area (hypersensitive response) protecting the rest of the plant from the infection (Thakur, 2007; Priyamvada *et al.*, 2011). Pathogens continue evolving by either changing (mutation) or losing (deletion) their effector molecules avoiding its recognition by host plant hence leading into more virulent strains. Wheat stem rust race *Ug99* is a good example whose mutation into more virulent strains has caused several wheat resistance genes to be overcome. For the plants to maintain their resistance, they must contain new R genes to recognize these new pathogen strains.

## 2.5 Effective stem rust resistance genes of wheat

Several effective stem rust resistance genes have been identified and successfully transferred to wheat from wheat related species (Singh *et al.*, 2006). However the rapid mutations of the pathogen has been a major challenge to wheat breeders as any newly found or incorporated resistance is broken down. The stem rust race *Ug99* and its variants have overcome most race-specific resistance genes present in the commercial varieties grown throughout the world. Stem rust resistance genes transferred from *Triticum aestivum* and effective against *Ug99* (*TTKSK*) are *Sr28* and *Sr29* genes (Singh *et al.*, 2006). The *Ug99* effective genes obtained from *Triticum turgidum* are *Sr2*, *Sr13* and *Sr14*. Of these, *Sr2* gene is the only non-race specific gene which was transferred into wheat from Yaroslav emmer. The gene does not confer adequate resistance under heavy disease pressure but is effective when combined with other genes as it only confers slow rusting resistance (Sunderwirth and Roelfs, 1980; Singh *et al.*, 2008). The *Sr2* gene is the most valuable adult plant gene for

breeding wheat with durable resistance. It is linked with *Pbc* gene that confers pseudo black chaff although a plant with high levels of pseudo black chaff expression is thought to be a low yielder (Brown, 1997).

There was successful transfer of stem rust resistance genes from wheat related species into cultivated bread wheat. Genes *Sr22* and *Sr35* were transferred from *Triticum monococcum* while *Sr37* was transferred into wheat from *Triticum timopheevi*. *Triticum speltoides* is the source of *Sr32* and *Sr39* genes which are now in cultivated wheat (Singh *et al.*, 2006). *Triticum tauschii* is the source of *Sr33* and *Sr45* genes while *Sr24*, *Sr25* and *Sr40* genes were transferred from *Thinopyrum elongatum* and *Triticum araraticum*, respectively (McIntosh and Luig, 1973; McIntosh, 1988; Dyck, 1992; Singh *et al.*, 2006). Effective genes such as *Sr22*, *Sr25*, *Sr32*, *Sr35*, *Sr39*, *Sr40* and *Sr44* confer resistance to the new *Ug99* variants but have not been fully utilized in our commercial wheat cultivars (Singh *et al.*, 2005; Jin *et al.*, 2007).

## 2.6 Development of durable resistance in wheat

One of the prerequisites for sustainable agriculture is use of disease resistant cultivars plants whose resistance serves as the most affordable way of controlling diseases such as stem rust, leaf rust and yellow rust caused by rapidly evolving pathogens. The development of varieties with durable resistance to stem rust is one of the aims of wheat breeding programs (Singh *et al.*, 2013). Durable resistance is usually associated with adult plant resistance due to the long lasting resistance conferred by the APR genes (Bariana *et al.*, 2001). The level of durable resistance can be increased by continuously selecting genotypes with lower levels of disease severity over seasons (Parlevliet and Van, 1988). The identified durable rust (stem, leaf, yellow and powdery mildew) resistance genes are *Lr34/Yr18/Pm38*, *Lr46/Yr29/Pm39*, *Sr2/Yr30* and *Yr36*. These genes which are located on wheat's D genome delay the infection process but do not provide complete resistance to the host plants. Genes *Lr34* and *Yr18* which carry the most durable forms of resistance confer slow rusting resistance to leaf and stripe rust, respectively and are completely linked to each other although they may not provide adequate resistance under high disease pressure when present alone (McIntoch, 1992; Singh and Huerta-Espino, 1997).

The leaf rust gene *Lr34* located on the short arm of chromosome 7D has been durable for more than 50 years. However, slow rusting *Lr46* gene is located in chromosome 1BL and is linked to *Yr29* gene that confers moderate levels of adult plant resistance to stripe rust

(William *et al.*, 2003). Resistance genes *Pm38* and *Pm39* have also been noted to confer durable resistance to wheat against powdery mildew (Lillemo *et al.*, 2008). Wheat stem rust APR gene *Sr2* has provided partial resistance to all stem rust races since its deployment in the 1920s and it still provides a certain degree of resistance to the *Ug99* strain and its variants. This gene is closely linked to the minor *Yr30* gene that confers resistance to yellow rust and its slow rusting ability is not adequate under heavy disease pressure but when combined with other minor genes, adequate resistance to the newly identified variants of *Ug99* race will be observed (Singh *et al.*, 2000). Accumulation of approximately five minor genes would lead to achievement of adequate levels of resistance to stem rust (Knott, 1988). Gene pyramiding in a variety leads to blocking of mutants virulent to one or two resistance genes (McIntosh, 1976; Park, 2007). Durable resistance can therefore be enhanced in adapted wheat varieties through several breeding approaches e.g single-backcross selected bulk method, repeated backcrosses among other methods (Singh *et al.*, 2006).

## **2.7 Qualitative and quantitative types of resistance**

Qualitative and quantitative resistances are the two genetic mechanisms for disease resistance in plants and are based on single and many genes, respectively (Line and Chen, 1995). Resistance based on single genes is also known as race specific, seedling or vertical resistance and the plants show complete resistance to some races while being susceptible to others. This type of resistance that is based on genes effective at both seedling and post seedling stage is easily overcome by mutating pathogens as illustrated by the long time protection provided by the now defeated stem rust resistance gene *Sr31* (Singh *et al.*, 2000; Chen, 2005; Priyamvada *et al.*, 2011). Screening done at seedling stage is very important as it helps one to postulate genes in test genotypes. About 30 major genes conferring resistance to *Ug99* races have been identified (Pumphrey, 2012).

Plants with non - race specific/ horizontal/ slow rusting resistance genes are resistant to all races that are known and this kind of resistance is not rapidly overcome by pathogens since it is based on many genes (Singh and Rajaram, 1992; Singh *et al.*, 2000; Dubcovsky *et al.*, 2010). Expression of quantitative resistance depends upon genotype and environmental conditions (Priyamvada *et al.*, 2011). Wheat cultivars with slow rusting genes are often susceptible at the seedling stage, but may be moderately to highly resistant to all pathotypes at the adult plant stage in the field (Singh *et al.*, 2000). Reduction in sizes of the uredinia, longer latent periods, low infection frequency, reduced duration and quantity of production of

spores are some of the slow rusting components (Caldwell, 1968; Wilcoxson, 1981; Priyamvada *et al.*, 2011). The recent epidemics due to the rapid spread of *Ug99* and its variants has drawn interest to partial resistance genes as sources of potentially more durable resistance (Schumann and Leonard, 2011).

High levels of adult plant resistance are provided by many minor genes (up to five) each contributing an additive effect to the host plant hence may not protect a plant from rust attack on their own (Singh *et al.*, 2008). There are at least five designated adult plant resistance genes that contribute to stem rust resistance and approximately ten quantitative trait loci (QTLs) have been identified to date (Pumphrey, 2012). The adult plant resistance gene *Sr2* located on chromosome 3BS, *Sr56* located on chromosome 5B of cultivar *Arina* confers resistance to stem rust (Bhavani *et al.*, 2011). Unpublished work by Singh *et al* is underway on APR genes *Sr57* having a pleiotropic effect with *Lr34* and *Yr18* whereas *Sr55* having pleiotropic effect with *Lr67* and *Yr46*. However, expression of adult plant resistance is influenced season to season by the environmental conditions (temperature and rainfall) and genetic aspect of the variety. The expression of APR genes is sensitive to temperature as its expression is more pronounced when temperatures are high than cold. Breeding for horizontal resistance is clearly supported by Van der Plank in his work on potato (*Solanum tuberosum*) varieties when they retained their resistance for 30 years from 1938 to 1968.

## **2.8 Stem rust management strategies**

Stem rust is known to be more difficult to control than leaf and yellow rusts. Destroying of volunteer wheat plants, use of fungicides, crop rotation and the development of resistant varieties are the current approaches in preventing the disease from causing losses to wheat crops (Todorovska *et al.*, 2009; Xue *et al.*, 2012). Volunteer wheat plants are destroyed by tillage or use of herbicides to reduce early season rust pressure, slow the development of new rust races and prevent the pathogens being carried over to the next growing season. Cultural practices for example early planting helps the crops escape the infection since the density of the inoculum is lower in the early planting season compared to planting late in the season (Xue *et al.*, 2012). Since many diseases are host specific and proliferate when the same crop is planted repeatedly, crop rotation disrupts the disease cycle. Avoiding planting of wheat plants next to a field that was severely diseased the previous year also helps in preventing the new crop being infected. Although timely application of fungicides such as AmistarXtra 280 SC (Azoxystrobin-200 g/l; cyproconazole - 80 g/l), Folicur 250 EC

(tebuconazole-250 g/l) and Orius 25 EW (tebuconazole 25%EW) among others prevent wheat losses to rust pathogens, it increases the production cost hence developing plants with very high levels of resistance under high disease pressure should be considered by pyramiding more effective resistance genes into susceptible wheat cultivars (Wanyera *et al.*, 2009; Dubcovsky *et al.*, 2010).

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## CHAPTER THREE

### IDENTIFICATION OF NOVEL SOURCES OF ADULT AND SEEDLING RESISTANCES AMONG KENYAN AND INTRODUCED WHEAT (*Triticum aestivum*) GERMPLASM

#### 3.1 Abstract

Stem rust caused by *Puccinia graminis* f.sp. *tritici* is an important foliar disease in wheat (*Triticum aestivum* L.) worldwide. Access to diverse sources of genetic resistance is important in building a broad base resistance to stem rust in Kenyan commercial wheat varieties. The objective of this study was to screen wheat genotype from diverse origins for both seedling (SPR) and adult plant resistance (APR) against predominant *Ug99* race and its variants in Kenya. Screening for seedling plant resistance was done under controlled greenhouse conditions while identification of genotypes with APR was done in the field at KALRO-Njoro, Kenya. This study was done during the 2012 and 2013 wheat growing seasons. Genotypes with low seedling infection types ('0' to '2') were considered as having SPR while those with low area under disease progress curve (AUDPC) and coefficient of infection (CI) values were considered as having APR/ slow rusting resistance to stem rust *Ug99* races. Under field conditions, there were variations in the disease severities and responses within and between seasons. AUDPC values ranged from 0 to 1,285, CI values from 0 to 100 and final disease severities (FDS) from 0 to 100S. In the greenhouse, infection types that ranged from '0' to 'X' were noted. Despite the high disease pressure in the field as expressed by the susceptible check (100S) and its high seedling infection type ('3<sup>+</sup>'), this study identified potential sources of adult plant (119 i.e 35%) and seedling (125 i.e 37% - *TTKSK* and 137 i.e 40% - *TTKST*) resistance against stem rust *Ug99* races. *Bounty*, *Kenya Swara*, *Kenya Nyati*, *Lenana*, *KSL18*, *PCB52*, *PCB62* and *PCB76* were among the most resistant genotypes bearing both resistance genes. These identified sources of resistances could be introgressed into Kenyan adapted wheat cultivars to achieve durable resistance against stem rust races.

### 3.2 Introduction

Stem rust (*Puccinia graminis* f. sp. *tritici*) of wheat (*Triticum aestivum*) continues to be a major constraint in wheat production globally. The yield losses of up to 100% have been registered in susceptible commercial varieties (Temesgen *et al.*, 1995; Njau *et al.*, 2010). Failure to monitor and prevent these losses will result in low production of wheat globally and therefore fail to meet its world demand (FAO, 2013; Olson *et al.*, 2013). The resistance in most of the newly released varieties is due to single major genes and therefore not durable as it is easily overcome by the continuously evolving pathogens (Singh *et al.*, 2008). In addition to this, the difficulty in control of *Puccinia graminis* f.sp *tritici* (*Pgt*) spread has been worsened by its long distance travel through wind and accidental human transmission (infected clothing or plant material) (Singh *et al.*, 2006). The current control strategies such as destruction of volunteer wheat plants, crop rotation and use of fungicides (only if done at early stages) help mitigate the effect of the disease. A long term and more effective approach involves gene pyramiding through which diverse resistances are introduced into adapted wheats (Todorovska *et al.*, 2009; Wanyera *et al.*, 2009; Dubcovsky *et al.*, 2010; Xue *et al.*, 2012).

Seedling plant resistance and slow rusting / adult plant resistances are the two kinds of resistances known. However, the former is easily overcome by new variants of pathogens as illustrated by the now defeated stem rust resistance gene *Sr31* (Singh *et al.*, 2000). However, plants with the latter type of resistance maintain their durability and are not easily overcome by mutating pathogens hence opted for by plant breeders and pathologists (Singh and Rajaram, 1992; Singh *et al.*, 2000; Dubcovsky *et al.*, 2010). So far, thirty seedling resistance genes and five genes for the adult plant resistances have been identified as effective to *Ug99* races (Pumphrey, 2012). Area under disease progress curve (AUDPC), coefficient of infection (CI) - which takes into consideration final disease severity and disease response are the measures which were used in identifying genotypes with slow rusting resistance. The virulence of *Ug99* variants in Kenyan commercial wheat varieties may be an indicator of narrow genetic base in them. Thus the objective of this study was to screen and identify other sources of resistance to wheat stem rust from diverse origins in the country. This was done at both seedling (greenhouse) and adult (field) plant stages against *Pgt Ug99* variants at KALRO-Njoro, Kenya in the 2012 and 2013 wheat growing seasons.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Experiment one: Field screening of wheat genotypes for adult plant resistance to stem rust

##### (a) Experimental site

This study was carried out at the Kenya Agricultural and Livestock Research Organization (KALRO) (0° 20'S, 29° N, 35° 56' 40" E) Centre, Njoro. This area experiences an average annual rainfall of 939.3 mm (average of 60 years) (Kenya Meteorological Station Identification Number 9031021) and average temperatures of 9 °C (Minimum) and 24 °C (Maximum). The soils are predominantly mollic phaeozems with a pH of 7.0.

##### (b) Genotypes

Three hundred and forty two (342) genotypes [(242 from 17 countries and 100 from CIMMYT i.e PCBWR- Parcela chica (small plots) bread wheat rainfed lines and KSL-selected Kenyan lines) (Appendices 2, 3)] were screened for adult plant resistance (APR) over two seasons (2012 and 2013) in the field. The cultivar *Cacuke* (CANADIAN/CUNNINGHAM/KENNEDY) was used as a susceptible check.

##### (c) Experimental procedure

The genotypes were planted in a field that previously had soyabean (*Glycine max*) crop. The field was prepared first by spraying it with a non selective herbicide; round up (glyphosate) at the rate of 360g ha<sup>-1</sup> and three weeks later, disc ploughing and harrowing to a fine tilth suitable for wheat planting was done. Each genotype was planted to two 1.5m long rows spaced 0.5m apart. The susceptible cultivar *Cacuke* was planted after every 20 genotypes to monitor rust build up. Several other cultivars susceptible to *TTKST* and *TTKSK* races were planted as spreaders perpendicular to the entries to supply adequate rust inoculum. The fertilizer di-ammonium phosphate (D A P) was applied at planting at the rate of 125 kg ha<sup>-1</sup> to supply an equivalent amount of 22.5 kg N ha<sup>-1</sup> and 25 kg P ha<sup>-1</sup>. Calcium ammonium nitrate (CAN) fertilizer as a top dress was applied at growth stage (GS) 20-29 (Zadoks *et al.*, 1974) at the rate of 100kg ha<sup>-1</sup> to provide 33 kg N ha<sup>-1</sup>. Pre-emergence herbicide, Buctril MC (*Bromoxynil octanoate*, 225 g ha<sup>-1</sup> and *MCPA Ethyl Hexyl Ester*, 225 g ha<sup>-1</sup>) was applied at GS 20-29 to further control annual broad leaved weeds. Also, Bulldock (*beta-cyfluthrin*), a systemic insecticide was sprayed at the rate of 31 g ha<sup>-1</sup> to control both sucking and chewing pests.



**(d) Stem rust variants *TTKST* and *TTKSK* pathogen build up.**

Epidemics were induced in the test plots through artificial inoculation with inoculum prepared from susceptible plants taken from the trap nurseries planted in Njoro stem rust screening field. Rusted stems were chopped into small pieces and soaked in a few drops of tween 20 and water. The inoculum was adjusted to provide a concentration of  $4 \times 10^6$  spores  $\text{ml}^{-1}$ . The spreaders were inoculated using a syringe at growth stage (GS) 30-49 (Zadoks *et al.*, 1974). To enhance stem rust infection and spread, the plants were irrigated severally.

**(e) Data collection**

Disease severities and responses were scored for each genotype between heading (GS 50-69) and plant maturity (GS 70-89) (Zadoks *et al.*, 1974). Data was collected when the spreader rows had attained 50% infection and repeated three times at an interval of 10 days. Severity was based on a modified Cobbs scale where 0%= immune (no uredinia or any other sign of infection) and 100%= completely susceptible (large uredinia without necrosis) (Peterson *et al.*, 1948). Disease responses were recorded as R= resistant (small uredinia surrounded by necrosis), MR= moderately resistant (medium-sized uredinia surrounded by necrosis), MS= moderately susceptible (medium-sized uredinia without necrosis), S= susceptible (large uredinia without necrosis), MSS= moderately susceptible to susceptible (medium to large-sized uredinia without necrosis) and MRMS= infection response that overlap the MR and MS categories (Roelfs *et al.*, 1992).

**(f) Data analysis**

Areas under disease progress curve (AUDPC) values were generated using the AUDPC CIMMYT software and the formula by Wilcoxson *et al.* (1975) as below:

$$AUDPC = \sum_{i=1}^{n-1} 0.5 (x_{i+1} + x_i) (t_{i+1} - t_i)$$

Where,  $X_i$  is the cumulative disease severity expressed as a proportion at the  $i^{\text{th}}$  observation,  $t_i$  is the time (days after planting) at the  $i^{\text{th}}$  observation and  $n$  is total number of observations.

The coefficient of infection (CI) was obtained by multiplying the final disease severity and the constant values for infection where; R= 0.2, MR= 0.4, M= 0.6, MS= 0.8, S= 1 (Stubbs *et al.*, 1986).

### **3.3.2 Experiment two: Greenhouse screening for seedling plant resistance to two stem rust races.**

#### **(a) Genotypes**

The entries under **section 3.3.1 (b)** were also tested in the greenhouse to identify genotypes with seedling plant resistance.

#### **(b) Preparation of pure *TTKST* spores**

Spores of the the variant *TTKST* of *Ug99* were purified and multiplied on a universal susceptible wheat cultivar *Kenya Mwamba* which bears *Sr24* gene. Planting was done in two batches; the second batch planted a week later for multiplication purposes of the pure spores collected from a single pustule. In the first batch, seeds of *Kenya Mwamba* were planted in five 6 × 6 cm diameter pots alongside with the differential sets [ (used to identify the *Pgt* races) Appendix 1] and placed in the growth chamber. When the seedlings were at growth stage (GS) 12, infected stems of the wheat plants were collected from trap nurseries in Njoro, chopped into small pieces and suspended in light mineral oil (Soltrol 170). The spore suspension obtained was then adjusted to a concentration of  $4 \times 10^6$  spores ml<sup>-1</sup> by adding more mineral oil. Seedlings of *Kenya Mwamba* were inoculated by atomizing with the spores solution using a hand sprayer in the inoculation chamber.

The seedlings were then air dried for 30 minutes in the inoculation chamber, then transferred into a dew chamber set at 16 -18 °C and about 100 RH for 48 hours for the spore germination and sporulation. Finally, these seedlings were transferred to a bench in the greenhouse maintained at 20 °C and monitored for disease development. After 14 days, a single pustule (referred as the pure spore) was collected into a capsule using atomizer machine. This pure spore was suspended in soltrol oil, adjusted to a concentration of  $4 \times 10^6$  spores ml<sup>-1</sup> and atomized onto the second batch of *Kenya Mwamba* seedlings for the multiplication of the spores. This set of *K. Mwamba* seedlings were exposed to similar conditions as the first set for the germination and sporulation of spores. The pure spores from the second batch were bulked in capsules and stored at -20 °C to ensure their viabilities.

#### **(c) Preparation of pure *TTKSK* spores**

The process of purifying and multiplication of *TTKSK* spores was done on cultivar *Kwale* which bears *Sr31* gene and the procedure as mentioned in **section 3.3.2 (b)** was followed.

#### **(d) Seedling screening using *TTKST* variant**

Ten seeds of each genotype were sown in square 6 × 6 cm diameter plastic pots in the growth chamber. At GS 12, purified spores were taken out from -20 °C, heat shocked in a water bath set at 45 °C for 15 minutes and the spores were suspended in soltrol oil to get a spore concentration of 4×10<sup>6</sup> spores ml<sup>-1</sup>. The prepared inoculum was sprayed on the seedlings using a hand sprayer. The plants were exposed to conditions above-mentioned in section 3.4.2. After 14 days, seedlings of the genotype were scored for infection types on a 0 to 4 scale (Zadoks *et al.*, 1974). This was based on uredinia size and presence or absence of necrotic regions. In this scale, '0'= no uredinia or any other sign of infections, '; - fleck'= presence of hypersensitive necrotic flecks but no uredinia, '1'= small uredinia surrounded by necrotic regions, '2'= small to medium size uredinia surrounded by necrosis, '3'= medium sized uredinia without necrosis and '4'= large uredinia without necrosis. Infection types '0', ';', '1' and '2' were categorized as resistant whereas '3' and '4' as susceptible. Infection types were confirmed by evaluating the second set of seedlings of each entry for the second time.

#### **(e) Seedling screening using *TTKSK* variant**

The screening procedure above-mentioned in **section 3.3.2 (d)** was applied in screening against purified *TTKSK* race.

### **3.4 Results**

Variations in the reaction of the genotypes to *Pgt* races in the field were observed between and within the seasons i.e ranging from 0 to 100 and responses ranging from resistant (R) to susceptible (S) in comparison to the susceptible variety *Caccuke* used as a control which exhibited a disease reaction of 100S (Appendices 2, 3). Infection types '1', '2' (categorized as resistant) and '3', '4' (categorized as susceptible) were observed in the greenhouse screening against *TTKSK* and *TTKST* races (Appendix 4). Evaluations against *TTKSK* race showed that 125 i.e 37% were resistant ('1', '2') and 217 i.e 63% were susceptible ('3', '4') while *TTKST* race was avirulent on 137 i.e 40% but virulence was observed on 205 i.e 60% (Fig. 3.1, Appendix 4). In both seasons, a hundred and nineteen (119) i.e 49% genotypes were identified as resistant and could be used as novel sources of adult plant/ slow rusting resistance (Appendices 2, 3). In the 2012 season, 5 lines (i.e 1%) namely; *Quamy*, *Bale*, *K6295-4A*, *RFN*, *Kenya Leopard* showed immune type of reaction (Fig. 3.2, Appendix 3). Additionally, 117 (i.e. 34%) were resistant, 159 (i.e 46%) showed

intermediate (moderately resistant and moderately susceptible) infection responses and 66 (i.e. 19%) were susceptible (Fig. 3.2, Appendix 3). In 2013 season, *Kenya Sungura* is the only genotype that had an immune type of infection. Genotypes with resistant (R) type of infection were 89 i.e 26% while those with intermediate and susceptible infection responses were 97 i.e 28% and 156 i.e 46%, respectively (Fig 3.2, Appendix 3).

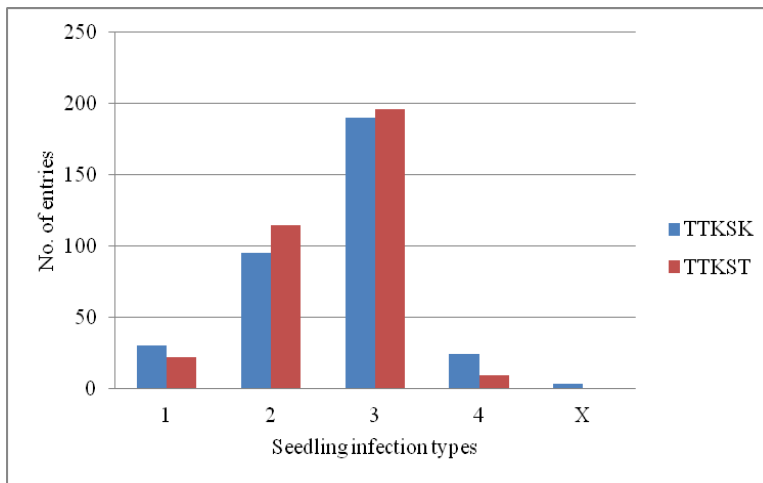


Fig. 3.1 Seedling stage evaluation of 342 wheat genotypes from diverse genotypes from origins against *TTKSK* and *TTKST Ug99* variants in Njoro, Kenya.

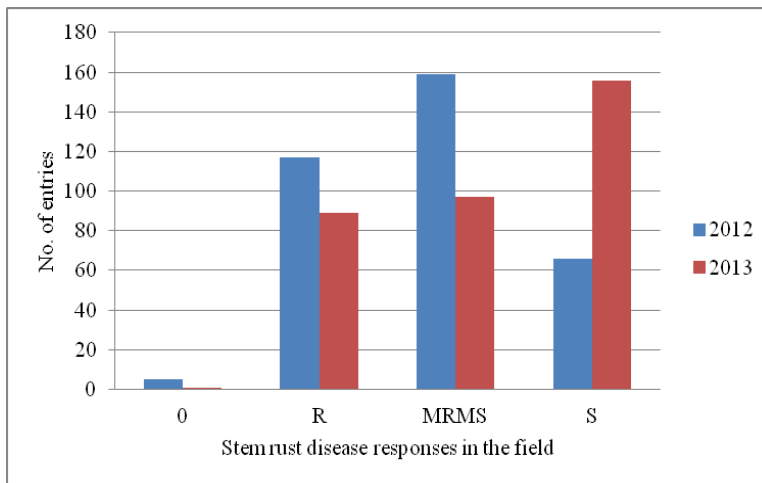


Fig. 3.2. Adult plant stem rust responses of 342 wheat from diverse origins evaluated at Njoro, Kenya during 2012 and 2013 seasons.

Genotypes judged to have high levels of adult plant resistance were identified based on area under disease progress curve (AUDPC) and coefficient of infection (CI) values. Genotypes with AUDPC values of 0-150, 151-300, 301-500 and > 500 were considered as having high, moderate, low and very low/ lack of slow rusting resistance genes, respectively (Appendices 2, 3). Similarly, these genotypes with CI values of 0-20, 21-40, 41-60 and > 60 were considered as having high, moderate, low and lack of slow rusting resistance genes, respectively. Genotypes from U.S.A (Minnesota) varied in their reactions to *Pgt* races based on AUDPC and CI values (slow rusting measures). Disease reactions of 5RMR to 60S, AUDPC of 75 to 760, and CI values of 0.4 to 60 were observed (Appendix 3). Genotypes such as *Crim*, *Chris*, *Polk*, *Timstein* among others exhibited high levels of adult resistance (5RMR-40MR) (Table 3.1, Appendix 3). Additionally, *Crim* variety had pseudo black chaff (pbc), a trait linked to *Sr2*. Virulence was observed in the above-mentioned genotypes from Minnesota against *TTKSK* and *TTKST* races (Table 3.2).

*Pgt* was virulent on genotypes of Ethiopian origin whereby susceptibility of up-to 80S as well as CI values ranging from 0 to 80 and maximum AUDPC values of 1,450 were manifested (Appendix 3). On the contrary, few accessions such as *Meraro*, *Digelu*, *Dure*, *Enkoy*, and *MG07762* among others showed high level of resistance i.e 5R-30MR (Table 3.1, Appendix 3). These genotypes showed infection types '2' to '2+' to both races other than *Meraro*, *Digelu*, *Dure*, *Inia 66* and *Enkoy* which were susceptible ('3+') to *TTKSK* but avirulence was observed when screened against *TTKST* race (Table 3.2, Appendix 4). Pbc trait was observed in *Mitike*, *K6290 bulk* and *Inia 66*, an indicator of the presence of *Sr2* gene (Appendix 3). *Bonza*, *Bonza 63* and *Frocor 2328* genotypes of Colombian origin varied in their reactions (5R to 50MSS) to stem rust races (Table 3.1). Avirulence to the two races was observed on variety *Bonza* ('2') while *Bonza 63* and *Frocor 2328* exhibited a high infection type ('3') (Table 3.2). *Gandum-i-Fasai*, an Iranian cultivar had an intermediate reaction of 20M in both seasons, which falls in resistant category considering the low CI and AUDPC values (Table 3.2). Both races were virulent to the genotype at seedling stage ('3') (Table 3.2). Virulence was observed on most genotypes from Mexico at adult (40MSS to 70S, AUDPC- 1,250, CI-70) and seedling stages ('3') while *Zaragoza 75* and *Bluebird* were resistant to *Pgt* races at adult (5MR-20M ) and seedling stages (;1 to '2+') (Appendix 3, Appendix4, Table 3.1, Table 3.2). Additionally, pbc was noted in *Bluebird* and *Bobwhite* varieties.

Table 3.1 Adult plant reactions of 18% screened wheat (*Triticum aestivum*) genotypes from diverse origins for resistance against stem rust (*Puccinia graminis* f.sp *Tritici*) Ug99 variants in the field at KALRO-Njoro, Kenya.

Genotype	Final Disease severity (FDS)		Coefficient of Infection (CI)		Area Under Disease Progress Curve(AUDPC)		Pseudo Black Chaff (PBC)	Postulated genes
	2012	2013	2012	2013	2012	2013		
<i>Crim</i>	15 RMR	15M	3	9	190	150	+	<i>Sr2</i>
<i>Chris</i>	5 RMR	5MR	1	2	75	80	-	<i>Sr5, Sr8a, Sr9g, Sr12, Sr7a, Sr8a, Sr9g, Sr12 lt</i>
<i>Polk</i>	5 MR	5MR	2	2	75	80	-	<i>Lt</i>
<i>Timstein</i>	10 MR	40MR	4	16	150	450	-	
<i>MG 07762</i>	30 MR	20MR	12	8	213	200	-	
<i>K6295-4A</i>	0	5R	0	1	0	25	-	
<i>Enkoy</i>	5 R	5RMR	1	1	75	100	-	
<i>Dure</i>	15 MR	15MR	6	6	115	125	-	
<i>Digelu</i>	5 RMR	15MR	0.4	6	20	125	-	
<i>Meraro</i>	5 R	5MR	1	2	20	100	-	
<i>Bounty</i>	5 R	5M	1	3	75	35	+	<i>Sr2 lt</i>
<i>Goblet</i>	5 MR	5RMR	2	1	75	75	+	<i>Sr2</i>
<i>Kenya Leopard</i>	0	5MR	0	2	0	25	-	
<i>Kenya Sungura</i>	5 MR	0	2	0	75	0	-	
<i>Kenya 6820</i>	5 RMR	5MR	0.4	2	75	100	+	<i>Sr2</i>
<i>Kenya Swara</i>	5 R	5MR	1	2	75	100	+	<i>Sr2</i>
<i>Kenya Nyati</i>	5R	5MR	1	2	75	100	+	<i>Sr2, Sr30, Sr5, Sr6, Sr7a, Sr7</i>
<i>Kenya Kanga</i>	5 R	5RMR	1	1	75	75	+	<i>Sr2</i>
<i>PCB 52</i>	5 RMR	20 MR	1	8	75	225	-	
<i>PCB 76</i>	50 MR	30 MR	20	18	368	225	-	
<i>Marquillo</i>	10 RMR	5MR	2	2	133	75	-	<i>Sr34, Sr3, Sr4, Sr9g, Sr12, Sr16</i>
<i>Zaragoza 75</i>	5 R	5MR	1	2	75	75	-	<i>Sr2, Sr36</i>
<i>Gabo</i>	5 MR	20MR	2	8	75	225	-	
<i>Bonza 63</i>	5 R	5RMR	1	1	75	75	-	<i>Sr8a, Sr9b, Sr6 lt</i>
<i>Giza 155</i>	5 MR	10MR	2	4	75	175	-	

Genotype	Final Disease Severity (FDS)		Coefficient of Infection (CI)		Area Under Disease Progress curve (AUDPC)		Pseudo Black Chaff (PBC)	Postulated genes
	2012	2013	2012	2013	2012	2013		
<i>Bluebird</i>	10 MR	20M	4	12	133	225	+	<i>Sr2, Sr 42, Sr5, Sr6, Sr8 , Sr11</i>
<i>Gandum-i-Fasai</i>	20 M	20M	12	12	80	225	-	
<i>Bonza</i>	5 RMR	10M	1	6	75	125	-	<i>Sr2, Sr42, Sr5, Sr6</i>
<i>Thatcher</i>	10M	10M	6	6	133	105	-	<i>Sr42, Sr26, Sr5,Sr9g, Sr12, Sr16</i>
<i>Bonny</i>	40 MS	60MSS	32	60	253	475	-	
<i>Kenya 155</i>	40 M	50S	24	50	455	750	-	
<i>Kenya- 8</i>	50 MSS	60S	50	60	423	475	-	
<i>Kenya-131</i>	70S	70S	70	70	800	800	-	
<i>Kenya Standard</i>	40 MSS	80S	40	80	418	1,300	-	
<i>Frocor 2328</i>	40 M	50MSS	24	50	513	625	-	
<i>Federation</i>	30 MSS	30MSS	30	30	380	425	-	
<i>Caccuke</i>	90S	100S	90	100	1,650	1,700	-	

R (resistant) = presence of hypersensitive necrotic flecks but no uredinia, MR (moderately resistant) = small pustules surrounded by necrotic areas, MS (moderately susceptible) = medium-sized pustules with no necrosis, M (MRMS) = moderately resistant, moderately susceptible, MSS (moderately susceptible to susceptible) = medium to large sized pustules without necrosis, S (susceptible) = large pustules with no necrosis, Pbc; + (positive) sign indicates presence of the trait and - (negative) sign indicates absence of the trait, *Ug99*= Stem rust strain first reported in Uganda in 1999. NB: Pbc trait is a morphological marker. Postulated genes were tracked using the pedigrees of the genotypes, <http://wheatpedigree.net> and wheat rust atlas of resistance genes by McIntosh *et al.*, 1995.

Table 3.2 Seedling plant infection types of 18% evaluated wheat (*Triticum aestivum*) genotypes against stem rust (*Puccinia graminis* f. sp. *tritici*) predominant *TTKST* and *TTKSK* races in the greenhouse at KALRO- Njoro.

Genotypes	<i>TTKSK</i>			<i>TTKST</i>		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
<i>Crim</i>	3	3, 2	3	2+	3-	3-
<i>Chris</i>	2+	2+	2+	2	2+	2+
<i>Polk</i>	3+	3	3+	3	3	3
<i>Timstein</i>	3	3+	3+	31	-	3
<i>MG 07762</i>	2	2	2	2+	2+	2+
<i>6295-4A</i>	3	3	3	3+	3	3+
<i>Enkoy</i>	3+	3	3+	3, 2+	2+	2+
<i>Digelu</i>	3+	3, 2	3+	2	2	2
<i>Meraro</i>	3+	3, 2+	3+	2+	2, 3+	2+
<i>Bounty</i>	2+	2+	2+	2	2	2
<i>Goblet</i>	3, 2+	3	3	3, 2+	3	3
<i>Kenya 155</i>	3, 2	3, 2	3	3, 2	3+	3+
<i>Kenya 8</i>	3, 2	3, 2+	3	3, 2	3	3
<i>Kenya Leopard</i>	3, 2+	3	3	3	3	3
<i>Kenya Sungura</i>	3+	3	3+	3, 2+	3+	3+
<i>Kenya-131</i>	3+	3	3+	3, 2	3-	3
<i>Kenya Standard</i>	3	3, 2+	3	3+	3, 2+	3+
<i>Kenya 6820</i>	2+	2+	2+	;1+	2+	2+
<i>KenyaSwara</i>	;1	;1	;1	0	;1	;1
<i>Kenya Nyati</i>	2+	;1+	2+	2+	2+	2+
<i>Kenya Kanga</i>	3	3-	3	3+	3+	3+
<i>Bonza 63</i>	3+	3+	3+	3	3	3
<i>Bonza</i>	2+	2, 2+	2+	2, 3	2+	2+
<i>Frocor 2328</i>	3-	3-	3-	3, 2+	3+	3+
<i>Gandum-i-Fasai</i>	3, 2+	3	3	3+	3+	3+
<i>Bluebird</i>	2+;1	2+	2+	;1+	2+	2+
<i>Zaragoza 75</i>	;1, 2+	2+	2+	;1+	2+	2+
<i>Marquillo</i>	2+	2+	2+	2+	2+	2+
<i>Bonza</i>	2+	2, 2+	2+	2, 3	2+	2+
<i>Thatcher</i>	;1	2+	2+	2+	2+	2+



Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
<i>Federation</i>	3+	3+	3+	3, 2+	3	3
<i>Gabo</i>	3-	3-	3-	3	3	3
<i>PCB52</i>	2+	2+	2+	2+	;1+	2+
<i>PCB62</i>	2	2	2	2	;1+	2
<i>PCB76</i>	3+	3+	3+	3	3, 2+	3
<i>KSL18</i>	2+	2+	2+	2	2	2
<i>Cacuuke</i>	3+	3	3+	3+	3+	3+

0= No signs of infection on the plant, ; = presence of hypersensitive necrotic flecks with no uredinia 1= small uredinia surrounded by necrosis, 2= small to medium sized uredinia surrounded by necrosis, 3= medium sized uredinia without necrosis, 4= large uredinia without necrosis, X= distribution of mixed type of reaction all over the leaf surface, positive (+) and negative sign (-) = larger and smaller uredinia, respectively than the normal size, esc = escape; due to symptoms observed in one or two plants and the rest of the plants in the same pot showing no symptoms (immune type of reaction). ITs 0, ;, 1, 2 = low seedling infection types, ITs 3 and 4 = high seedling infection types, *Ug99* = Stem rust strain first reported in Uganda in 1999.

The pathogen was avirulent to *Marquillo* and *Thatcher* varieties at seedling ('2') and adult stages with a disease reaction of 10RMR to 10M, AUDPC values < 133 and CI values < 24 (Table 3.1, Table 3.2). *Federation* variety from Australia exhibited high disease severity of 30MSS in both seasons and high seedling infection type ('3') unlike *Gabo* which was resistant showing disease responses ranging from 5MR to 20MR though virulence at seedling stage ('3') was observed (Table 3.1, Table 3.2). The AUDPC and CI values observed on the above-mentioned varieties were 425 and 30, respectively. *Pgt* was avirulent on *Giza 155*, an Egyptian genotype which exhibited low seedling infection type ('2+') and high adult plant resistance; 5MR-10MR reaction, CI and AUDPC values of 4 and 175, respectively (Table 3.1, Table 3.2). Genotype *Impala* of South African origin maintained a disease reaction of 30M, AUDPC and CI values of 550 and 18, respectively in both seasons though virulence was observed at seedling stage ('3') (Table 3.1, Table 3.2). Concurrently, *pbz* trait was observed on its stem and spike.

Most Kenyan wheat varieties such as *Kenya Nyati*, *Kenya Kanga*, *Kenya Swara*, *Kenya 6820*, *Lenana*, *Bounty*, *Goblet* among others maintained high levels of resistance with disease reactions that ranged from 5R to 15MR, maximum AUDPC and CI values of 250 and 20, respectively (Table 3.1, Appendix 3). Variations in the reactions of the varieties against the evaluated races at seedling stage were observed ranging from ';1+' to '3' (Table 3.2, Appendix 4). On the contrary, the pathogen was virulent on varieties such as *Bonny* (60MSS), *Kenya 155* (50S), *Kenya 8* (60S), *Kenya 131* (70S), *Kenya standard* (80S) among others with AUDPC and CI values of 1,300 and 80, respectively (Table 3.1, Appendix 3). Most of the Kenyan wheat varieties that lacked adult stage resistance also exhibited high seedling infection types '3' (Table 3.2, Appendix 4).

Evaluations of wheat lines that originated from CIMMYT- *Pacela chica* (small plots) bread wheat rainfed lines (PCBWR) and selected Kenyan lines- (KSL) discovered lines such as *KSL18*, *PCB52*, *PCB62*, *PCB76* among others as potential sources of resistance (disease reaction- 5R to 50MR, AUDPC-400, CI-20) to *Pgt* races (Table 3.1, Appendix 3). Observed seedling infection types varied from ';1+' to '4' among these lines (Table 3.2, Appendix 4). Despite the high disease severity observed in the second season of screening (2013), most of these lines maintained their resistance over the two seasons (Appendix 2). Lines such as *PCB2*, *PCB8*, *PCB9*, *PCB3* and *PCB69* among others share the same parentage though variations in disease reactions to *Pgt* were observed at both screening stages (Appendices 2,

4). Presence of *Pbc*, a trait linked to *Sr2* was observed on lines *PCB19*, *PCB 20*, *KSL 5*, *KSL 9*, and *KSL11* among others (Appendix 2).

### 3.5 Discussion

The avirulence of the currently predominant *Ug99* variants of stem rust at seedling and adult stages in some genotypes demonstrated the presence of major and minor/slow rusting resistance gene(s), respectively. Resistance depicted by most genotypes at seedling stage was not expressed at adult stage and this meant that most of the evaluated genotypes have single genes that could not withstand the high disease pressure in the field on their own. Evaluations of wheat and barley (*Hordeum vulgare*) genotypes against powdery mildew (*Blumeria graminis* f.sp *hordei*), barley leaf rust (*Puccinia hordei*), tan spot (*Tritici-repentis Drechs*) and stem rust at seedling and adult plant stages have shown variations in their reactions to the diseases (Wang *et al.*, 2005; Pathan and Park, 2007; Tadesse *et al.*, 2011; Singh *et al.*, 2013). Adult plant/ slow rusting resistance have been preferred by most plant breeders and pathologists because it is long lasting. Combinations of multiple APR and/or seedling resistance genes have led to high levels of leaf rust (*Puccinia triticina*), yellow rust (*Puccinia striiformis* f.sp. *tritici*) and stem rust resistance in wheat (Wang and Kou, 2010; Rutkoski *et al.*, 2011).

Genotypes with low AUDPC values i.e < 500, low CI values i.e < 20 and those that exhibited resistant category disease responses i.e R, MR were considered to be having high levels of adult plant/ slow rusting resistance in this study. Measures such as AUDPC and CI values used in identifying genotypes with slow rusting resistance to stem rust *Ug99* variants have been found to be positively correlated (Qamar *et al.*, 2007; Bux *et al.*, 2012; Safavi *et al.*, 2013; Denbel *et al.*, 2013). Weather related factors such as temperatures and rainfall could have favoured the severity of *Pgt* in the second year of screening (2013) compared to the first year (2012) where more genotypes were susceptible in the former than latter year (Table 3.3, Table 3.1, Appendices 2, 3).

Table 3.3 Mean temperatures and rainfall experienced during field evaluation period of 2012 and 2013 at KALRO-Njoro.

Season	January	February	March	April	May
2012					
Min. Temp. °C (Mean)	10.0	16.0	18.0	14.0	12.0
Max. Temp. °C (Mean)	23.0	18.0	22.0	24.0	22.0
Mean rainfall (mm)	0.0	13.6	11.0	295.0	183.7
2013	August	September	October	November	December
Min. Temp. °C (Mean)	8.0	9.0	10.0	10.0	10.0
Max. Temp. °C (Mean)	22.0	23.0	21.0	22.0	23.0
Mean rainfall (mm)	110.6	173.3	73.9	60.6	137.5

Min. = Minimum, Max. = Maximum, Temp. = Temperature, mm= Millimeters.

Ideal temperatures for the germination of the urediniospores are; minimum (2 °C), optimum (15 - 24 °C) and maximum (30 °C) while for spore sporulation, the favorable temperatures are; minimum (5 °C) , optimum (30 °C) and maximum (40 °C) (Hogg *et al.*, 1969; Roelfs *et al.*, 1992). Temperatures noted in this study were within the range that favours the establishment and development of the pathogen hence the high rainfall experienced in the second year may have contributed and favoured *Pgt* severity (Table 3.3, Table 3.1). Genotypes *Quamy*, *Bale*, *K6295-4A*, *RFN*, *Kenya Leopard* and *Kenya Sungura* showed complete resistance to *Pgt* and this possibly meant that either the basis of resistance in these varieties was complex adult plant resistance due to their susceptibility at seedling stage or the pathogen infective units were present when the host was not at its receptive stage and vice versa (Table 3.1) (Priyamvada *et al.*, 2011). Variations in the expression of resistance genes in both stages could also imply that there was presence of gene diversity amongst evaluated genotypes (Fininsa *et al.*, 2007; Safavi and Afshari, 2012; Newcomb *et al.* 2013). Although identification and location of the genes conferring resistance to *Pgt* in the evaluated genotypes have not been done, parents used in deriving these genotypes have been used in tracking and postulating the possible effective genes to the predominant *Ug99* variants in this study.

In regard to slow rusting parameters, all the genotypes from Minnesota could be considered as sources of resistance in improving Kenyan adapted cultivars though virulence was observed in most of them at seedling stage ('3'), an indication that major genes were absent (Table 3.2). Despite the presence of ineffective resistance genes to stem rust races in genotypes *Chris* (*Sr8a*, *Sr9g* and *Sr12*), *Newthatch* (*Sr5*, *Sr7b*, *Sr12* and *Sr1*) and *Ceres* (*Sr7b* and *Sr28*-effective) (McIntosh *et al.*, 1995; <http://wheatpedigree.net>) there could be presence of more unidentified genes conditioning resistance in these varieties due to the avirulence observed in the field (Table 3.1). The resistance exhibited by *Chris* variety derived from the cross FRONTANA/3\*THATCHER/3/ KENYA 58 may have been contributed by the parents involved in the crosses. Though Thatcher has *Sr5*, *Sr9g*, *Sr12* and *Sr16* ineffective genes, effective *Sr2*, *Sr22* and *Sr42* genes tracked back from *Hope*, *Marquis* and *Kanred* varieties, respectively could have resulted to the observed high levels of APR and seedling resistance in *Chris* variety (Fig 3.3, Table 3.1, Table 3.2, Knott, 2000; Kolmer *et al.*, 2011; <http://wheatpedigree.net>).

Additionally, inheritance studies on *Chris* variety at the adult plant stage revealed that resistance is controlled by two complementary recessive genes (Knott, 1997). Resistance

observed in *Ceres* variety at seedling and adult stages to both variants under the study is thought to have been contributed by the tracked *Sr22* and *Sr28* effective genes from *Marquis* and *Kota* varieties, respectively (Table 3.1, Table 3.2, <http://wheatpedigree.net>). Virulence of *Newthatch* to both races at seedling stage depicted that there was absence of effective major genes for resistance (Table 3.2). The resistance exhibited by *Polk* derived from THATCHER/SUPREZA/3/ KENYA 58 / NEWTHATCH // FRONTANA cross may have been contributed by the parents involved in the crosses. Despite the ineffective *Sr5*, *Sr9g*, *Sr12* and *Sr16* genes, the tracked *Sr2*, *Sr22* and *Sr42* effective genes could have resulted to the incompatibility of the host and pathogen in both stages in *Polk* variety (Table 3.1, Table 3.2, <http://wheatpedigree.net>).

Virulence of the pathogen to most Ethiopian genotypes was manifested in both stages (Table 3.1, Table 3.2). On the contrary, cultivars such as *Meraro*, *Digelu*, *Dure*, *Enkoy*, *MG07768*, *Mitike*, *K6290 bulk*, *K6295-4A* and *MG07762* had both major and minor genes though virulence was observed against *TTKST* variant in some varieties (Table 3.1, Table 3.2). Ethiopia being among the hotspot regions of stem rust disease, *K6295-4A*, *Meraro* and *Digelu* cultivars had also been considered as good sources of adult resistance due to the low final disease severity and AUDPC values they depicted though *Dure* cultivar, one of the high yielding wheat cultivars in Ethiopia had been reported as being susceptible despite being resistant in this study (Denbel *et al.*, 2013). Additionally, high levels of adult plant resistance observed in *Enkoy* cultivar which is known of carrying *Sr36* effective gene was maintained in earlier as well as in this study (Table 3.1) (Denbel *et al.*, 2013; <http://wheatpedigree.net>).

There was compatibility of *Pavon 76* and pathogen at adult stage in this study as well as in the previous study done in Ethiopia and this variety is being utilized in CIMMYT wheat crossing program (Singh *et al.*, 2011; Denbel *et al.*, 2013). Its resistance may be ascribed to *Sr2* gene present (due to the observed pbc trait) and other uncharacterized slow rusting genes. The variety lacks major resistance gene (s) due to the virulence observed against *TTKSK* and *TTKST* races (Table 3.2). *Bonza* and *Bonza 63* genotypes, all of Colombian origin were resistant in the field while *Frocor 2328* lacked the resistance genes (Table 3.1). Resistance shown by *Bonza* in both stages could be due to *Sr2*, *Sr22* and *Sr42* effective genes tracked back from *Hope*, *Marquis* and *Kanred* varieties, respectively (<http://wheatpedigree.net>). Furthermore, single recessive gene has been found to confer resistance to *Bonza* variety (Knott, 1997). Lack of effective major gene (s) in *Bonza 63* could have led to its

susceptibility at seedling stage though there could be unidentified APR resistance genes that conferred its resistance at adult stage (Table 3.1, Table 3.2).

Combination of the effective *Sr2*, *Sr42* and *Sr36* genes could have contributed to the resistance depicted by *Zaragoza75* and *Bluebird* varieties at seedling and adult stages (<http://wheatpedigree.net>). *Marquillo* derived from MARQUIS/ (TR.DR) IUMILLO cross has *Sr22*, *Sr42* and *Sr34* effective major genes which are suspected to have led to the avirulence of *Pgt* to the predominant *Ug99* variants in both stages (McIntosh *et al.*, 1995; <http://wheatpedigree.net>). *Sr34* gene was introgressed from wild relatives but has not been commercially utilized (Ghazvini *et al.*, 2012). Avirulence of *Pgt* at seedling and adult stages on *Marquis*, a derivative of HARD-RED-CALCUTTA cross could possibly be due to *Sr22* effective gene postulated in the variety. Thatcher variety derived from (MARQUIS/ (TR.DR) IUMILLO // MARQUIS / KANRED) cross has *Sr26*, *Sr22* and *Sr42* effective genes (<http://wheatpedigree.net>; Kolmer *et al.* 2011). Since major genes are expressed at both seedling and adult stages, the observed low seedling infection type and adult resistance could have been contributed by the combination of these resistance genes (Table 3.1, Table 3.2).

Stem rust *Pgt* was avirulent on *Giza 155*, an Egyptian genotype at both seedling and adult stages (Table 3.1, Table 3.2). Unidentified major and minor effective resistance genes may have conferred the observed resistance. Variety *Impala* from South Africa showed some resistance at adult stage though virulence was observed at seedling stage to both races. Other than the unidentified APR genes, *Sr2* minor gene was postulated to contribute to this resistance because of the observed morphological marker (pbc) on Hope variety which is one of its parents (McIntosh *et al.*, 1995; Singh *et al.*, 2006). Presence of *Sr2* gene alone may not provide adequate level of resistance to a variety under high disease pressure, thus the virulence observed in *Hope* variety (Singh *et al.*, 2011; Bhavani *et al.*, 2011).

Most of the Kenyan wheat varieties evaluated were also considered as novel sources of resistance to the emerging strains of stem rust disease. Despite the outbreak of destructive strains of stem rust since 2001, most of these varieties had both major and slow rusting resistance genes to *Ug99* and its variants (Table 3.1, Table 3.2). Some of them released in the same period might have had their resistance broken down by the new virulent races. The resistance showed by *Kenya Nyati* in both stages could have been contributed by its parents (AFRICA-MAYO/2\*ROMANY). Romany has an effective *Sr30* gene while *Africa mayo* has *Sr2* gene which could have been due to the observed pbc trait (Fig. 3.5; <http://wheatpedigree.net>). Avirulence to variety *Kenya Nyati* could have been conferred by

the combination of the two resistance genes. The resistance observed in both stages in *Kenya 6820* variety, a derivative of KENYA 4500-35 / KENYA SWARA cross is thought to have been conferred by *Sr2* gene because of the observed pbc trait on the variety and possibly unidentified effective resistance genes (Fig. 3.7).

Genes that led to seedling and adult plant resistance portrayed by varieties *Timstein* and *Yaqui-50* could have also contributed to the avirulence of *Pgt* in Trophy genotype. Ineffective *Sr8a* and *Sr9b* genes were tracked in *Kenya Kanga* from *Frontana* variety, one of its parents. The resistance exhibited by *Kenya Kanga* could be from unidentified effective resistance gene (s) (Fig. 3.6). Variety *Bounty* has several ineffective genes to *Pgt* but the tracked *Sr2*, *Sr22* and *Sr42* effective genes from *Hope*, *Marquis* and *Kanred*, respectively could have resulted to the resistance observed in this variety (Fig.3.8; <http://wheatpedigree.net>). In some varieties such as *Kenya Ploughman*, the susceptibility is attributed to either of its parents involved in deriving it. Despite the tracked *Sr22* and *Sr28* effective genes, these genes may have not been passed on to the variety hence the observed moderate levels of virulence by *Pgt* (Fig. 3.4)

The avirulence of *Pgt* on most lines of CIMMYT origin at both seedling and adult stages postulates the presence of unknown major and minor genes, respectively (Table 3.1, Table 3.2). Due to the observed pseudo black chaffs on spikes and stems on some lines, their high levels of resistance have been attributed to *Sr2* gene and possibly unidentified effective resistance genes. Wheat lines having either *Kingbird* and / or MUU varieties among their parents expressed high levels of adult plant resistance but virulence was observed at seedling stage (Table 3.1, Table 3.2). Variety *Kingbird* which is among the resistant wheat lines used in wheat improvement programs against stem rust disease is known of carrying *Sr2* gene and other minor genes contributing an additive effect. In *Kingbird*, adult plant resistance quantitative trait loci (QTL) are located on chromosome 1A, 3BS, 5BL, 7A and 7DS. In addition, 3 to 4 independent resistant genes were found. On the other hand, MUU variety carried QTLs on chromosome 5BL and 2 to 3 resistance genes revealed. Furthermore, lines that carried QTLs on chromosome 5BL had lower stem rust severities that ranged from 15 to 30% (Bhavani *et al.*, 2011).

Lines derived from KACHU/KIRITATI cross are believed to be carrying slow rusting resistance genes due to the reactions observed at adult stage though virulence observed at seedling stage revealed the lack of major genes in these lines (Table 3.1, Table 3.2). In addition, QTL mapping on KIRITATI identified QTLs on chromosomes 2D, 3BS, 5BL and



7DS (Bhavani *et al.*, 2011). Wheat lines with CHIBIA // PRLII / CM65531 / 3 / SKAUZ / BAV92\*2 / 4 / QUAIU cross maintained their reactions to *Pgt* at adult stage though susceptibility was exhibited at seedling stage, an indication of the presence of several minor genes in their background and lack of major genes. Lines that shared the same parentage i.e having BABAX / LR42 // BABAX\*2 / 3 / TUKURU\*2 / 4 / HEILO cross exhibited presence of adult resistance genes though variations were observed at seedling stage (Table 3.1, Table 3.2). This meant that some lines picked up the major genes while others did not during their development. The resistance observed in wheat lines derived from the cross BABAX/LR42//BABAX\*2/3/TUKURU could have been conferred by *Sr2* and *SrTmp* effective genes to stem rust *Ug99* variants (Table 3.1).

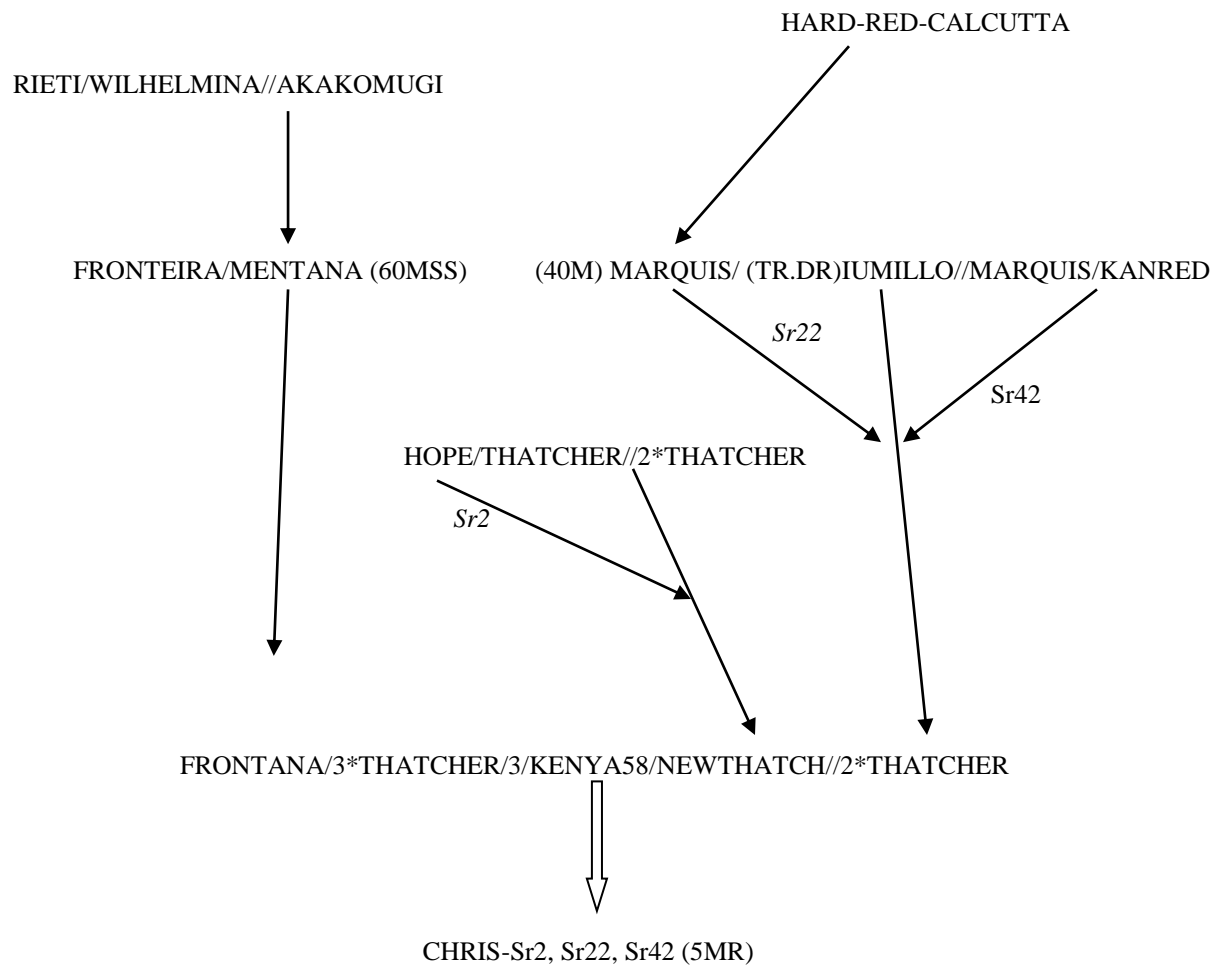


Figure 3.3 Flow of *Sr2*, *Sr22* and *Sr42* effective adult and seedling resistance genes conferring resistance to stem rust race *Ug99* in Chris variety.

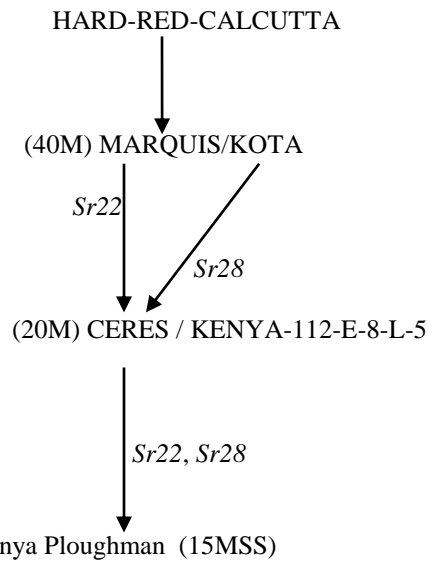


Fig. 3.4 Flow of *Sr22* and *Sr28* adult resistance genes which could have led to the moderate levels of susceptibility in Kenya Ploughman.

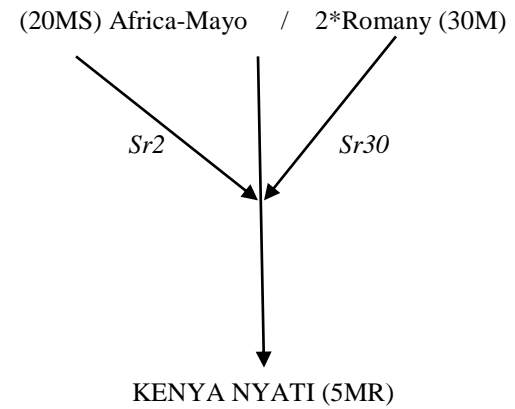


Fig. 3.5 Combination of *Sr2* and *Sr30* effective resistance genes could have contributed to the resistance observed.

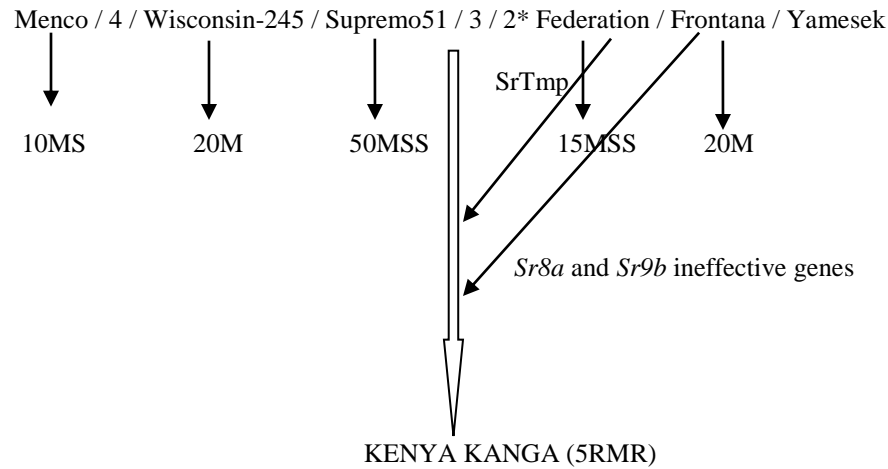


Fig. 3.6 Flow of *SrTmp* and unidentified effective resistance gene (s) that contributed to the observed resistance.

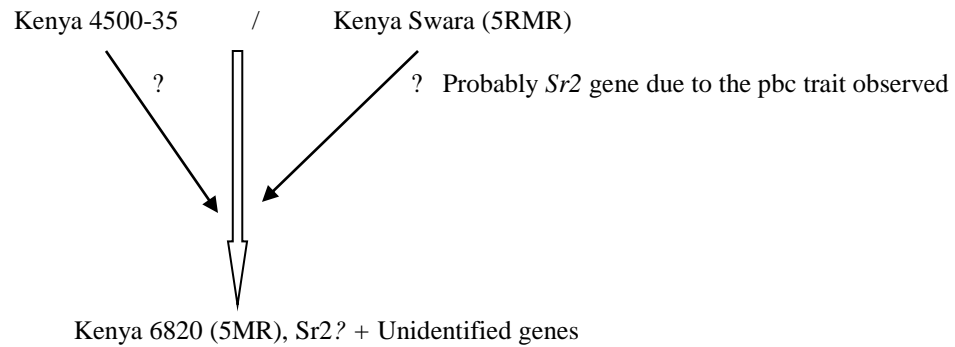


Fig. 3.7 Flow of *Sr2* and unidentified effective resistance gene (s) in Kenya 6820 variety might have conferred the high levels of resistance observed.

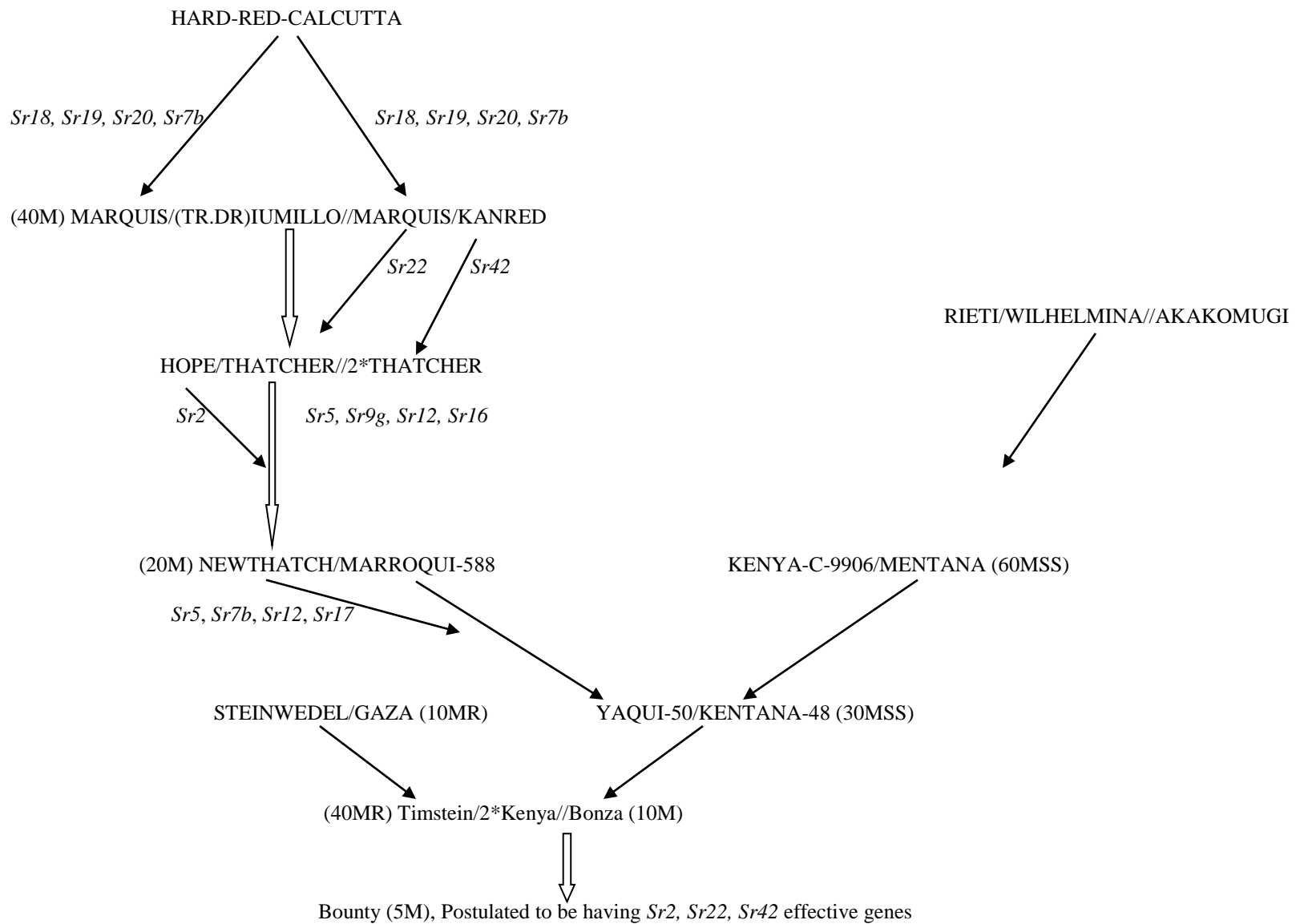


Fig. 3.8 Flow of *Sr2*, *Sr22*, *Sr28*, *Sr30*, *Sr42* and *SrTmp* effective resistance genes could have led to the observed resistance in Bounty variety.

### 3.6 Conclusion

Despite the high disease pressure in regard to susceptible check's level of resistance (100S), this study identified potential sources of adult plant resistance and seedling plant resistance such as *Kenya Swara*, *Kenya Nyati*, *Bounty*, *Lenana*, *K6290 Bulk*, *KSL18*, *PCB52*, *PCB62* and *PCB76* against stem rust *Ug99* variants. The resistances in such genotypes should be deployed into susceptible Kenyan wheat cultivars. The study further showed that there was genetic diversity among the tested genotypes due to the level of resistance that ranged from immune to susceptible (0-100S). These diverse resistances should also be utilized to improved the narrow based kind of resistance exhibited in most of the Kenyan wheat cultivars.

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## CHAPTER FOUR

### INHERITANCE OF STEM RUST (*Puccinia graminis* PERS. f. sp. *tritici* ERICKS AND E. HEN) RACE *TTKST* IN BREAD WHEAT (*Triticum aestivum* L.) LINES

#### 4.1 Abstract

Stem rust caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*) is currently one of the major biotic constraints in wheat (*Triticum aestivum*) production worldwide. The objective of this study was to determine the type of resistance to stem rust in *KSL18*, *PCB52*, *PCB62* and *PCB76* wheat lines which were previously selected from experiments one and two in chapter three. These lines depicted high levels of resistance in both seedling and adult plant stages. The resistant lines were crossed with two known susceptible cultivars *Kwale* and *Duma*. The resulting F<sub>1</sub> and F<sub>2</sub> populations alongside the parents were then tested in the greenhouse against stem rust race *TTKST*. The four selected wheat lines exhibited infection types ‘;’ to ‘2’ confirming resistance while *Kwale* and *Duma* had infection types ‘3+’ to *TTKST*. Results from evaluated infection types on the F<sub>2</sub> population derived from *Kwale* × *PCB52* ( $\chi^2 = 0.8881$ ) showed that resistance is due to a single dominant gene. In all other F<sub>2</sub> populations (*Kwale* × *KSL18*, ( $\chi^2 = 0.9900$ ); *Kwale* × *PCB76*, ( $\chi^2 = 0.6796$ ); *Kwale* × *PCB62*, ( $\chi^2 = 0.3608$ ); *Duma* × *PCB52*, ( $\chi^2 = 2.3179$ ); *Duma* × *KSL18*, ( $\chi^2 = 0.1668$ ); *Duma* × *PCB76*, ( $\chi^2 = 2.6840$ ) and *Duma* × *PCB62*, ( $\chi^2 = 1.3593$ ), resistance was found to be conferred by two genes that complemented each other (i.e duplicate recessive epistasis) hence the ratios 9R:7S. These four resistant lines are recommended for further evaluation for other qualities and proposed as potential varieties and/or used as sources of valuable stem rust resistance in wheat breeding programs.

## 4.2 Introduction

The sources of resistance genes to stem rust (*Puccinia graminis* f.sp *tritici*) and inheritance in wheat (*Triticum aestivum* L.) are important to all wheat breeders in their endeavour to develop disease resistant varieties. Increased and sustainable productivity of this crop both in Kenya and globally will be achieved largely if there is adoption of resistance to the rusts (yellow, leaf and stem) (Leonard, 2001; Njau *et al.*, 2010). The resurgence of stem rust has received high attention from plant breeders and pathologists of wheat breeding programs due to the yield losses incurred worldwide (Singh *et al.*, 2011). Spraying with fungicides against stem rust is expensive, hazardous and a short term solution to the damage caused by this foliar disease. A foreseeable and feasible best option is bioresistance (Singh *et al.*, 2006; Singh *et al.*, 2008). A number of novel sources of resistance to stem rust have been reported. However, little has been reported on the kind and mode of the resistances they carry. Such information is very important to all breeders wishing to design breeding strategies for its use. The mode of action of such genes is either dominant or recessive; single (monogenic) or multigenic (polygenic); autosomal or sex-linked (Singh and Huerta-Espino, 1995; Singh *et al.*, 1997, Beaver *et al.*, 1999, Walker and Bosland, 1999; Aladele and Ezeaku, 2003; Odeny *et al.*, 2009).

Inheritance studies on barley (*Hordeum vulgare*), oat (*Avena sativa*), beans (*Phaseolus vulgaris*), rye (*Secale cereal*) and pigeon pea (*Cajanus cajan*) have shown that major genes and/or modifying genes are vital in conferring resistance to various pathogens at the seedling and adult plant stages. For example, resistance to *Puccinia coronata avenae* in spring oats is mainly conferred by major genes (Staletic *et al.*, 2009). Wheat lines possessing *Lr28* gene showed dominant genes governing resistance to leaf rust races 77-1, 77-2 and 77-5 (Bansal *et al.*, 2008). However, the resistance to stem rust in wheat with *Tr129* background to race *MCCF* is conditioned by two dominant genes (Ghazvini *et al.*, 2012). In barley, resistance to isolate 76-32-1335 of *Puccinia graminis* f. sp. *secalis* is conferred by one recessive gene (Steffenson *et al.*, 1985). With such an array of resistances to stem rust, it is proposed that pyramiding these genes may offer a more lasting and broad based resistance. To achieve this, it is essential that the breeder is equipped with knowledge on the kind of gene action underlying such resistance. The objective of this study was to determine the nature and number of genes conferring this resistance in selected wheat lines.

### **4.3 Materials and methods**

#### **4.3.1 Experimental site**

This study was conducted at Kenya Agricultural and Livestock Research Organization (KALRO) (0° 20' 29" N, 35° 56' 40" E) Njoro centre-Kenya. The area experiences an average annual rainfall of 939.3 mm (average of 60 years) (Kenya Metereological Station Identification Number 9031021) and average temperatures of 9 °C (minimum) and 24 °C (maximum). The soils are predominantly mollic phaeozems with a pH of 7.0.

#### **4.3.2 Parental stock and development of F<sub>1</sub> and F<sub>2</sub> populations**

Four resistant wheat lines *KSL18*, *PCB52*, *PCB62* and *PCB76* were selected from screened wheat genotypes (at both seedling and adult plant stages) in **sections 3.3.1 and 3.3.2**. These lines together with known susceptible cultivars *Kwale* and *Duma* were used in this study. *KSL18*, *PCB52*, *PCB62* and *Kwale* are hard white spring wheats while *Duma* and *PCB76* are hard red spring wheats. Also, *KSL18*, *PCB62* and *PCB76* are early maturing while *PCB52*, *Kwale* and *Duma* are medium in maturing. The resistant and susceptible parents were crossed using straight cross method to develop the hybrids. The hybrids were selfed to produce F<sub>2</sub> populations which were used for the study.

#### **4.3.3 Preparation of pure race *TTKST* of stem rust**

Stem rust race *TTKST* was purified, stored and prepared for inoculation as described in **section 3.3.2 (b)**.

#### **4.3.4 Screening of parents, F<sub>1</sub> and F<sub>2</sub> populations in the glasshouse**

Five seeds of each purified inbred parents, F<sub>1</sub>s and a hundred and fifty seeds of each F<sub>2</sub> population of eight crosses were sown in square 6 × 6 cm diameter plastic pots in the growth chamber. At GS 12, purified spores were taken out from -20 °C, heat shocked in a water bath set at 45 °C for 15 minutes and the spores were suspended in soltrol oil to get a spore concentration of 4 × 10<sup>6</sup> spores ml<sup>-1</sup>. The prepared inoculum was sprayed on the seedlings using a hand sprayer. The plants were exposed to conditions mentioned in **section 3.3.2 (b)**.

#### 4.3.5 Data collection

After 14 days, seedlings of the parents, F<sub>1</sub>s and F<sub>2</sub> populations were scored for infection types using a 0 to 4 scale (Zadoks *et al.*, 1974). Refer to **section 3.3.2 (d)** on evaluation of the seedlings.

#### 4.4 Data analysis of F<sub>2</sub> populations

The infection types observed on parents, F<sub>1</sub> and F<sub>2</sub> genotypes were categorized into resistant ('0', '1', '2', '2+') and susceptible ('3', '3+', '4'). The data of the F<sub>2</sub> populations were analyzed using the following procedure of SAS version 9.4 (SAS, 2012);

```
Title 'Name of cross;
Data Chisquare;
Input type $ count;
Cards;
R
S
;
Proc freq;
Tables type/testp= (0.75, 0.25);
Tables type/testp= (0.5625, 0.4375);
Tables type/testp= (0.562, 0.4375);
Tables type/testp= (0.625, 0.375);
Tables type/testp= (0.8125, 0.1875);
Tables type/testp= (0.9375, 0.0625);
Tables type/testp= (0.5625, 0.25, 0.1875);
Tables type/testp= (0.5625, 0.375, 0.0625);
Tables type/testp= (0.625, 0.1875, 0.1875);
Tables type/testp= (0.75, 0.1875, 0.0625);
Weight count;
Run;
```

In order to determine a fit into the 3:1 ratio or other ratios using the following formular:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where  $\chi^2$  = Chi Square value,  $\sum$  = Summation, O = Observed numbers in each category and E = Expected numbers in the corresponding category according to hypothesis

Phenotypic correlation was performed between seedling infection types observed on F<sub>2</sub> populations that conformed to 9:7 and 3:1 resistant: susceptible ratio using SAS version 9.4 (SAS, 2012) basing on the following formula:

$$r = \frac{n(\sum XY) - (\sum X)(\sum Y)}{\sqrt{[n(\sum X^2) - (\sum X)^2][n(\sum Y^2) - (\sum Y)^2]}}$$

Where  $r$  = Pearson correlation coefficient,  $x$  = values in set of infection type data from one population,  $y$  = values in the set of infection type data from the next population and  $n$  = Total number of values (Ott and Longnecker, 2001).

## 4.5 Results

### 4.5.1 Genetics of resistance to stem rust race *TTKST* for seedling resistance

To establish the genetics of resistance in the four resistant lines, it was found that the F<sub>1</sub>s from the eight crosses were all resistant (Table 4.1). Hybrids from *Kwale* × *PCB52*, *Kwale* × *KSL18*, *Kwale* × *PCB76* and *Kwale* × *PCB62* crosses with resistant genes placed in *Kwale* background showed infection types ‘;1<sup>+</sup>’, ‘2’ from *PCB52*, ‘2’, ‘2<sup>+</sup>’ from *KSL18*, ‘; 1<sup>+</sup>’ from *PCB76* and ‘; 1<sup>+</sup>’, ‘2’ from *PCB62* (Table 4.1). F<sub>1</sub>s derived from a cross when *Duma* was used as recipient parent exhibited ‘2’ to ‘2<sup>+</sup>’ infection types compared to ‘; 1<sup>+</sup>’, ‘2’ infection types observed on the F<sub>1</sub>s derived from crosses involving susceptible cultivar *Kwale* (Table 4.1). The F<sub>2</sub> population derived from *Kwale* × *PCB52* fitted segregation ratio of 3:1 (R: S) ( $\chi^2 = 0.6881$ ) (Table 4.1). In this cross, 72% of the progenies exhibited ‘0’ to ‘2’ infection types. Among the resistant genotypes, 39% of the progenies showed resistant reactions of ‘0’ and ‘;’ infection types whereas 28% showed susceptible reaction of ‘3’ to ‘4’ infection types (Figure 4.1). There was no association between the infection types observed in this cross with those depicted by the other crosses (Table 4.2).

The F<sub>2</sub> genotypes derived from *Kwale* × *KSL18*, *Kwale* × *PCB76* and *Kwale* × *PCB62* crosses showed infection types that conformed to 9:7 ( $\chi^2 = 0.9900, 0.6796, 0.3608$ , respectively) ratio of resistant to susceptible (Table 4.1). From these three crosses, 61, 56 and 59% of the genotypes fall in resistant class of ‘0’ to ‘2’ infection types, respectively (Figure 4.1). An association was observed between infection types of crosses *Kwale* × *KSL18* and *Kwale* × *PCB76* ( $r = 0.979^{**}$ ) (Table 4.2). Additionally, infection types shown by *Kwale* × *PCB62* and *Kwale* × *KSL18* crosses were also associated ( $r = 0.842^*$ ) (Table 4.2). Considering ‘0’ and ‘;’ infection types, 30, 27 and 18% of the individuals, respectively, were considered highly resistant. All the F<sub>2</sub> progenies that involved *Duma* as the female parent (*Duma* × *PCB52*, *Duma* × *KSL18*, *Duma* × *PCB76* and *Duma* × *PCB62*) conformed to 9:7 ratio of resistant to susceptible, ( $\chi^2 = 2.3179, 0.1668, 2.6840$  and  $1.3593$ ), respectively (Table



4.1, Figure 4.1). The crosses showed proportions of 63, 58, 64 and 55%, respectively of resistant genotypes with the infection types ranging from '0' to '2' (Figure 4. 1). The progenies of the cross *Duma* × *PCB52* had 16% of the genotypes exhibiting '0' and ';' infection types while 37% of the genotypes were susceptible (Figure 4. 1).

The observed infection types from *Duma* × *PCB52* and *Duma* × *KSL18* crosses were associated ( $r = 0.925^{**}$ ) (Table 4.2). Progenies of the cross *Duma* × *KSL18* had the least proportion (9%) of the genotypes exhibiting '0' and ';' infection types while 42% were susceptible (Figure 4.1). Positive association between infection types observed on *Duma* × *KSL18* and *Duma* × *PCB76* crosses was also observed ( $r = 0.876^*$ ) (Table 4.2). F<sub>2</sub> progenies developed from *Duma* × *PCB76* progenies had 16% showing '0' and ';' infection types and 30% of the genotypes were susceptible exhibiting '3' to '4' infection types (Figure 4.1). The infection types observed on *Duma* × *PCB76* and *Duma* × *PCB62* crosses were also positively associated ( $r = 0.920^{**}$ ) (Table 4.2). The progenies of the cross *Duma* × *PCB62* had 11% of the genotypes exhibiting '0' and ';' infection types and the highest proportion of 36% showed a '3' infection type (Figure 4. 1).

Table 4.1 Infection types (IT) of parents, F<sub>1</sub> plants and segregation in F<sub>2</sub> populations to pathotype *Pgt-TTKST* of *Puccinia graminis* f.sp.*tritici* at seedling stage.

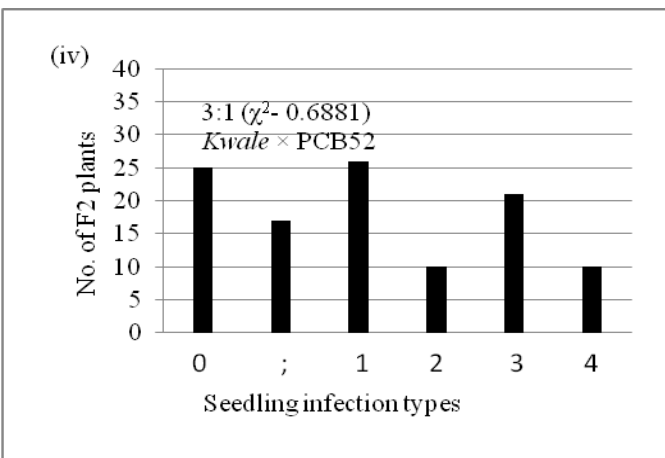
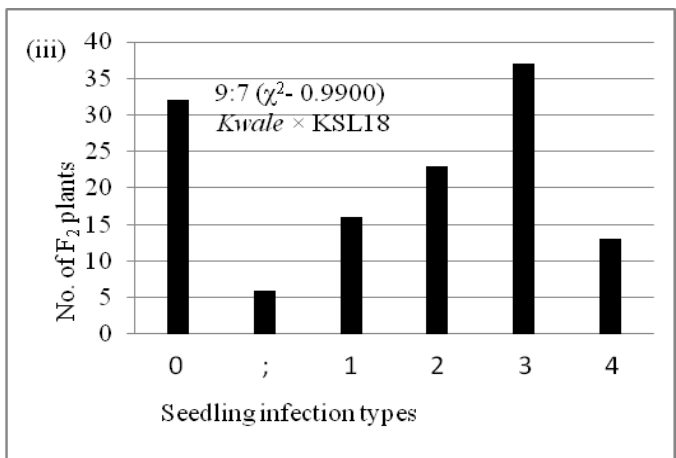
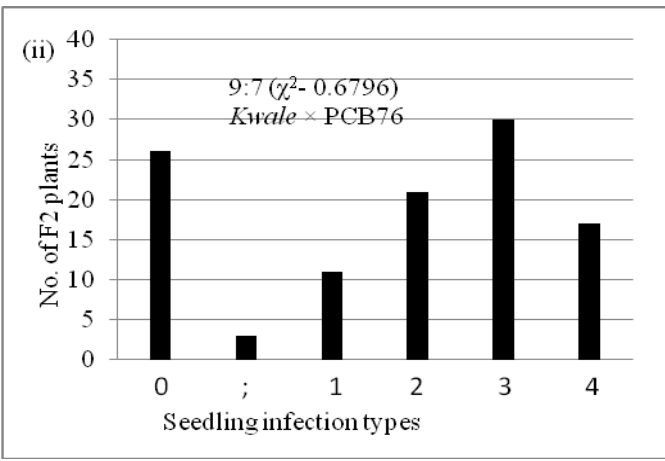
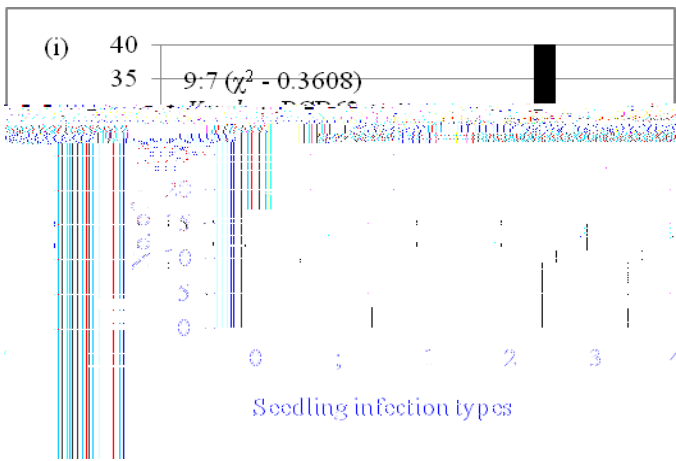
Parents	Observed Infection types	Resistant	Susceptible	Observed ratio (R:S)	Chi-square ( $\chi^2$ )	p- value
<i>Kwale</i>	3 <sup>+</sup>					
<i>Duma</i>	3 <sup>+</sup>					
PCB52	2 <sup>+</sup>					
KSL18	2 <sup>+</sup>					
PCB76	;1					
PCB62	2 <sup>+</sup>					
Crosses with <i>Kwale</i> as susceptible parent						
F <sub>1</sub> ( <i>Kwale</i> × PCB52)	;1 <sup>+</sup> , 2					
F <sub>2</sub> ( <i>Kwale</i> × PCB52)		78	31	3:1	0.6881	0.4068
F <sub>1</sub> ( <i>Kwale</i> × KSL18)	2, 2 <sup>+</sup>					
F <sub>2</sub> ( <i>Kwale</i> × KSL18)		77	50	9:7	0.9900	0.3197
F <sub>1</sub> ( <i>Kwale</i> × PCB76)	;1 <sup>+</sup>					
F <sub>2</sub> ( <i>Kwale</i> × PCB76)		61	47	9:7	0.6796	0.4097
F <sub>1</sub> ( <i>Kwale</i> × PCB62)	;1 <sup>+</sup> , 2					
F <sub>2</sub> ( <i>Kwale</i> × PCB62)		62	48	9:7	0.3608	0.5481
Crosses with <i>Duma</i> as susceptible parent						
F <sub>1</sub> ( <i>Duma</i> × PCB52)	2 <sup>+</sup>					
F <sub>2</sub> ( <i>Duma</i> × PCB52)		68	39	9:7	2.3179	0.1279
F <sub>1</sub> ( <i>Duma</i> × KSL18)	1 <sup>+</sup> , 2, 2 <sup>+</sup>					
F <sub>2</sub> ( <i>Duma</i> × KSL18)		64	46	9:7	0.1668	0.6830
F <sub>1</sub> ( <i>Duma</i> × PCB76)	2, 2 <sup>+</sup>					
F <sub>2</sub> ( <i>Duma</i> × PCB76)		62	49	9:7	2.6840	0.1014
F <sub>1</sub> ( <i>Duma</i> × PCB62)	2					
F <sub>2</sub> ( <i>Duma</i> × PCB62)		61	40	9:7	1.3593	0.2437

Infection type (IT) was based on the scale described by Stakman *et al.* (1962) with ITs ; 1, 2 considered resistant and 3 considered susceptible. Positive (+) = larger uredinia than the normal size and negative (-) = smaller uredinia than the normal size.

Table 4.2 Correlation among observed seedling infection types on F<sub>2</sub> populations evaluated for resistance to race *TTKST*.

	<i>Kwale</i> × PCB62	<i>Duma</i> × PCB62	<i>Duma</i> × PCB76	<i>Kwale</i> × PCB76	<i>Kwale</i> × KSL18	<i>Duma</i> × KSL18	<i>Duma</i> × PCB52	<i>Kwale</i> × PCB52
<i>Kwale</i> × PCB62		0.888 *	0.811 *	0.753	0.842*	0.847*	0.786	0.215
<i>Duma</i> × PCB62			0.920**	0.481	0.564	0.912*	0.716	-0.190
<i>Duma</i> × PCB76				0.239	0.369	0.876*	0.701	-0.023
<i>Kwale</i> × PCB76					0.979 ***	0.453	0.531	0.246
<i>Kwale</i> × KSL18						0.527	0.586	0.354
<i>Duma</i> × KSL18							0.925 **	-0.065
<i>Duma</i> × PCB52								0.169
<i>Kwale</i> × PCB52								

\*, \*\*, \*\*\* = significant at 0.05, 0.01 and 0.001, respectively.



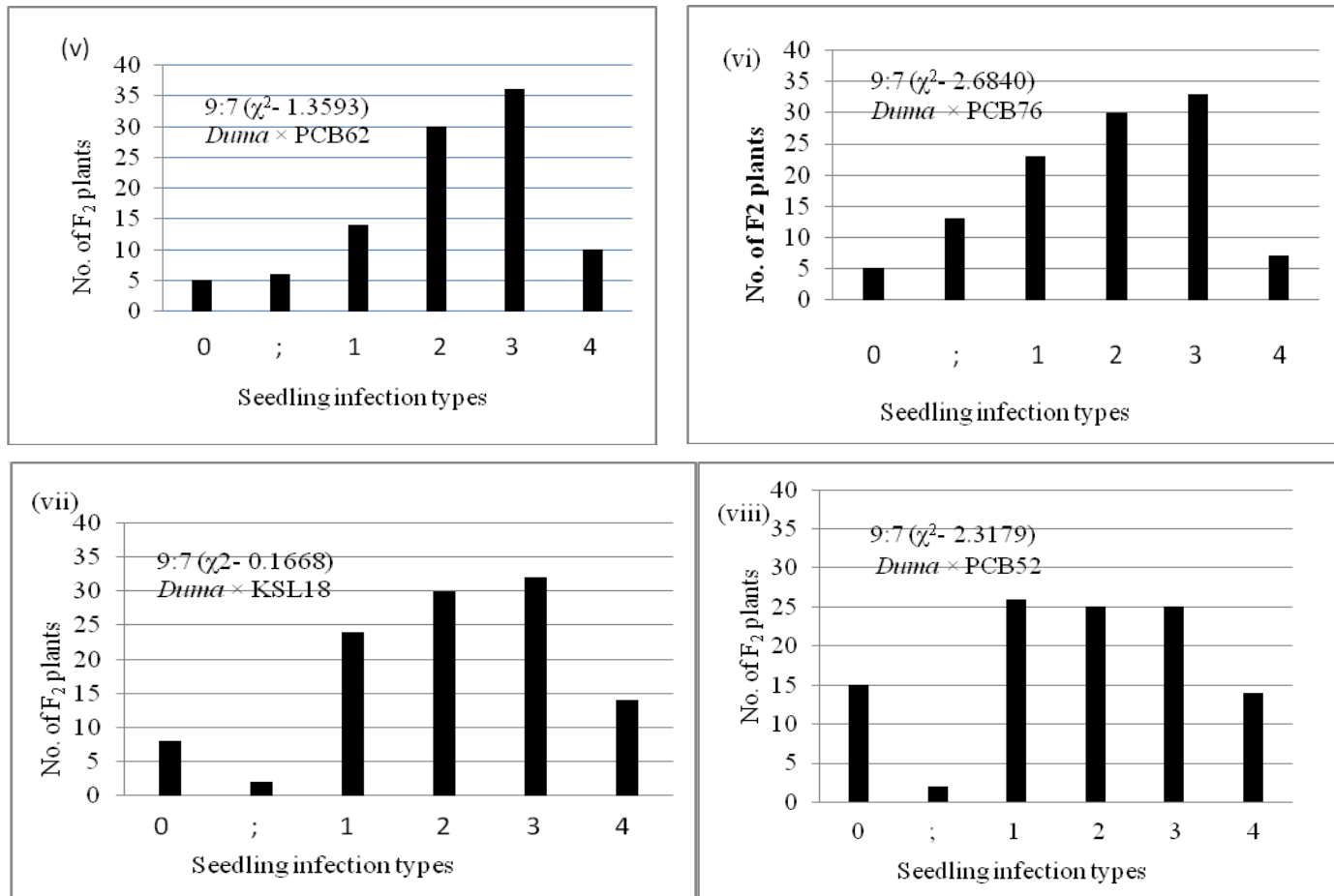


Figure 4.1 Proportion of seedling infection types of F<sub>2</sub> wheat (*Triticum aestivum*) populations developed from eight crosses and evaluated in the greenhouse for *Puccinia graminis* f.sp. *tritici* race TTKST (category: resistant= 0, ;, 1, 2; susceptible= 3 and 4).

## 4.6 Discussion

All the male parental genotypes and hybrids were confirmed resistant to *Pgt* race *TTKST* by the '1' to '2' infection types (Table 4.1). The responses of F<sub>1</sub>S and segregation of F<sub>2</sub> genotypes is an indication that the resistance at F<sub>1</sub> is conferred by major gene(s). This suggested that these lines possess resistance genes that could be useful in the improvement of cultivated wheat varieties. The segregation ratios for resistance to *Pgt* suggested that major genes that are modified due to epistatic effect are involved in conferring resistance to race *TTKST*. Nevertheless, apart from *Kwale* × *PCB52* cross whose segregation ratio conformed to 3:1(R:S), the 9:7 ratio observed on the other crosses demonstrated that modifying genes that influenced the resistance genes to *Pgt TTKST* may be due to duplicate recessive epistasis (Staletic *et al.*, 2009). The observed 9R:7S ratio in this study was also depicted by barley F<sub>2</sub> progenies derived from Steptoe × Q21861 cross when they were screened against stem rust *Pgt MCC* and *QCC* races (Jin *et al.*, 1994). However, double recessive genes (9S:7R) conferred resistance to head bug in populations developed from different sorghum genotypes (Aladele and Ezeaku, 2003).

Other than the resistance to *Pgt* in wheat lines noted in this study, single dominant gene also conferred resistance to leaf rust and powdery mildew (*Blumeria graminis* f.sp *hordei*) in barley, chocolate spot (*Botrytis fabae*) in faba bean (*Vicia faba* L.), leaf rust in wheat cultivars and Asian soybean rust (*Phakopsora pachyrhizi*) in soybean (*Glycine max*) (Naghavi *et al.*, 2002; Abbasi *et al.*, 2004; Charan *et al.*, 2006; Noorka and El-bramawy, 2011; Ghazvini *et al.*, 2012; Iwo *et al.*, 2012; Park *et al.*, 2012). In addition, two dominant independent (15R:1S) and three dominant genes (63R:1S) conferred resistance to leaf rust races 0R9 (106), 29R23 (104B), 109R31-1 (77-2), HD 2285, Vaishali and HD2189 in wheat (Bahadur *et al.*, 2002; Charan *et al.*, 2006). Crosses that involved *Kwale* cultivar (*Kwale* × *PCB62*, *Kwale* × *PCB76* and *Kwale* × *KSL18*) had more or less the same frequencies of '0' infection type in the resistant categories, an indication that a major/dominant gene was expressed in these crosses (Figure 4.1). When infection types of *Kwale* × *PCB76* and *Kwale* × *KSL18* populations were correlated, positive associations were noted between the infection types in all categories (Figure 4. 1, Table 4. 2). This showed that the effect of resistant and modifying genes were the same in *Kwale* background because of expression of 9:7 ratios in F<sub>2</sub> population.

The infection types in *Kwale* × *PCB62* and *Kwale* × *KSL18* crosses were also positively correlated and this association indicated that mode of gene action was the same because the

proportion of infection types in the resistant (';', '1', '2') and susceptible ('3', '4') categories were more or less the same (Table 4.2). Crosses that involved cultivar *Duma* (*Duma* × *KSL18*, *Duma* × *PCB76* and *Duma* × *PCB62*) had less number of resistant categories ('0', ';', '1', '2') compared to susceptible categories ('3', '4') (Figure 4.1) and this suggests that probably some recessive genes were involved in the modification of the resistance genes. However, single recessive genes (3S:1R) conferred resistance to *Pgt* races (ND8702 and ND89-3) in barley, resistance to fusarium wilt in pigeon pea and head bug resistance in sorghum (Jin and Steffenson, 1994; Aladele and Ezeaku, 2003; Karimi *et al.*, 2010).

Among the crosses, only *Kwale* × *PCB52* exhibited 3:1 ratio without modifying effects. In addition, there were more infection types in the resistant category ('0', ';', '1', '2') compared to those in the susceptible category ('3', '4') in *Kwale* × *PCB52* cross (Figure 4.1). This was an indication that *PCB52* donated a major gene for resistance to *Pgt* at seedling stage. A single dominant gene also conferred resistance to *Pgt* f.sp. *hordei* in barley and wheat populations derived from four resistant and one susceptible bread wheat cultivars against three different leaf rust races; 0R9 (106), 29R23 (104B) and 109R31-1 (77-2) (Jin and Steffenson, 1994; Charan *et al.*, 2006).

#### **4.6 Conclusion**

The inheritance of resistance in the wheat lines to *Puccinia graminis* f.sp. *tritici* was conditioned by a single major gene due to the observed 3R:1S ratio in *Kwale* × *PCB52* cross and epistatic effect of two interacting major genes (9R:7S) in crosses derived from *PCB62*, *PCB76* and *KSL18* lines. *Duma* cultivar could be having recessive modifying genes for *Pgt* resistance.

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## CHAPTER FIVE

### General discussion, conclusions and recommendations

The first experiment focused on identifying best performing genotypes in both seedling and adult plant stages. The avirulence of the current predominant *Ug99* variants of stem rust at seedling and adult stages in noted genotypes demonstrated the presence of major and minor/slow rusting resistance gene(s), respectively. Preferences of plant breeders, pathologists, agronomists and farmers are on a variety having both genes combined. Measures such as low area under disease progress curve (AUDPC) and coefficient of infection (CI) values have been used in identifying such type of genotypes suggested to have slow rusting resistance to stem rust *Ug99* (Bux *et al.*, 2012; Denbel *et al.*, 2013). Weather related factors such as temperatures and rainfall play a major role in identifying a resistant genotype. In this study, the temperatures noted were within the range that favours the establishment and development of stem rust pathogen hence the high rainfall experienced in the second year of evaluation is believed to have contributed and favoured *Pgt* severity which minimized the chances of disease escapes in screened genotypes. Therefore, the identified genotypes such as *Bounty*, *Lenana*, *K6290 Bulk*, *Kenya Swara*, *Kenya Nyati*, *KSL18*, *PCB52*, *PCB62* and *PCB76* were among the most resistant ones that depicted presence of both major and minor resistance genes.

Variations in the expression of the resistance genes was shown by the different levels of the severities and responses registered in the resistant genotypes, which imply that there was presence of gene diversity amongst them (Newcomb *et al.*, 2013). Wheat lines *KSL18*, *PCB52*, *PCB62* and *PCB76* that proved to be resistant in both stages together with the susceptible cultivars *Kwale* and *Duma* were used in developing crosses for the inheritance studies in the second experiment. This was very important since the kind of gene action and the number of genes that conferred the resistance was revealed. Resistance depicted by low infection types on  $F_1$  and segregation of  $F_2$  genotypes was an indication that the resistance at  $F_1$  was conferred by major gene(s). The segregation ratios for resistance to *Pgt* suggested that major genes that are modified due to epistatic effect were involved in conferring resistance to race *TTKST*. Nevertheless, apart from *Kwale*  $\times$  *PCB52* cross whose segregation ratio conformed to 3:1(R: S), the 9:7 (R: S) ratio observed on the crosses developed from *Kwale*  $\times$  *KSL18*, *Kwale*  $\times$  *PCB76*, *Kwale*  $\times$  *PCB62*, *Duma*  $\times$  *PCB52*, *Duma*  $\times$  *KSL18*, *Duma*  $\times$  *PCB76* and *Duma*  $\times$  *PCB62*

demonstrated that modifying genes that influenced the resistance to *Pgt TTKST* may be due to duplicate recessive epistasis (Staletic *et al.*, 2009).

The identified genotypes with seedling and adult plant resistance should be further characterized and the resistance genes accumulated in Kenyan commercial wheat varieties through intercrosses. Additionally, further mapping of the adult plant resistance (APR) quantitative trait loci (QTLs) should be done on genotypes that showed high levels of resistance in the field. Further testing should be done on the most resistant lines such as *KSL18*, *PCB62*, *PCB76* and *PCB52* in different environments. Other agronomic parameters like yield and height should be taken into consideration and the best performing lines considered for release as new varieties. Results of the second experiment suggests the need for further research to locate the genes that conferred resistance in the wheat genomes. The gene action and number of genes involved in resistance at seedling stage should be verified at advanced generations.

## APPENDICES

Appendix 1. BGRI International Core Differential Set – Stem Rust (ordered by sets used in North American nomenclature system).

Set	Gene	Low infection type	Differential line for world distribution, April 2009	Origin/Pedigree	Source
1	Sr5	0	ISr5-Ra CI 14159	Thatcher/Chinese Spring	Jin, USDA
	Sr21	1-	T monococcum/8*LMPG-6 DK13	Einkorn CI 2433	Fetch, AAFC
	Sr9e	1- to 2-	Vernstein PI 442914	Little Club //3* Gabo /2* Charter /3/3* Steinwedel / CI 7778	Jin, USDA
2	Sr7b	2	ISr7b-Ra CI 14165	Hope/Chinese Spring	Jin, USDA
	Sr11	; to 2-	Yalta PI 155433	Kenya C6402/Pusa4//Dundee	Park, Australia
	Sr6	0;	ISr6-Ra CI 14163	Red Egyptian/Chinese Spring	Jin, USDA
	Sr8a	2- to 2	Mentana W1124 PI 221154	Rieti / Wilhelmina // Akagomughi	Park, Australia
	Sr9g	2-	Acme CI 5284	Selection from Kubanka(CI 1516)	Pretorius, SA
3	Sr36	0;	W2691SrTt-1 CI 17385	CI 12632 T. timopheevii	Jin, USDA
	Sr9b	2	Prelude*4/2/Marquis*6/Kenya 117A	Kenya 117A	Fetch, AAFC
	Sr30 Sr17	1+ to 2 ;1	Festiguay W2706 PI 330957 Prelude/8*Marquis*2/2/Esp 518/9	Festival / Uruguay C10837 Esp 518/9	Park, Australia Fetch, AAFC
4	Sr9a	1- to 2-	ISr9a-Ra CI 14169	Red Egyptian/Chinese Spring	Jin, USDA
	Sr9d	1- to 1	ISr9d-Ra CI 14177	Hope/Chinese Spring	Jin, USDA
	Sr10	;1N to 3C	W2691Sr10 CI 17388	Marquis*4/Egypt NA95/2/2*W2691	Jin, USDA
	SrTmp	2-	CnsSrTmp	Triumph 64 (CI 13679)/Chinese Spring	Jin, USDA
5	Sr24	1- to 2-	LcSr24Ag	Little Club/Agent (CI 13523)	Jin, USDA
	Sr31	1- to 2	Kavkaz/Federation4	Kavkaz	Pretorius, SA
	Sr38	X=	Trident	Spear*4/VPM (PI 519303)	Park, Australia
	SrMcN	2-	McNair 701 (CI 15288)		Jin, USDA

Source: Prof. Z. A. Pretorius, University of the Free State, South Africa.

Appendix 2. Adult plant reactions of CIMMYT wheat (*Triticum aestivum*) lines to stem rust (*Puccinia graminis f. sp. tritici*) race Ug99 at KALRO- Njoro in 2012 and 2013 seasons.

Line	Pedigree	Final Disease Severity (FDS)		Coefficient of Infection (CI)		Area Under Disease Progress Curve (AUDPC)		Pseudo Black Chaff (PBC)
		2012	2013	2012	2013	2012	2013	
PBC1	KACHU/KIRITATI	10 M	15 M	6	9	133	200	+
PCB68	KACHU/KIRITATI	15 M	10 M	9	6	153	125	+
PCB36	KACHU/BECARD//WBLL1*2/BRAMBLING	20 M	30 MSS	12	30	173	325	+
PCB2	KIRITATI//ATTILA*2/PASTOR/3/AKURI	15 M	30 MSS	9	30	153	275	+
PCB3	KIRITATI//ATTILA*2/PASTOR/3/AKURI	15 M	15 MSS	9	15	153	200	+
PCB69	KIRITATI//ATTILA*2/PASTOR/3/AKURI	30 M	20 M	18	12	250	350	+
PCB53	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/FRNCLN	50 M	15 MSS	30	15	415	275	+
PCB13	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/HUIRIVIS #1	10 MR	10 M	4	6	95	125	+
PCB4	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KIRITATI//ATTILA*2/PASTOR	30 M	40 MSS	18	40	305	450	-
PCB5	WBLL1*2/BRAMBLING/3/KIRITATI//PBW65/2*SERI.1B	20 M	30 MSS	12	30	173	325	-
PCB60	BLL1*2/BRAMBLING//CHYAK	25 M	30 MSS	15	30	268	500	-
PCB27	WBLL1*2/KUKUNA/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA/2*WBLL1	40 M	50 MSS	24	50	495	475	+
PCB28	WBLL1/KUKUNA//TACUPETO F2001/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	20 MR	20 MSS	8	20	210	275	+
PCB6	ONIX/KBIRD	30 MR	40 MR	12	16	213	325	-
PCB45	ONIX/KBIRD	30 MR	30 MR	12	12	305	275	-
PCB73	ONIX/KBIRD	20 MR	10 MR	8	4	173	100	-
PCB46	ONIX/KBIRD	10 MR	30 MR	4	12	133	275	-
PCB7	FRANCOLIN #1/MESIA//MUNAL #1	25 M	25 MSS	15	25	230	300	+
PCB10	FRANCOLIN #1*2/KINGBIRD #1	10M	5M	6	3	133	100	+
PCB9	ALTAR 84/AE.SQUARROSA (221)/3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KI I//PBW65/2*SERI.1B	30 M	15 MSS	18	15	288	200	-
PCB11	FRNCLN*2/TECUE #1	30 MR	20 M	12	12	288	225	+

Appendix 2 *continued*

PCB12	PFAU/SERI.1B//AMAD/3/WAXWING/4/TECUE #1/5/PFAU/SERI.1B//AMAD/3/WAXWING	25 MR	25 MSS	10	25	230	325	+
PCB37	PFAU/SERI.1B//AMAD/3/WAXWING*2/4/TECUE #1	40 M	20 M	24	12	420	275	-
PCB14	MUNAL//WBLL1*2/BRAMBLING	20 M	20 MSS	12	20	210	275	-
PCB41	MUNAL*2//WBLL1*2/BRAMBLING	25 M	30 MSS	15	30	230	450	+
PCB42	MUNAL/3/KIRITATI//PRL/2*PASTOR/4/MUNAL	40 M	30 MSS	15	30	403	475	+
PCB30	MUNAL/3/HUW234+LR34/PRINIA//PFAU/WEAVER	40 M	30 MSS	24	30	420	425	+
PCB15	KINGBIRD #1/INQALAB 91//INQALAB 91*2/KUKUNA	40 M	80 S	24	80	513	925	-
PCB16	INQALAB 91*2/KUKUNA*2//JUCHI	50 MR	60 MSS	20	60	478	775	+
PCB17	TAM200/PASTOR//TOBA97/3/HEILO/4/PAURAQ	30 M	15 MSS	18	15	398	200	+
PCB47	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/DANPHE #1	30 MR	10 MSS	12	10	250	125	+
PCB72	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/ CM65531/3/SKAUZ/BAV92	60 MSS	60 MSS	60	60	685	650	-
PCB18	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/DANPHE #1	5 MR	10 M	2	6	75	125	-
PCB19	TUKURU//BAV92/RAYON/3/FRNCLN	30 M	40 M	18	24	343	425	+
PCB20	TUKURU//BAV92/RAYON/3/FRNCLN	30 M	20 MSS	18	20	360	350	+
PCB21	FRNCLN/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	25 MR	30 M	10	18	230	325	+
PCB22	BECARD#1/5/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ	15 M	15 MSS	9	15	190	200	+
PCB23	BECARD#1/5/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ	15 M	15 MSS	9	15	153	275	+
PCB24	BECARD/FRNCLN	40 M	30 MSS	24	30	403	375	+
PCB25	BECARD/FRNCLN	40 M	30 MSS	24	30	420	325	+
PCB26	BECARD/FRNCLN	30 M	30 MSS	18	30	325	325	+
PCB29	KISKADEE #1//KIRITATI/2*TRCH	20 M	40 MSS	12	40	210	450	+
PCB31	PBW65/2*PASTOR/3/KIRITATI//ATTILA*2/PASTOR/4/DANPHE #1	30 M	60 MSS	18	60	363	625	-
PCB32	PBW65/2*PASTOR/3/KIRITATI//PBW65/2*SERI.1B/4/DANPHE #1	10 M	10 MSS	6	10	95	105	+
PCB33	ATTILA/3*BCN*2//BAV92/3/KIRITATI/WBLL1/4/DANPHE	5 M	10 MSS	3	10	75	105	+
PCB34	ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETO F2001*2/BRAMBLING/5/PAURAQ	10 M	15 MSS	6	15	133	250	+
PCB35	ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETO F2001*2/BRAMBLING/5/PAURAQ	15 M	20 MSS	9	20	153	275	+

Appendix 2 *Continued*

PCB52	MUU/KBIRD	5 RMR	20 MR	1	8	75	225	-
PCB38	MUU/3/KIRITATI//ATTILA*2/PASTOR/4/MUU	15 M	15 M	9	9	153	150	+
PCB39	FRANCOLIN #1/AKURI #1//FRNCLN	30 M	15 M	18	9	305	250	+
PCB40	PCAFLR/KINGBIRD #1//KIRITATI/2*TRCH	30 M	30 M	18	18	363	400	+
PCB43	ND643/2*WBLL1/5/2*WAXWING/4/SNI/TRAP#1/3/ KAUZ*2/TRAP//KAUZ	40 M	40 M	24	24	420	425	+
PCB44	ND643/2*TRCH//BECARD/3/BECARD	40 M	60 MSS	24	60	513	675	-
PCB48	KACHU/KINDE	20 M	10 MSS	12	10	173	125	-
PCB49	KACHU/KINDE	20 M	20 MSS	12	20	173	275	-
PCB50	KACHU/KINDE	10 MR	15 M	4	9	95	200	-
PCB51	KACHU/KINDE	15 MR	20 MSS	6	20	95	275	-
PCB54	WBLL1/KUKUNA//TACUPETO F2001/3/QUAIU #2	25 MR	30 MR	10	12	190	325	-
PCB55	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL 1/4/QUAIU	10 RMR	30 MR	2	12	95	325	-
PCB56	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL 1/4/QUAIU	15 RMR	30 MR	3	12	115	275	-
PCB57	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL 1/4/QUAIU	10 RMR	30 MR	2	12	95	325	-
PCB58	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL 1/4/QUAIU	15 MR	20 MR	6	8	95	225	-
PCB59	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL 1/4/QUAIU	5 MR	30 MR	2	12	75	225	-
PCB61	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/QU AIU	30 MR	30 MR	12	12	250	225	-
PCB62	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/QU AIU	30 MR	30 MR	12	12	268	225	-

Appendix 2 continued

PCB 63	SITE/MO//PASTOR/3/TILHI/4/MUNAL #1/5/MUNAL	30 M	20 MSS	18	20	305	350	+
PCB 64	ND643/2*WBLL1//ATTILA*2/PBW65/3/MUNAL	50 MR	20 MR	20	8	478	275	-
PCB65	ND643//2*ATTILA*2/PASTOR/3/WBLL1*2/KURUKU/4/WBL L1*2/BRAMBLING	40 M	50 M	24	30	383	475	-
PCB66	ND643//2*ATTILA*2/PASTOR/3/WBLL1*2/KURUKU/4/WBL L1*2/BRAMBLING	40 M	60 MSS	24	60	345	650	-
PCB67	GAN/AE.SQUARROSA (408)//2*OASIS/5*BORL95/3/TACUPETO F2001*2/BRAMBLING	40 M	20 M	24	12	328	375	+
PCB70	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/4/KINGBIRD #1	10 M	10 MR	6	4	95	125	+
PCB71	HUIRIVIS #1/MUU//WBLL1*2/BRAMBLING	10 MR	20 M	4	12	133	175	+
PCB74	KACHU//WBLL1*2/KUKUNA/3/BRBT1*2/KIRITAT	50 MSS	20 MSS	50	20	403	275	-
PCB75	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WB LL1*2/BRAMBLING	50 MR	15 MR	20	6	330	200	-
PCB76	BABAX/LR42//BABAX*2/3/TUKURU*2/4/HEILO	50 MR	30 MR	20	18	368	225	-
PCB 77	BABAX/LR42//BABAX*2/3/TUKURU*2/4/HEILO	20MR	40 M	8	24	135	325	-
PCB78	FRET2/KUKUNA//FRET2/3/HEILO/4/BABAX/LR42//BABAX *2/3/KURUKU	20MR	20 MSS	8	20	135	350	-
PCB79	PICUS/3/KAUZ*2/BOW//KAUZ/4/KKTS/5/T.SPELTA PI348530/6/2*FRANCOLIN #1	20M	40 MSS	12	40	210	450	-
KSL1	SSERI/CHIBIA/4/BAV92//IRENA/KAUZ/3/HUITES	30 MSS	50 MSS	30	50	250	425	-
KSL4	WBLL1*2/BRAMBLING/5/BABAX/LR42//BABAX*2/4/SNI/T RAP#1/3/KAUZ*2/TRAP//KAUZ	10 MR	15 MR	4	6	95	200	-
KSL2	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/PFAU/ WEAVER//BRAMBLING/6/BAV92//IRENA/KAUZ/3/HUITES	20 MSS	25 MSS	20	25	135	250	-
KSL7	WBLL1*2/BRAMBLING/5/BABAX/LR42//BABAX*2/4/SNI/T RAP#1/3/KAUZ*2/TRAP//KAUZ	20 MR	15 MR	8	6	173	150	-
KSL17	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABA X	10 MR	10 MR	4	4	133	125	-
KSL18	WBLL1*2/KURUKU/4/BABAX/LR42//BABAX*2/3/KURUKU	15 MR	10 MR	6	4	153	125	-



Appendix 2 continued

KSL10	WBLL1*2/TUKURU/7/CNDO/R143//ENTE/MEXI_2/3/AE GILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/FRET2	15 MR	15 MR	6	6	153	150	+
KSL3	BAV92//IRENA/KAUZ/3/HUITES*2/4/CROC_1/AE.SQU ARROSA (224)//KULIN/3/WESTONIA	20 MSS	40 MSS	20	40	159	325	-
KSL5	BECARD/5/PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/CIRCUS	10 M	10 M	6	6	95	125	+
KSL6	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KA UZ	20 MR	50 MR	8	20	210	400	-
KSL8	BABAX/LR42//BABAX*2/4/SNI/TRA P#1/3/KAUZ*2/TRAP//KAUZ/5/WBLL1*2/TUKURU	15 MR	40 MR	6	16	190	275	-
KSL9	TRCH/6/HPO/TAN//VEE/3/2*PGO/4/MILAN/5/SSERI1	5 R	15 MR	1	6	75	200	+
KSL11	KBIRD//INQALAB 91*2/TUKURU	10 RMR	20 M	2	12	95	225	+
KSL12	PBW343*2/KUKUNA/3/PGO/SERI//BAV92	10 RMR	5 MR	2	2	95	100	+
KSL13	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	10 RMR	10 M	2	6	81	125	-
KSL14	WAXWING/KIRITATI*2//YANAC	15 MR	5 RMR	6	1	115	75	
KSL15	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAU Z/6/PASTOR/7/YANAC/8/CAL/NH//H567.71/3/SERI/4/C AL/NH//H567.71/5/2*KAUZ/6/PASTOR	5 R	5 MR	1	2	75	80	-
KSL16	TACUPETOF2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGI LOPSSQUARROSA (TAUS)/4/WEAVER/5/PASTOR/7/ROLF07	5 RMR	5 MR	1	2	75	100	+
KSL19	KSW/7/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/ 2*KAUZ/6/PASTOR/8/CAL/NH//H567.71/3/SERI/4/CAL/ NH//H567.71/5/2*KAUZ/6/PASTOR	10 RMR	5 M	2	3	95	100	-
KSL20	KENYA NYANGUMI/3/2*KAUZ/PASTOR//PBW343	40 MSS	5 MR	40	2	365	100	-
KSL21	TILILA/JUCHI/4/SERI.1B//KAUZ/HEVO/3/AMAD	15 M	5 MR	9	2	153	100	+
CACCUKE		90S	100S	90	100	1630	1700	-

CI= Coefficient of Infection, AUDPC= Area under disease progress curve, PBC= Pseudo black chaff where (+) and (-) indicates presence and absence of PBC trait, respectively, Ug99= Stem rust strain first reported in Uganda in 1999, R= Resistant; presence of hypersensitive necrotic flecks but no uredinia, (M= MRMS); moderately resistant moderately susceptible, MR= Moderately resistant; small pustules surrounded by necrotic areas, MS= moderately susceptible; medium-sized pustules with no necrosis, MSS= moderately susceptible to susceptible; medium to large sized pustules without necrosis, S= Susceptible; large pustules with no necrosis, KALRO = Kenya agricultural and livestock research organization.

Appendix 3. Adult plant reactions of the Bread wheat collections from different regions to stem rust race *Ug99* at KALRO- Njoro in 2012 and 2013 seasons.

Variety	Pedigree	Genes Present	Year of Release	Country	Final Disease severity (FDS)		Coefficient of Infection (CI)		Area Under Disease Progress Curve (AUDPC)		Pseudo Black Chaff (PBC)
					2012	2013	2012	2013	2012	2013	
Crim	KLEIN-TITAN/3*THATCHER/3/II-44-29/2*THATCHER		1963	Minnesota	15 RMR	15M	3	9	190	150	+
Chris	FRONTANA/3*THATCHER/3/KENYA A-58/NEWTATCH/2*THATCHER	<i>Sr5 Sr8a Sr9g Sr12 Sr7a Sr8a Sr9g Sr12</i>	1965	Minnesota	5 RMR	5MR	1	2	75	80	-
Polk	THATCHER / SUPREZA /3/ KENYA 58 / NEWTHATCH // FRONTANA		1968	Minnesota	5 MR	5MR	2	2	75	80	-
Era	II-55-10/4/PEMBINA/II-52-329/3/II-53-388/III-58-4//II-53-546		1970	Minnesota	10 M	10MS	6	8	95	125	-
Fletcher	II-55-10/4/PEMBINA/II-52-329/3/II-53-388/III-58-4//II-53-546		1970	Minnesota	5 MR	10M	2	6	75	125	-
Kitt	II-55-14/II-60-15		1975	Minnesota	15 M	15M	9	9	115	130	-
Anahuac	II-12300//LERMA-ROJO-64/SIETE-CERROS-66/3/NORTENO-67		1978	Minnesota	15 M	60S	9	60	208	675	-
Marshall	ERA/WALDRON		1982	Minnesota	20 MS	40S	16	40	210	475	-
Wheaton	CRIM/2*ERA//BUITRE/GALLO		1983	Minnesota	15 M	50M	9	30	190	425	-
Minnpro	MN-72299/MN-74115		1990	Minnesota	10 M	20MR	6	8	133	155	-
2375					60 S	40S	60	40	760	550	-
Verde	MN-7663/SBY-354-A		1995	Minnesota	30 M	50MSS	18	50	343	425	-
Ciano F67	KLEIN-TITAN/3*THATCHER/3/II-44-29/2*THATCHER		1963	Minnesota	10 MR	20MS	4	16	150	225	-
Newthatch	HOPE/THATCHER//2*THATCHER	<i>Sr5 Sr7b Sr12 Sr17</i>	1944	Minnesota	5 MR	20M	2	12	75	275	-
Timstein	STEINWEDEL/GAZA		1939	Minnesota	10 MR	40MR	4	16	150	450	-
Borah	NO-58/THATCHER//THATCHER/KENYA -FARMER/3/MN-III-58-1//FRONTANA/3*THATCHER		1974	Minnesota	5 MR	15M	2	9	20	125	-

Appendix 3 *continued*

Norm Shield	MN-73167/MN-81070 COTEAU(CI-17749,s)/(CI-17801,w)DAWN	1992	Minnesota	20 M	15M	12	9	248	150	—
A99ar	GLENLEA / ZARAGOZA	1982	Minnesota	40 M	50S	24	50	438	650	—
Mcvey	NING-8331/MN-87029//MN-89068	1999	Minnesota	20 M	30MSS	12	30	173	400	—
Justin	CONLEY/ND-40-2	1962	Minnesota	30 M	30MSS	18	30	343	450	—
Mida	MERCURY/RL-625	1944	Minnesota	20 M	15M	12	9	248	250	—
Wared	II-55-10/4/PEMBINA/II-52-329/3/II-53-388/III-58-4//II-53-546	1974	Minnesota	20 M	40MSS	12	40	210	425	—
Yaqui 50	NEWTATCH/MARROQUI-588	1950	Minnesota	25 M	10MR	15	4	248	125	—
Ceres	MARQUIS/KOTA	1924	Minnesota	20 M	20M	12	12	173	275	—
MG 07762			Ethiopia	30 MR	20MR	12	8	213	200	—
MG 07782			Ethiopia	60 M	30MSS	36	30	703	275	—
MG 07793			Ethiopia	60 MSS	70S	60	70	483	725	—
Sindi 1			Ethiopia	40 M	50M	24	30	565	750	—
Sindi 2			Ethiopia	50 MSS	80S	50	80	645	1,200	—
Sindi 3			Ethiopia	50 MSS	80S	50	80	645	1,350	—
Sindi 5			Ethiopia	40 MSS	80S	40	80	565	1,050	—
Sindi 6			Ethiopia	40 MSS	80S	40	80	495	1,450	—
Sindi 7			Ethiopia	50 MSS	80S	50	80	605	1,150	—
Sindi 8	Landrace		Ethiopia	60 S	80S	60	80	645	1,200	—
Laketch	PENJAMO-62/GABO 55	1970	Ethiopia	10 M	30MSS	6	30	133	550	—
K-6106-8	CI-8154/2*FROCOR/3/2*GABO-54/36896//II-53-526	1977	Ethiopia	5 R	missing	1		75	0	—
Enkoy	—		Ethiopia	5 R	5RMR	1	1	75	75	—
K 6290	AFRICA MAYO/*ROMANY	1977	Ethiopia	10 RMR	10M	0.8	6	95	100	+
Bulk			Ethiopia	0	5R	0	1	0	25	—
K6295-4A	ROMANY/GABO-GAMENYA	1980	Ethiopia	5 M	15M	3	9	75	150	—
Pavon 76	VICAM 571//CIANO F67//SIETE CERROS T	1982	Ethiopia	5 M	15M	3	9	75	150	—
Batu	GALLO/CUCKOO//KAVKAZ/SUPER X	1984	Ethiopia	25 M	20MSS	15	20	193	375	—
Dashen	KAVKAZ/BUHO//KALYANSONA/BL UEBIRD	1984	Ethiopia	15 M	15M	9	9	115	150	—
HAR-407	KAVKAZ/BUHO//KALYANSONA/BL UEBIRD	1987	Ethiopia	5 M	10M	3	6	75	125	—

Appendix 3 *continued*

KKBB	KAVKAZ/KALYANSONA/BLUEBIR D		1982	Ethiopia	30 M	30MSS	18	30	213	325	-
Gara	AVRORA//KALYANSONA/BLUEBIR D/3/(SIB)WOODPECKER		1984	Ethiopia	15 M	15MSS	9	15	190	150	-
Mitike	BOBWHITE/REICHENBACHII		1993	Ethiopia	10RMR	10M	2	6	95	125	+
Galema	4777*2//FKN/GABO-AUS/3/PAVON F 76		1995	Ethiopia	20 M	20M	12	12	155	175	-
Kubsa	NORD-DESPREZ/VG- 9144//KALYANSONA/BLUEBIRD/3/ YACO/4/VEERY		1994	Ethiopia	40 M	20MSS	24	20	438	275	-
Wabe	MIRLO/BUCKBUCK		1994	Ethiopia	30 M	25MSS	18	25	305	450	-
Abola	BOBWHITE/BUCKBUCK		1997	Ethiopia	20 M	15MSS	12	15	210	300	-
Megal	F371/TRM//BUC''S''/3/LIRA''S''		1997	Ethiopia	30 M	20MSS	18	20	415	375	-
Tusie	COOK/VEERY//DOVE/SERI M82		1997	Ethiopia	40 M	30MSS	24	0	403	400	-
Katar	COOK/VEE''S''//DOVE''S''/SERI/3/B JY''S''		1999	Ethiopia	20 M	20MSS	12	20	210	375	-
MG 07759				Ethiopia	30 M	50MSS	18	50	288	475	-
MG 07768				Ethiopia	5 RMR	15M	1	9	75	150	-
MG 07795				Ethiopia	40 M	60MSS	24	60	365	675	-
Sindi 4				Ethiopia	40 M	60M	24	36	455	1,100	-
Inia66	LERMA ROJO 64/SONORA 64	<i>Sr2 Sr8a Sr11.</i>	1971	Ethiopia	5 R	15M	1	9	75	150	+
Enkoy	HEBRAND SEL/WISCONSIN 245/SUPRESA/3/2*FROCOR//FRONT ANA/YAQUI/4/AGUILERA		1974	Ethiopia	5 R	5RMR	1	1	75	100	-
Romany	COLOTANA 261-51 / YAKTANA 54A	<i>Sr5 Sr6 Sr7a Sr30 sr9b</i>	1970	Ethiopia	25 M	30M	15	18	230	325	-
Sonara 63	YAKTANA-54//NORIN- 10/BREVOR/3/2*YAQUI-54		1975	Ethiopia	70 S	80S	70	80	1015	1,450	-
Shina	GOLDEN-VALLEY(GOV)/AZTECA- 67//MUSALA/3/R-37/GHL- 121//KALYANSONA/BLUEBIRD/4/A NI		1998	Ethiopia	40 M	30MSS	24	30	455	400	-

Appendix 3 continued

Tura	ARO-YR-SEL-60-1989	1999	Ethiopia	10 M	15MSS	6	15	133	200	—
Hawi	CHILERO/PARULA	2000	Ethiopia	60 M	40MSS	36	40	593	450	—
Madda walabu Simba	TANORI F 71/3/Fn/Th/Nar59 *2/4/Bol'S' PARULA/VEERY #6//MYNA/VULTURE	2000	Ethiopia	15 M	20MSS	9	20	190	225	—
Sofumar	4777(2)//FKN/GB/3/PAVON F 76	2000	Ethiopia	40 MSS	40MSS	40	40	438	400	—
Wetera	MONCHO''S''-BUCKBUCK''S''	2000	Ethiopia	70 S	60MSS	70	60	835	675	—
Dodota	BLUEJAY/COCORAQUEF 75//PARULA/BOBWHITE	2001	Ethiopia	70 S	70S	70	70	910	1,150	—
Dure	—	2001	Ethiopia	15 MR	15MR	6	6	115	125	—
CI 14393	FROCOR*2/4/COMETA/3/ NEWTATCH// MENTANA/ MENKEMEN	1975	Ethiopia	10 RMR	20M	0.8	12	133	155	—
Sirbo	VS73.600/MRL/3/BOBWHITE//YECOR A F 70/TRIFON	2001	Ethiopia	10 M	10MSS	6	10	78	125	—
Bobicho	PEREGRINE/PF70354/KALYANSONA /BLUEBIRD/ALONDRA/3/MARINGA	2002	Ethiopia	60 S	60MSS	60	60	593	525	—
Dereseglen	CI8154//2*FEDERATION	1974	Ethiopia	20 MR	30M	8	18	265	325	—
ET-12-D4	MAMBA/UQ105	1981	Ethiopia	15 M	15M	9	9	115	150	—
Digelu	SHANGHAI #7//KAUZ		Ethiopia	5 RMR	15MR	0.4	6	20	125	—
Meraro	—	2005	Ethiopia	5 R	5MR	1	2	20	100	—
Jiru		2006	Ethiopia	30 MSS	70S	30	70	398	1,000	—
Bobin	THEW/STEINWEDEL	1925	Australia	40 M	40MSS	24	40	455	525	—
McMurachy	RC-1373/RED-EGYPTIAN	1958	Canada	40 M		24		455	m	—
Reward	MARQUIS/PRELUDE	1928	Canada	30 M	70S	18	70	415	900	—
Olescens dwarf	NORIN-10/MARA,ITA//ANGOLAN-X- 2-50	1970	Zimbabwe	25 M	40MSS	15	40	285	475	—
Bonanza	PITIC-62/(SIB)CHRIS//SONORA-64	1969	Kansas	5 MR	10M	2	6	75	125	+
Exchange	WARDEN,GBR/HYBRID-ENGLISH	1963	Indiana	40 MSS	60MSS	40	60	345	675	—
Wis. 245	PD-2666-A-2-2-2-15-6-3*3/(TR.TI)D- 357-1	1954	Wisconsin	5 MR	20M	2	12	75	325	—
1010 AM 2(L)	II-50-17/KENYA-184-P	1969	Kenya	15 M	20M	9	12	153	225	—

Appendix 3 Continued

1076.D.7	LEE/FRONTANA//KENYA-184-P	1969	Kenya	20 M	20MSS	12	20	248	275	+
1200.M 291	CI 12632 /3* La PREVISION		Kenya	0 25 MS	5RMR 80S	0 20	1 80	0 285	75 1,100	- -
1061.K.1	MIDA // McMURACHY / EXCHANGE /3/ RIO NEGRO		Kenya	5 RMR	5MR	1	2	58	35	-
338 AA 1 690F4	AUSTRALIAN-27//KENYA-192-Q KENYA-360-	1951	Kenya	30 M	50S	18	50	268	475	-
SEL.D.1	H//2*MARQUIS/AGROPYRON ELONGATUM	1969	Kenya	15 M	20M	9	12	153	205	+
1010 F3 SEL.4	II-50-17//KENYA-184-P	1969	Kenya	20 M	20MSS	12	20	173	275	-
Africa Mayo	AFRICA/MAYO-48	1960	Kenya	20 M	20MS	12	16	210	350	+
B F2 36 Bailey			Kenya	50 M	80S	30	80	605	1,350	-
	/4*THATCHER/3/THATCHER//KENY A- 58/NEWTATCH/4/THATCHER/5/FR ONTANA/4*THATCHER	1966	Kenya	5 RMR	10M	1	6	20	105	-
Beacon- Ken	FRONTANA / KENYA 58 // NEWTATCH /3/3* BONZA	1968	Kenya	5 R	5RMR	1	1	75	75	-
Bailey	/4*THATCHER/3/THATCHER//KENY A- 58/NEWTATCH/4/THATCHER/5/FR ONTANA/4*THATCHER	1966	Kenya	5 RMR	10M	1	6	20	105	-
Beacon- Ken	FRONTANA / KENYA 58 // NEWTATCH /3/3* BONZA	1968	Kenya	5 R	5RMR	1	1	75	75	-
Bonny	YAQUI-53/2*BONZA	1966	Kenya	40 MS	60MSS	32	60	253	475	-
Bounty Brewster	TIMSTEIN/2*KENYA//BONZA	1966	Kenya	5 R	5M	1	3	75	35	+
	FRONTANA/4*THATCHER/3/THATC HER//KENYA- 58/NEWTATCH/4/THATCHER/5/FR ONTANA/4*THATCHER	1966	Kenya	5 RMR	50MSS	1	50	58	575	-

Appendix 3 *continued*

Catcher	THATCHER/SANTA-CATALINA//FROCOR	<i>Sr2 Sr6 Sr8a Sr9g Sr12</i>	1963	Kenya	40 M	30MSS	24	30	383	275	-
Fanfare	FROCOR//FRONTANA/YAQUI		1964	Kenya	15 M	15M	9	9	115	200	+
Fronthatch	FRONTANA / KENYA58 // NEWTHATCH		1963	Kenya	5 M	10MR	3	4	58	100	-
Gabrino	KENTANA/RIO-NEGRO//GABO-54		1963	Kenya	15 MR	10MR	6	4	115	100	-
Goblet	GABO-54/LERMA-52//GABO/3/KENYA/GENERAL-URQUIZA		1967	Kenya	5 MR	5RMR	2	1	75	75	+
H 441	REWARD/ CI 12632			Kenya	5 MR	5RMR	2	1	75	75	+
Kentana	KENTANA-48/YAQUI-48		1960	Kenya	5 RMR	5M	1	3	75	100	+
Yaqui				Kenya	40 M	50S	24	50	455	750	-
Kenya 155	RELIANCE/KENYA-73-D		1951	Kenya	30 M	30M	18	18	323	275	-
Kenya-184-P				Kenya	25 MS	15MSS	20	15	230	250	-
Kenya-360-H	KENYA 294M7C6C / KENYA 184P2A1E			Kenya	10MS	10MS	8	8	133	175	-
Kenya -362-B-1	EQUATOR / KENYA 294.M . 7.C.6 .C.		1956	Kenya	30 M	60M	18	36	398	1,100	-
Kenya 7	-				20 M	30M	12	18	248	275	-
1010 F3	II-50-17/KENYA-184-P		1969	Kenya	5 MR	5RMR	2	1	75	80	-
SEL.7				Kenya	15 MR	20M	6	12	190	155	-
1012 B.1.	MENTANA/KENYA//BAGE/3/KENYA-184-P		1969	Kenya	50 MSS	60S	50	60	423	475	-
1016 P.1	KENYA-360-H/II-50-17		1969	Kenya	5 R	20M	1	12	75	150	-
CACUKE					5 R	5RMR	1	1	75	25	-
Kenya 8	-				20 S	20MSS	20	20	173	225	-
Kenya Cheetah	WARIGO/STERLING			Kenya	5MR	5MR	2	2	58	35	-
Kenya Civet	CI 12632 /3* KENYA 354		1966	Kenya							
Kenya Grange	KENYA-360-F/GRANADERO-KLEIN		1966	Kenya							
Kenya Hunter	EQUATOR II / KENYA 310.0 . 33.2 // HOPE / TIMSTEIN /3/ REGENT		1964	Kenya							

Appendix 3 Continued

Kenya Jay	EQUATOR/KENYA-318	1966	Kenya	15 S	10MS	15	8	115	175	-
Kenya Kudu	KENYA-131/KENYA-184-P	1966	Kenya	20 MR	15MS	8	12	173	250	-
Kenya	LAGAEDINHI /3* KENYA 381P //	1966	Kenya	0	5MR	0	2	0	25	-
Leopard	CI 12632 /3* KENYA 354P									
Kenya	CI-8154/2*FROCOR/3/2*GABO-	1974	Kenya	25M	30MSS	15	30	285	525	-
Mbweha	54/36896//II-53-526									
Kenya page	MENTANA/KENYA-58//BAGE/3/KENYA-184-P	1963	Kenya	5 R	5MR	1	2	58	75	-
	CERES/KENYA-112-E-8-L-5	1950	Kenya	30 MSS	15MSS	30	15	250	130	-
Kenya	ID 1877/MORRIS	1969	Kenya	5 MR	0	2	0	75	0	-
Ploughman										
Kenya	MARQUIS/AGUILERA 8		Kenya	40 MSS	60MSS	40	60	455	525	-
Sungura	FLORENCE / AGUILERA 8		Kenya		70S	Missin g	70	.	800	-
Kenya-122	AUSTRALIAN-26-A/KENYA-117-A		Kenya	40 S	70S	40	70	363	900	-
Kenya-131	KENYA-112/CERES		Kenya	30 MSS	15MS	30	12	323	200	-
Kenya-294-B-2 A-3	KENYA-112/CERES		Kenya	30 MSS	5M	30	3	288	80	-
Kenya-318.O.3B.2										
Kenya-318-AJ										
Lenana	YAQUI- 48 / KENTANA- 48	1963	Kenya	5 R	5M	1	3	75	80	+
Menco	MENTANA / KENYA // FRONTANA / CINCO	1963	Kenya	10 M	30MSS	6	30	133	425	-
Kenya-5	S)LV-KEN	1960	Kenya	30 M	70S	18	70	360	1,150	-
Mentor	MENGAVI/3/SPICA/KODA//GABO	1967	Kenya	5 R	10MR	1	4	75	100	-
Morris	THATCHER//KENYA-117 A/MIDA/3/FRONTANA/4*THATCHER/4/THATCHER/5/FRONTANA /4*THATCHER		Kenya Kenya	5R	5MR	1	2	20	75	-
P.Walker	-		Kenya	5 MR	10M	2	6	75	175	-
Pitcher	-			10 MR	15M	4	9	133	150	-
Primex	90875,MEX	1969	Kenya	5 RMR	5M	1	3	75	80	+
R 64	DIENTE DE CAMELLO/CERES	1953	Kenya	20 M	15M	12	9	173	130	-



Appendix 3 *continued*

Regent	H44/REWARD	<i>Sr7b</i>	1939	Kenya								
		<i>Sr9d</i>			5 R	15M	1	9	75	200	-	
		<i>Sr17 sr2</i>										
Reliance	KANRED/MARQUIS	<i>Sr5 Sr16</i>	1933	Kenya	50 MSS	40MSS	50	40	460	375	-	
		<i>Sr20</i>										
Reliance 261M	RELIANCE / KENYA 68		-	Kenya	50 MSS	15M	50	9	478	200	-	
RFN Rhodesia	(S)SABANERO		1949	Kenya	0	5RMR	0	1	0	75	-	
Santa					30 MSS	30MSS	30	30	75	375	-	
Tama	YAKTANA-54/LERMA-52		1963	Kenya	5 R	5MR	1	2	75	100	-	
Token- Ken	TIMSTEIN/2*KENYA//YAQUI- 50		1966	Kenya	10 M	15MR	6	6	133	250	+	
Trophy	TIMSTEIN/2*KENYA-RF- 324//2*YAQUI-50		1968	Kenya	5 RMR	10MR	1	4	75	125	+	
1016.P.2	360H /3/Frontana // KENYA 58 / NEWTHATCH		1969	Kenya	15 MS	20MS	12	16	153	225	-	
1061.K.4	MIDA // McMURACHY / EXCHANGE /3/ RIO NEGRO		-	Kenya	5 MS	15M	4	9	75	200	-	
Egyptian Na 95	KENYA-U / KENYA 9MIA-3			Kenya	30 M	50M	18	30	305	900	-	
1010 F3 SEL. 13 C	II-50-17/KENYA-184-P		1969	Kenya	15 M	10M	9	6	98	125	-	
688 F4 SE 3	KENYA-294M //2* MARQUIS/ AGROPYRON ELONGATUM		1969	Kenya	5 M	15M	3	9	75	300	-	
Equator 3	AUSTRALIAN-VARIETY MARQUIS / AGUILERA 8		1920	Kenya	60 S	40MSS	60	40	518	600	-	
		<i>Sr7a</i>										
		<i>Sr10</i>			50 MSS	30MSS	50	30	368	375	-	
		<i>sr9b</i>										
Kenya B- 256-G	KENYA-U/KENYA-9-M-1-A-3			Kenya	20 M	30MS	12	24	173	425	-	
Kenya Farmer	AUSTRALIAN-27/KENYA-192	<i>Sr9b</i>	1954	Kenya								
		<i>Sr7a</i>			30 M	30MSS	18	30	250	475	-	
		<i>Sr10</i>										
		<i>Sr11.</i>										

Appendix 3 Continued

Kenya Governor	-		1925	Kenya	10 M	30MR	6	12	133	325	-
Kenya Nungu	WISCONSIN-245/II-50-17//CI-8154/2*FEDERATION/3/2*TOBARI-66		1975	Kenya	20 M	20MR	12	8	190	225	-
Kenya Plume	MIDA/MCMURACHY//EXCHANGE/3/KENYA-184-P	<i>Sr2 Sr5 Sr6 Sr7a Sr8a Sr12 Sr17</i>	1965	Kenya	30 M	15M	18	9	288	200	-
Kenya Nyoka	CI-8154/2*FEDERATION//3*ROMANY		1975	Kenya	5 RMR	15M	1	9	75	150	-
Kenya Standard			1930	Kenya	40 MSS	80S	40	80	418	1,300	-
Kenya-58	RED EGYPTIAN / KENYA BF3B10V1				50 S	50MSS	50	50	440	575	-
Pewter Salmayo	PW-327,USA/5*THATCHER SALLES/MCMURACHY//MAYO-48		1964	Kenya	25 M	30MSS	15	30	248	425	-
			1963		15 M	15M	9	9	153	150	-
Aggia					25 M	30MSS	15	30	340	425	-
Kenya 6820 RL 1377	KENYA 4500-35 / KENYA SWARA MARQUIS / AGUILERA 8			Kenya	5 RMR	5MR	0.4	2	75	100	+
Fury	FROCOR/MENTANA/KENYA-2/MCMURACHY/YAQUI-50		1964	Kenya	10 RMR	20MSS	0.8	20	133	375	-
Gem Bonito	BT908 / FRONTANA // CAJEME 54 BONZA 'S'4/ FRONTANA // THATCHER/ TRIGO GLUTINOSA/3/ MENTANA/5/2* LERMA// SELKIRK/ LERMA /3/ WISCONSIN 245/4/ MENKEMEN 626		1964	Kenya	20 MR	5RMR	8	1	173	100	-
			1973	Kenya	5 R	1MR	1	0.2	75	15	-
PW Thatcher Kenya Nyangumi	THATCHER/AGENT TEZANOS-PINTOS-PRECOZ//SELKIRK-ENANO*6/LERMA-ROJO-64/3/AFRICA-MAYO-48/4/KENYA-SWARA/K-4500-6		1979	Kenya	5 MR	10M	2	6	75	125	-
					5 M	10M	3	6	75	125	-

Appendix 3 Continued

Kenya Tembo	WISCONSIN-245/II-50-17//CI-8154/2*TOBARI-66	1975	Kenya	5 M	10MR	3	4	75	125	-
Kenya Fahari	TOBARI-66/3/SRPC-527-67//CI-8154/2*FROCOR	1977	Kenya	10 MR	5M	4	3	95	40	-
Breadwheat 23	-			15 M	5MR	9	2	190	80	-
Kenya Yombi	MBUNI/SRPC-64//YRPC-5	1998	Kenya	20 MSS	25MSS	20	25	210	500	-
Kenya Heroe B192	MBUNI/SRPC-64//YRPC-1	1999	Kenya	20 MSS	25MSS	20	25	210	500	-
Kenya 117C Kruger	MARQUIS / AGUILERA 8 KAVKAZ/3/SONORA 64/CIANO F 67//INIA F 66/4/MAYA 74//BLUEBIRD/INIA F 66	1987	Kenya Kenya	20 M 50 MSS	50M	12 50	30	265 628	950 0	- -
Kenya Kongon	CI-8154/2*FROCOR//3*ROMANY/4/WISCONSIN-245/II-50-17/CI-8154//2*FROCOR/3/TOBARI-66	1981	Kenya	5 RMR	5MR	1	2	75	75	+
Kenya Swara	CI-8154/2*FROCOR/3/TIMSTEIN/2*KENYA//Y-59.2.B	1972	Kenya	5 R	5MR	1	2	75	100	+
Kenya Paka	WISCONSIN-245/II-50-17//CI-8154/2*TOBARI-66	1975	Kenya	10 MR	10MR	4	4	133	100	-
Kenya Zabadi	CORRECAMINOS/INIA-67//K-4500-2/3/KENYA-SWARA//TOBARI-66/CIANO-67	1979	Kenya	20 M	15M	12	9	210	150	-
Kenya Ngiri	CI-8154/2*FROCOR//WHEAT-RYE-TRANSLOCATION/SANTA-CATALINA/3/MANITOU/4/2*TOBARI-66	1979	Kenya	20 M	10M	12	6	248	125	+
Duma	AURORA/UP301//GALLO/SUPER X/3/PEWEE/4/MAIPO/MAYA 74//PEWEE	1993	Kenya	25 M	30MSS	15	30	268	375	-
Mbega	BONANZA/YECORA-70/3/F-35-75//KALYANSONA/BLUEBIRD	1993	Kenya	15 M	15MS	9	12	98	275	-

Appendix 3 *continued*

Mbuni	ZARAGOZA-75/3/LD-357- E/THATCHER//GALLO	1987	Kenya	25 M	20MS	15	16	230	300	_
Ngamia	BUCKY/MAYA-74/4/BLUEBIRD//HD- 832/OLESENS DWARF/3/CIANO 67 /PENJAMO 62		Kenya	15 M	25MSS	9	25	190	450	_
Chozi	F-12-7/COCORAQUE 75//GENARO 81	1998	Kenya	30 M	30M	18	18	288	475	_
Njoro	IAS-	2007	Kenya							
BW II	58/4/KALYANSONA/BLUEBIRD//CAJEME-F- 71/3/ALONDRA/5/BOBWHITE			10 M	20M	6	12	95	175	_
Kenya- Ibis	KWALE/DUMA	2008	Kenya	10 M	5MR	6	2	95	75	_
Kenya Popo	KLEIN-ATLAS/TOBARI- 66//CENTRIFEN/3/BLUEBIRD/4/KENYA- FAHARI	1982	Kenya	5 MS	15M	4	9	58	125	_
BTC Barce	-			40 M	30MSS	24	30	513	500	_
FL I Kenya 9	-		Kenya		5RMR		1	missing	25	_
Quamy	-			0	5MR	0	2	0	100	+
Kenya Nyati	AFRICA-MAYO/2*ROMANY	1973	Kenya	5 R	5MR	1	2	75	100	+
Kenya Kanga	MENCO/4/WISCONSIN-245/SUPREMO 51/3/2*FEDERATION/FRONTANA/YAMESEK	1977	Kenya	5 R	5RMR	1	1	75	75	+
Kenya Tumbili	KTB/GIZA-155//NADADORES-63/T-238-1-5-8- 17-10/3/KLEIN-ATLAS /TOBARI-66//CENTRIFEN/BLUEBIRD	1984	Kenya	20 MR	10MS	8	8	173	175	_
Kenya Chiriku	KTB/(SIB)CARPINTERO	1989	Kenya	5 RMR	10MR	1	4	75	75	_
Pasa	BUCK BUCK/CHLAT	1989	Kenya	15 M	20MS	9	16	153	175	_
Kenya Paa	KAVKAZ/3/CIANO-67/CHRIS//OLESENS- DWARF	1981	Kenya	5 MR	15MR	2	6	75	150	_
Kenya Kifaru	TOBARI-66*3/3/WISCONSIN-245//CI- 8154/2*FROCOR	1977	Kenya	15 MR	20M	6	12	153	175	_
321 BT 11 B1 BW21	AUSTRALIAN-45-C-5/KENYA-117-A	1960	Kenya	10 M	15M	6	9	95	275	_
				40 S	30MSS	40	30	340	400	

Appendix 3 *Continued*

8952						40 S	30MSS	40	30	415	575	
Bonza 63	RIO-NEGRO/2*BONZA-55		<i>Sr8a Sr9b sr6</i>	1963	Colombia	5 R	5RMR	1	1	75	75	
Bonza	YAQUI-50/KENTANA-48			1970	Colombia	5 RMR	10M	1	6	75	125	
Frocor 2328	FRONTANA // C.O. / C.R.			1951	Colombia	40 M	50MSS	24	50	513	625	-
Gandum- i-Fasai	LV-IRN				Iran	20 M	20M	12	12	80	225	-
NP761	PUSA-52/PUSA-165			1941	India	30 M	30MSS	18	30	415	425	-
Kalyanoso na	FRONTANA // KENYA 58/ NEWTATCH/3/NORIN 10 /BREVOR/4/ GABO 55			1967	India	25 M	80S	15	80	340	1,200	-
Bluebird	CIANO-67(SIB)//SONORA- 64/KLEIN-RENDIDOR/3/II-8156			1969	Mexico	10 MR	20M	4	12	133	225	+
Lerma rojo	LERMA-50/YAQUI-48//MARIA- ESCOBAR*2/SUPREMO-211			1955	Mexico	40 M	70S	24	70	548	1,250	-
Kentana 48	KENYA-C-9906/MENTANA			1948	Mexico	40 M	30MSS	24	30	420	475	-
Penjamo 62	FKN/NORIN 10 BREVOR				Mexico	15 M	30MSS	9	30	208	400	-
Red Egyptian	RED EGYPTIAN				Mexico	40 MSS	40MSS	40	40	420	625	-
Mentana	RIETI/WILHELMINA//AKAKOMU GI			1913	Mexico	40 M	60MSS	24	60	328	775	-
Gradenero					Mexico	40 M	40MSS	24	40	438	475	-
Yaktana 54A	YAQUI-48/KENTANA- 48//FRONTANA			1954	Mexico	25 M	30MSS	15	30	230	425	-
Zaragoza 75	MENGAVI/II-8156		<i>Sr36</i>	1975	Mexico	5 R	5MR	1	2	75	75	-
Tobari 66	TEZANOS-PINTOS- PRECOZ/SONORA-64-A			1966	Mexico	20 M	30MS	12	24	283	350	-
Supremo	SURPRESA//HOPE/MEDITERRAN EAN			1948	Mexico	30 MSS	50MSS	30	50	380	500	-
Cocoraqu e 75	II-12300//LERMA-ROJO-64/II- 8156/3/NORTENO-67			1975	Mexico	40 M	60S	24	60	438	850	-

Appendix 3 Continued

Bobwhite	AVRORA//KALYANSONA/BLUEBIRD/3/(SIB)WOODPECKER		1977	Mexico	20 M	15MS S	12	15	210	200	+
Bage	1068.36/LA-ESTANZUELA-2787-C		1938	Brazil	30 M	60S	18	60	360	800	-
Impala	KOALISIE/HOPE		1954	South Africa	30 M	30M	18	18	433	550	+
Frontana	FRONTEIRA/MENTANA	<i>Sr8a sr9b</i>	1930	East Africa	20 M	15MS S	12	15	173	225	-
Marquis	HARD-RED-CALCUTTA	<i>Sr18 Sr19 Sr20 Sr7b</i>	1907	North America	15 M	40M	9	24	208	525	-
Hard Federation					50 MS	80S	40	80	460	1,400	-
Hope		<i>Sr2 Sr7b Sr9d Sr17</i>		North America	20 M	60S	12	60	210	525	-
Marquillo	MARQUIS/(TR.DR)IUMILLO		1926	North America	10 RMR	5MR	2	2	133	75	-
Thatcher	MARQUIS/(TR.DR)IUMILLO//MARQUIS/KANRED	<i>Sr5 Sr9g Sr12 Sr16</i>	1934	North America	10 M	10M	6	6	133	105	-
Federation	YANDILLA/PURPLE-STRAW[113];PURPLE-STRAW/YANDILLA		1901	Australia	30 MSS	30MS S	30	30	380	425	-
Gabo	TIMSTEIN/KENYA-58//GABO		1955	Australia	5 MR	20MR	2	8	75	225	-
Giza 155	GIZA-144/3/MIDA,USA/CADET,USA//2*HI NDI-62		1968	Egypt	5 MR	10MR	2	4	75	175	-
II-50-17					15 RMR	15M	1.2	9	153	150	-
MN72131					15 MS	60S	12	60	115	625	-
Waldron	JUSTIN/ND-81		1968		20 M	30MS	12	24	173	275	-
Angus					5 M	10M	3	6	58	125	-
Beltista	-				60 M	70S	36	70	553	1,050	-
HRS 55	-				60 MSS	60S	60	60	778	800	-
Bale	-				0	20M	0	12	0	225	-
Vance					10 MR	10M	4	6	133	105	-
869					5 RMR	10M	1	6	58	105	-

Appendix 3 *continued*

Lahota	5 R	5RMR	1	1	75	25	-
CACCUKE	90S	100S	90	100	1650	1,700	-

CI= Coefficient of Infection, AUDPC= Area under disease progress curve, PBC= Pseudo black chaff where (+) and (-) indicates presence and absence of PBC trait, respectively, *Ug99*= Stem rust strain first reported in Uganda in 1999, R= Resistant; presence of hypersensitive necrotic flecks but no uredinia, (M= MRMS); moderately resistant moderately susceptible, MR= Moderately resistant; small pustules surrounded by necrotic areas, MS= moderately susceptible; medium-sized pustules with no necrosis, MSS= moderately susceptible to susceptible; medium to large sized pustules without necrosis, S= Susceptible; large pustules with no necrosis, KALRO = Kenya agricultural and livestock research organization.

Appendix 4. Greenhouse seedling infection types of wheat (*Triticum aestivum*) accessions to stem rust (*Puccinia graminis f. sp. tritici*) predominant *TTKSK* and *TTKST* races at KALRO- Njoro.

Genotypes	<i>TTKSK</i>			<i>TTKST</i>		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
PBC1	3-, 2	3+	3+	3	3	3
PCB68	3+	3+	3+	3+	3	3+
PCB36	3+	3+	3+	3+	3+	3+
PCB2	;1+	;1+	;1+	2+	2+	2+
PCB3	3-	3, 2	3	2, 3	3-	3-
PCB69	; 1+	;1+	;1+	;2+	2+	2+
PCB53	3+	3, 2+	3+	3+	3+	3+
PCB13	3-, 2	3-	3-	3, 2+	3+	3+
PCB4	3+	2, 3+	3+	3, 2+	3+	3+
PCB5	2-	2-	2-	2+	2, 3+	2+
PCB60	2+	2+	2+	2+	2+	2+
PCB27	3+	3+, 2	3+	3, 2+	3+	3+
PCB28	3+	3	3+	3+	3, 2+	3+
PCB6	3-	3, 2+	3-	3, 2+	3	3
PCB45	3+	3+	3+	3+	3+	3+
PCB73	3, 2+	3	3	3+	3+	3+
PCB46	3+	3, 2+	3+	3-	3	3-
PCB7	4	4	4	3, 2	3+	3+
PCB10	X	3, 3-	X	4	4	4
PCB8	3-	3, 2+	3-	3, 2+	3, 2+	3
PCB9	3+	3-	3+	3	3	3
PCB11	2+	2+	2+	;1+	;1	;1+
PCB12	3+	3+	3+	2, 3+	3	3
PCB37	2+	2+	2+	2	2	2
PCB14	3+	3+	3+	3, 2+	3	3
PCB41	3-	3-	3-	3+	3+	3+
PCB 42	3+	3+	3+	3, 2+	3, 2+	3
PCB30	4	4, 3+	4	3+	3+	3+
PCB15	3-	3-	3-	3-	3+	3+
PBC1	3-, 2	3+	3+	3	3	3
PCB68	3+	3+	3+	3, 2	3, 2+	3
PCB36	3+	3+	3+	3+	3+	3+
PCB2	;1+	;1+	;1+	;1+	2+	2+
PCB3	3-	3	3-	3+	3+	3+
PCB69	; 1+	;1, 2	;1+	2, 3+	2+	2+
PCB53	3+	3, 2+	3+	3, 2+	3+	3+
PCB13	3-, 2	3-	3-	2, 3+	3+	3+
PCB4	3+	3, 2+	3+	3+	3+	3+
PCB5	2+	2-	2+	2+	2	2+
PCB60	2+	2, 2+	2+	2, 3+	2+	2+
PCB27	3+	3+, 2	3+	3, 2+	3, 2+	3
PCB28	3+	3, 3-	3+	2, 3+	3+	3+
PCB6	3-	3, 2+	3	3	3	3
PCB45	3+	3+	3+	3+	3+	3+
PCB73	3, 2+	3	3	3+	3	3+
PCB46	3+	3+, 2	3+	3, 2+	3, 2+	3
PCB7	4	4	4	3+	3+	3+



Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
PCB10	X	3+	X	4	3+	4
PCB8	3-	3, 2+	3-	3, 2+	3+	3+
PCB9	3+	3	3+	2, 3+	3+	3+
PCB11	2+	2+	2+	2	2+	2+
PCB12	3+	3+	3+	3	3+	3+
PCB37	2+	2+	2+	2+	2, 3+	2+
PCB14	3+	3+	3+	32+	3+	3+
PCB41	3-	3-	3-	3+	X	3+
PCB42	3+	3+	3+	3, 2+	3+	3+
PCB30	4	4	4	3+	3+	3+
PCB15	3-	3-	3-	3	3	3
PCB16	2+	2+	2+	;1+	2+	2+
PCB17	2+	2, 1+	2+	;1+	;1	;1+
PCB47	4	3+	4	3+	3+	3+
PCB72	3-	3-	3-	3-	3+	3+
PCB18	3+	3+	3+	3+	3, 2+	3+
PCB19	3+	3+	3+	3+	3, 2+	3+
PCB20	2+	2+	2+	2+	2+	2+
PCB21	3+	4	4	4	3+	4
PCB22	3+	3, 2+	3+	3, 2+	3	3
PCB23	3+	3, 2+	3+	3+	3+	3+
PCB24	3-	3-	3-	3	3	3
PCB25	3-	3-, 2	3-	3, 2+	3	3
PCB26	3-	3-, 2	3-	3-	3, 2+	3
PCB29	;1+	2+	2+	2+	2+	2+
PCB31	3+	3+	3+	3	3	3
PCB32	4	4	4	3+	3+	3+
PCB33	3-	3, 2+	3	3, 2	3, 2+	3
PCB34	3, 2+	3+	3+	3, 2+	3	3
PCB35	4	4	4	3+	3+	3+
PCB52	2+	2+	2+	2+	;1+	2+
PCB38	3, 2	3+	3+	3	3	3
PCB39	2+	2+	2+	2+	2+	2+
PCB40	3+	3-	3+	3+	3-	3+
PCB43	3, 2+	3+	3+	3	3	3
PCB44	3+	3+	3+	3	3	3
PCB48	3+	3-	3+	3-	3	3
PCB49	3+	3+	3+	3+	3+	3+
PCB50	4	4	4	3+	3+	3+
PCB51	3	3, 2	3	3, 2+	3+	3+
PCB54	2+	2+	2+	;1	0	;1
PCB55	2	2+	2+	2	2	2
PCB56	2+	2+	2+	3, 2+	2+	2+
PCB57	;1+	;1, 2+	;1+	;1+	2+	2+
PCB58	2+	2+	2+	2+	2+	2+
PCB59	2+	2	2+	2+	2	2+
PCB61	2	2+	2+	2	2	2
PCB62	2	2	2	2	;1+	2
PCB63	3+	3+	3+	3+	3+	3+
PCB64	3+	3, 2+	3+	3	3+	3+

Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
PCB65	;1+	2	2	2	2	2
PCB66	3, 2	3	3	3+	3, 2+	3+
PCB67	2	2	2	2	2+	2+
PCB70	3, 2+	3+	X	3	3	3
PCB71	;1	2-	2-	;1+	2-	2-
PCB74	2+	2+	2+	2+	2+	2+
PCB75	;1	;1	;1	2+	2	2+
PCB76	3+	3+	3+	3	3, 2+	3
PCB77	3+	3+	3+	3	3+	3+
PCB78	2	2	2	2+	2+	2+
PCB79	2+	21	2+	2+	2+	2+
KSL1	3, 2+	3+	3+	3	3	3
KSL4	3-	3-	3-	3+	3+	3+
KSL2	3+	3, 2	3+	3, 2+	3	3
KSL7	;1	;1+	;1+	2+	;1+	2+
KSL17	2+	2+	2+	;1+	;1	;1+
KSL18	2+	2+	2+	2	2	2
KSL10	3+	3+	3+	3	3	3
KSL3	2+	2+	2+	2+	2+	2+
KSL5	2+	2+	2+	2+	;1	2+
KSL6	1	1	1	2+	2+	2+
KSL8	1	1, 2+	1	1, 2	1	1
KSL9	3, 2	3, 2	3	3	3, 2	3
KSL11	3, 2	3+	3+	3+	3+	3+
KSL12	4, 3+	3+	4	4	4, 3+	4
KSL13	2	2+	2+	2+	2+	2+
KSL14	;1, 2	2, 1	;1	;1	;1, 2+	;1
KSL15	;1	;1	;1	;1	;1	;1
KSL16	;1	;1	;1	;1	;1, 2+	;1
KSL19	2+	2+	2+	2+	2+	2+
KSL20	3+	4	4	4	4	4
KSL21	3, 2	3	3+	3+	3, 3+	3+
Crim	3	3	3	2+	3-	3-
Chris	2+	2+	2+	2	2+	2+
Polk	3+	3	3+	3	3	3
Era	2+	2	2+	2+	2+	2+
Fletcher	3, 2+	3+	3+	3+	3+	3+
Kitt	3	3	3	3	3	3
Anahuac	2	2+	2+	2+	2	2+
Marshall	3+	4	4	4	4	4
Wheaton	3, 2	3+	3+	3+	3+	3+
Minnpro	3	3+	3+	3+	3	3+
2375	3+	3+	3+	3+	3+	3+
Verde	2+	2	2+	2, 2+	2+	2
Ciano F67	2, 1	2+	2+	;1+	2+	2+
Newthatcher	3+	3+	3+	3+	3+	3+
Timstein	3	3+	3+	31	-	3
Borah	2+	2+	;1+	;1+	1+	;1+
Norm	2	2+	2+	2+	2+	2+

Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
Shield	;1	;1	;1	;1	0	;1
A99ar	3, 2+	3	3	3+	3+	3+
Mcvey	3, 2	3+	3+	3, 2+	3	3
Justin	3+	3+	3+	3	3	3
Mida	3+	4	4	3+	3+	3+
Wared	3, 2	3	3	3+	3+	3+
Yaqui 50	2+	2+	2+	2	2	2
Ceres	2+	2+	2+	2, 3+	2	2
MG 07762	2	2	2	2+	2+	2+
MG 07782	3, 2	3	3	3+	3, 2	3+
MG 07793	3+	3+	3+	2, 3+	3-	3-
Sindi 1	3+	4	4	3+	3+	3+
Sindi 2	4	4	4	3+	4	4
Sindi 3	3, 2	3	3	3+	3	3+
Sindi 5	3	3	3	3	3	3
Sindi 6	4	4	4	4	4	4
Sindi 7	3+	3, 2	3+	3+	3, 2	3+
Sindi 8	3+	4	4	3+	3+	3+
Laketch	3+	3, 2	3	3	3	3
K-6106-8	;1+	0	;1+	2+	2+	2+
Enkoy	2	2	2	2+	2+	2+
K6290 Bulk	2+	2+	2+	;1+	2+	2+
K6295-4A	3	3	3	3+	3	3+
Pavon 76	3+	3+	3+	3	3+	3+
Batu	3-	3	3	3-	3-	3-
Dashen	3+	3+	3+	2	2	2
HAR-407	2, 3+	2	2	;1	2	2
KKBB	3+	3+	3+	3, 2+	3	3
Gara	3+, 2	3+	3+	2, 3	2+	2+
Mitike	2+	2, 3	2+	2, 3	2, 3	2
Galema	3+	3+	3+	3, 2+	2+	2+
Kubsa	3, 2	3	3	3+	3	3+
Wabe	3, 2	3, 2	3	3+	3, 2	3+
Abola	3+	3+	3+	3, 2+	3	3+
Megal	3+	3, 2	3+	2+	2+	2+
Tusie	3+	3+	3+	3+	3+	3+
Katar	4	4	4	4	3+	3+
MG 07759	3, 2	3+	3+	2	2	2
MG 07768	2+	2	2+	2	2+	2+
MG 07795	3+	3+	3+	2, 3+	2+	2+
Sindi 4	3+	3, 2	3+	3+	3+	3+
Inia66	3, 2	3+	3+	3	3+	3+
Enkoy	3+	3	3+	3, 2+	2+	2+
Romany	2	2	2	2+	2+	2+
Sonara 63	3+	3+	3+	3	3	3
Shina	4	4	4	3+	3+	3+
Tura	3	3, 2	3	3+	3+	3+
Hawi	4	3+	4	3+	3+	3+
Madda walabu	3+	3+	3+	2+	2+	2+
Sirbo	3+	3, 2+	3+	3+	3, 2	3+
Bobicho	3+	4	4	3	3, 2+	3

Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
Dereseqlen	3-	3, 2	3	3, 2+	2+	2+
ET-12-D4	3+	3-	3+	3, 2+	3	3
Digelu	3+	3, 2	3+	2	2	2
Meraro	3+	3, 2+	3+	2+	2, 3+	2+
Jiru	3+	3+	3+	3	3	3
Bobin	3-	3-	3-	3+	3+	3+
Reward	32	3+	3+	3	3+	3+
Olescens dwarf	3+	3+	3+	3+	3+	3+
Bonanza	2	2	2	2+	2	2+
Exchange	4	4	4	3	3+	3+
Wis. 245	2	2	2	2+	2+	2+
1010AM 2(L)	2+	2+	2+	;1+	2+	2+
1076.D.7	3+	3, 2	3+	3	3-	3
1200.M	2+	2+	2+	2+	2+	2+
291	3-	3, 2	3	3	3, 2	3
1061.K.1	2+	2+	2+	;1+	2+	2+
338 AA 1	3+	3, 2	3+	3, 2+	3+	3+
690F4	3+	3+	3+	3+	3+	3+
SEL.D.1	3+	3+	3+	3+	3+	3+
1010 F3 SEL.4	3+	3+	3+	3+	3+	3+
AfricaMayo	2+	2+	2+	1	;1	;1
B F2 36	3	3+	3+	3+	3+	3+
Bailey	2+	2+	2+	;1+	2+	2+
Beacon-Ken	2+	3, 2	2+	2+	2+	2+
Bonny	3	3	3	3	3, 2+	3
Bounty	2+	2+	2+	2	2	2
Brewster	3, 2	3	3	3+	3, 2+	3+
Catcher	3, 2	3, 2	3	3	3	3
Fanfare	3	3+	3+	3	3-	3
Fronthatch	2	;1+	2	2	2	2
Gabrino	2+	2+	2+	;1	2+	2+
Goblet	3, 2+	3	3	3, 2+	3	3
H 441	;1	2	;1	;1	;1	;1
Kentana Yaqui	2+	;1+	2+	2+	;1	2+
Kenya 155	3, 2	3, 2	3	3, 2	3+	3+
Kenya-184-P	3, 2	3, 2	3	3+	3+	3+
Kenya-360-H	3	2, 3	3	3+	3	3+
Kenya -362-B-1	3, 2+	2+	2+	;1+	2+	2+
Kenya 7	3+	3+	3+	3+	3+	3+
1010 F3 SEL.7	2	2+	2+	2	2, 2+	2
1012 B.1. (L)	2	2	2	2+	2+	2+
1016 P.1	;1	2+	2+	2+	2	2+
Kenya Mbweha	3	3, 2+	3	3+	3, 2+	3+
Kenya page	;1	0	;1	2, 3	2+	2+
Kenya Ploughman	3, 2	3, 2+	3	3-	3+	3+
Kenya Sungura	3+	3	3+	3, 2+	3+	3+

Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
Kenya-122	3+	4	4	3+	3+	3+
Kenya-131	3+	3	3+	3, 2	3-	3
Kenya-294-B-2 A-3	3, 2	3+	3+	3+	3, 2+	3+
Kenya-318.O.3B.2	3	3	3	3-	3	3
Kenya-318-AJ-4	3, 2+	3	3	3	3, 2+	3
Lenana	;1	2+	2+	2, 3	2+	2+
Menco	2	2	2	2-	2	2
Kenya-5	3	3, 2	3	3, 2+	3+	3+
Mentor	2+	2+	2+	2+	2+	2+
Morris	;1+	;1+	;1+	;1+	2+	2+
P. Walker	2+	2+	2+	;1+	2	2
Pitcher	2	2+	2+	2+	2	2+
Primex	;1	;1	;1	;1+	2+	2+
R 64	2+	2+	2+	2	3, 2+	2
Regent	3	3, 2+	3	3	3, 2+	3
Reliance	4	4	4	3+	3, 2+	3+
Reliance 261M	3+	3+	3+	3+	4	4
RFN	3, 2+	3	3	3+	3	3+
Rhodesia	3	3, 2	3	3	3	3
Santa	3, 2	3+	3+	3	3+	3+
Tama	;1+	;1	;1+	2+	2, 3+	2+
Token- Ken	;1+	;1+	;1+	2+	;1, 2+	2+
Trophy	;1	;1+	;1+	2+	2+	2+
1016.P.2	3-	3-	3-	3, 2+	3	3
1061.K.4	3-	3, 2	3-	3+	3, 2	3+
Egyptian Na 95	3+	2, 3+	3+	3	3	3
1010 F3 SEL. 13 C	2+	3, 2+	2+	2+	2+	2+
688 F4 SEL 3	2	2, 3-	2	2+	2+	2+
Equator	3, 2	3	3	23+	3	3
Salmayo	3-	3-	3-	3, 2+	3	3
Aggia	3+	3+	3+	3+	3+	3+
Kenya 6820	2+	2+	2+	;1+	2+	2+
RL 1377	3+	3	3+	3, 2+	3+	3+
Fury	3, 2+	3+	3+	3+	3+	3+
Gem	;1+	;1+	;1+	2+	2+	2+
Bonito	;1	;1	;1	2+	2+	2+
PW Thatcher	2+	3, 2+	2+	2+	2+	2+
Kenya Nyangumi	3+	3+	3+	3	3	3
Kenya Tembo	3, 2	3, 2	3	3+	3+	3+
Kenya Fahari	3	3, 2	3	3	3, 2+	3
Breadwheat 23	2+	2+	2+	2+	2+	2+
Kenya Yombi	3-	3-	3-	3+	3+	3+
Kenya Heroe	3	3, 2	3	3+	3+	3+
B192	3+	4	4	3+	3+	3+
Kenya 117C	3+	3+	3+	3, 2+	3	3

Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
Kruger	3+	3, 2+	3+	3+	3+	3+
Kenya Kongon	3, 2+	2, 3+	3	3	2, 3+	3
KenyaSwara	;1	;1	;1	0	;1	;1
Kenya Paka	2	2	2	2	2	2
Kenya Zabadi	2+	2	2+	2+	2+	2+
Kenya Ngiri	2+	2+	2+	2+	2	2+
Duma	3+	3+	3+	3+	3, 2+	3+
Mbega	3+	3, 2+	3+	3, 2+	3	3
Mbuni	3+	3, 2+	3+	3	3-	3
Ngamia	2+	2+	2+	3, 2+	2+	2+
Chози	3, 2	3	3	3+	3+	3+
Njoro BW II	3	3, 2+	3	3	3+	3+
Kenya-Ibis	3+	3+	3+	3	3	3
Kenya Popo	3, 2	3	3	3-	3-	3-
BTC Barce	3+	3+	3+	3+	3+	3+
FL I Kenya 9	2+	;2+	2+	2+	2	2
Quamy	3, 2	3-	3	3	3, 2+	3
Kenya Nyati	2+	;1+	;1+	2+	2+	2+
Kenya Kanga	3	3-	3	3+	3+	3+
Kenya Tumbili	3-	3, 2+	3	3	3, 2+	3
8952	3, 2+	3+	3+	3	3	3
Kenya Chiriku	3, 2+	3-	3-	3+	3+	3+
Pasa	3+	3+	3+	3	3	3
Kenya Paa	2+	2+	2+	2-	2	2
Kenya Kifaru	3, 2	3	3	3	3, 2+	3
321 BT 11 B1	2	2+	2+	2	2+	2+
BW21	3+	3, 2+	3+	3+	3	3+
Kenya Farmer	3, 2	3, 3+	3	3+	3, 2	3+
Kenya Governor	2	;1, 2+	2	;1	2	2
Kenya Nungu	2+	2	2	2+	2+	2+
Kenya Plume	3, 2+	3+	3+	3, 2+	3+	3+
Kenya Nyoka	2+	2, 3-	2+	3+	2+	2+
Kenya Standard	3	3, 2+	3	3+	3, 2+	3+
Kenya-58	3+	3+	3+	3, 2+	3	3
Pewter	3, 2	3, 2+	3	3, 2+	3	3
Kenya 8	3, 2	3, 2+	3	3, 2	3	3
Kenya Cheetah	3, 2+	3, 2+	3	3	3	3
Kenya Jay	2+	2, 3	2+	;1+	2+	2+
Kenya Kudu	2+	2+	2+	;1	;1	;1
Kenya Leopard	3, 2+	3	3	3	3	3
Kenya B-256-G	2+	3, 2+	3	2, 3-	2	2

Genotypes	TTKSK			TTKST		
	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score	1 <sup>st</sup> score	2 <sup>nd</sup> score	Most severe score
Bonza 63	3+	3+	3+	3	3	3
Bonza	2+	2, 2+	2+	2, 3	2+	2+
Frocor 2328	3-	3-	3-	3, 2+	3+	3+
Gandum-i-Fasai	3, 2+	3	3	3+	3+	3+
NP761	4	4, 3+	4	X	3+	X
Kalyanosona	3+	4	4	3+	3+	3+
Bluebird	2+, ;1	2+	2+	;1+	2+	2+
Lerma rojo	3+	3+	3+	3, 2+	3+	3+
Kentana 48	3, 2+	3+	3+	3	3	3
Penjamo 62	2, 2+	3-	3-	2+	3	3
Red Egyptian	3+	3+	3+	3, 2+	3	3
Mentana	3+	3+	3+	3+	3	3+
Gradenero	3	3, 2+	3	3	3	3
Yaktana 54A	3-	3-	3-	3+	3+	3+
Zaragoza 75	;1, 2+	2+	2+	;1+	2+	2+
Tobari 66	3+	3+	3+	3, 2+	3+	3+
Supremo	3-	3, 2+	3-	3, 2+	3	3
Cocoraque 75	3+	3+	3+	3+	3+	3+
Bobwhite	3-	3-	3-	3-	3-	3-
Bage	3, 2+	3+	3+	3, 2+	3	3
Impala	3+	3+	3+	3, 2+	3	3
Frontana	2+	2+	2+	2+	2+	2+
Marquis	2+	2+	2+	2+	;1+	2+
Hard Federation	3+	3, 2+	3+	3, 2+	3	3
Hope	3, 2+	3+	3+	3-	3	3
Marquillo	2+	2+	2+	2+	2+	2+
Thatcher	;1	2+	2+	2+	2+	2+
Federation	3+	3+	3+	3, 2+	3	3
Gabo	3-	3-	3-	3	3	3
Giza 155	2+	2+	2+	2+	2+	2+
II-50-17	;1	2+	2+	;1	2	2
MN72131	2+	3+	3+	3, 2+	3	3
Waldron	X	3+	3+	4	3+	3+
Angus	4	4	4	X	4	X
Beltista	3	3	3	3+	3+	3+
HRS 55	3	3	3	3, 2+	3+	3+
Bale	3, 2	3-	3	3+	3-	3+
Vance	2	3, 2	3	2	2+	2+
869	3, 2+	3+	3+	X	3, 2+	X
Lahota	2+	2+	2+	;1+	;1+	;1+

;= Presence of hypersensitive necrotic flecks with no uredinia, 0= no signs of infection on the plant, 1= small uredinia surrounded by necrosis, 2= small to medium sized uredinia surrounded by necrosis, 3= medium sized uredinia without necrosis, 4= large uredinia without necrosis, X= distribution of mixed type of reaction all over the leaf surface, positive (+) and negative sign (-) = larger and smaller uredinia, respectively than the normal size.