EFFECTIVENESS OF ADULT AND SEEDLING RESISTANCE IN MANAGEMENT OF STEM RUST (*Puccinia graminis* f.sp. *tritici*) OF WHEAT (*Triticum aestivum* L.) IN CIMMYT LINES

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A Thesis Submitted to the Graduate School in Partial Fulfilment for the Requirements of the Award of Master of Science Degree in Plant Breeding of Egerton University

EGERTON UNIVERSITY

OCTOBER, 2018

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been previously submitted or presented for examination in this or any other University for any degree.

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Recommendation

This thesis has been submitted for the degree of Master of Science in Plant Breeding with our approval as the supervisors.

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DEDICATION

This thesis is dedicated to my beloved parents Grace Akinyi Ndiege and Meshack Otieno Odemba, my siblings: Kevin Billy Ochieng', Everlyne Achieng, and Erick Omondi and my husband Albert Orwa Akuno to whom I attribute my success. They have been a pillar in my life and a constant source of encouragement in my academic and social life.

ACKNOWLEDGEMENT

First and for most, I want to thank the Almighty God for giving me the courage, endurance and power for the completion of this study. I would also like to thank all staffs and students of Egerton University for their support, advice and encouragement. My appreciation too to my supervisors, Prof. J. Owuoche and Prof. M. Okiror, and to Ms R. Wanyera for their technical support and constructive criticisms. I would like to express my appreciation to CIMMYT for providing seeds and KALRO, Njoro, for providing facilities to undertake this study and to KALRO staff, particularly those in the Pathology Section for technical assistance.

ABSTRACT

Stem rust of wheat (*Triticum aestivum L.*) caused by *Puccinia graminis* f.sp. *tritici* had been controlled globally through the use of resistant varieties. However, the emergence of a new and more virulent race *Ug99* (designated *TTKSK*) and its variants reversed these gains. The objectives of this study therefore were: to assess the progress in using adult plant resistance (APR) in controlling stem rust in CIMMYT wheat lines and to evaluate CIMMYT wheat lines for seedling and adult plant resistance to stem rust races *TTKSK*, *TTKST*, *TTTSK* and *TTKTK*. Both field and greenhouse experiments were conducted at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro (0º 20' S, 35º 56' E). The field experiment involved testing 744 wheat lines originating from 1st, 3rd, 5th, 7th and 9th CIMMYT stem rust resistance screening nurseries and four checks. The experiment was laid in an *Alpha lattice* design. Seedling and adult resistance to the stem rust races *TTKST*, *TTKSK*, *TTKTK* and *TTTSK* were determined in the greenhouse, where lines were inoculated artificially. Progress in using APR was assessed using disease severity data from the nurseries between years 2005 and 2016. The proportion of lines which showed severities of ≤30% were higher across all the nurseries in all years of evaluation compared to those that had severities of ≥35%. The number of lines which exhibited low severities (≤30%) increased in the first, third and fifth nurseries in the first three years of evaluation but later reduced in 2015. In 2016, these proportions increased again. In the seventh and ninth nurseries, the proportions declined in 2015 but later increased in 2016 from the initial proportion. The number of lines which revealed high disease severities (≥35%) kept on reducing and increasing with time from the first to the ninth nurseries but these proportions were still lower than those for the lines which showed severities of ≤30%. Out of the 39 lines evaluated for seedling resistance, only three of them showed high infection type (IT) of 3 with race *TTKST*, one showed IT 3 with race *TTKTK*, four showed IT 3 with race *TTKSK* and two showed IT 3 with race *TTTSK,* the rest showed infection types of between 0 and 2 which is considered low. For APR, only 0.13% of the lines showed a severity of ≤5% to race *TTKST* while 99.87% showed a severity of ≥10%. To race *TTKTK*, 43.59% of the lines revealed a severity of ≤5% while 56.41% showed a severity of ≥10%. To race *TTKSK,* 43.59% of the lines revealed a severity of ≤5% while 56.41% showed a severity of ≥10% and to race *TTTSK,* 46.15% of the lines exhibited a severity of ≤5% while 53.85% showed a severity of ≥10%. APR is a good breeding strategy as 75.08% of the lines showed severity of ≤30%. Lines *SRG7*, *SRG13*, *SRG24* and *SRG35* showed the lowest final disease severities, infection types and AUDPC in all the four races, therefore can be used as sources of new resistant genes and also be released as new varieties for farmers to adopt.

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LIST OF ABBREVIATIONS AND ACRONYMS

APR- Adult plant resistance

AUDPC - Area under disease progress curve

CAN- Calcium ammonium nitrate

CIMMYT - International maize and wheat improvement centre

DAP- Diammonium phosphate

FAO - Food and agriculture organization

KALRO- Kenya Agricultural and Livestock Research Organization

IT- Infection Type

Pgt- Puccinia graminis f.sp. *tritici*

RCBD- Randomized complete block design

SAS- Statistical Analysis Software

SRG- Stem rust gain

SRRSN- Stem rust resistance screening nurseries

CHAPTER ONE
INTRODUCTION

1.1 Background information

Stem rust of wheat caused by *Puccinia graminis f.sp. tritici* (*pgt*) Eriks and Henning is one of the three rusts that infects wheat. The causal pathogen is a fungus of the division Basidiomycota, class Pucciniomycetes and order Pucciniales (Kirk *et al*., 2001). It is capable of causing immense damage to the wheat crop worldwide. Stem rust occurs in warm and moist environments that are characteristics of most wheat growing areas in Kenya, however, the pathogen is virulent on some varieties even in high altitudes (Wanyera *et al*., 2006). Wheat is grown in many agro-ecological zones with diverse planting dates and cropping seasons, this ensures significant amount of airborne inocula that initiate early epidemics (Wanyera *et al*., 2009). Stem rust has largely been controlled by the deployment of resistance genes, designated as *Sr* genes to various wheat cultivars grown in Kenya and other countries (Ellis *et al*., 2014). However, in 1999, the emergence of a new race of stem rust from Uganda named *Ug99* (*TTKSK*) (Pretorious *et al*., 2000) rendered previously resistant cultivars susceptible. This race has since spread to several countries including Kenya (2001); Ethiopia (2003); Sudan (2006); Yemen (2006) and Iran (2007) (Singh *et al*., 2008; Nazari *et al*., 2009). Its identification in Yemen confirmed that this race had crossed to the Middle East and Asia. Since 1999, thirteen variants under the *Ug99* race lineage have been identified in 13 other countries in Northern and Eastern African and in the Middle East (Singh *et al*., 2011; Singh *et al*., 2015; Patpour *et al*., 2016).

The effectiveness of the previously deployed resistance genes has been short lived due to the rapid emergence of more virulent variants of the pathogen following mutations (Valkuon, 2001). This is because most of these genes were race-specific, and qualitative resistance is usually short-lived, owing to frequent changes in the pathogen population. Due to the fast break down of commercial varieties carrying such resistance genes, search for and characterization of diverse sources of resistance is continually needed to replace the defeated genes. Stem rust resistance genes (*Sr*) are numerically designated in wheat genetics catalogue (McIntosh *et al*., 2011) however, the race *TTKSK* is virulence to most of them (Singh *et al*., 2006). The emergence and rapid evolution of *TTKSK* into several other variants is further proof that the pathogen is virulent to the existing stem rust resistance genes such as *Sr31, Sr24, Sr36*, and *Sr9h* (Jin *et al*., 2009; Pretorious *et al*., 2012; Rouse *et al*., 2014). Consequently, it is essential to broaden the genetic base of local cultivars by incorporating novel resistance genes from both alien sources and wheat progenitors (Valkuon, 2001). Resistance genes last an average of five

to six years before they succumb to new races (Kilpatrick, 1975; Wellings and McIntosh, 1990; Jeffrey *et al*., 2014). This problem of newly emerged races of pathogens has led to the adoption of alternative forms of resistance that are durable (Singh *et al*., 2000). The alternative option is to deploy adult plant resistance (APR) genes that confer quantitative resistance, it involves accumulation of minor resistance genes in the same background resulting into more durable resistant cultivars (Robinson, 1980; Singh *et al*., 2000). Since adult plant resistance (APR) conditions non-specific resistance, it is usually characterized by low infection frequencies, reduced size of urediniospores, and diminished urediniospore production (Stuthman *et al*., 2007).

Wheat cultivars with slow rusting are characteristically moderately to highly resistant to most of races at the adult stage in the field but susceptible at the seedling stage (Singh *et al*., 2000). For example, *Sr2* derived from the variety *Hope* in combination with other genes provided the foundation for durable resistance to stem rust in CIMMYT germplasm (Roelfs, 1988; Van-Ginkel and Rajaram, 1992; Singh *et al*., 2008). In addition, *Sr36* derived from *Triticum timopheevii* that confers a slow rusting resistance due to low receptivity appears to offer useful protection in combination with genes for specific resistance (Rowell, 1982; McIntosh, 1992; Rubiales *et al*., 2000).

How effective and durable any heritable trait is in a cultivar is gauged by measuring the genetic progress of such a trait. Therefore, estimates of genetic progress can be used as one of the variables to analyse the performance of any genetic breeding strategy, for instance the progress in using APR. Genetic progress together with heritability estimates offers the most effective criteria for identifying breeding methods that are most effective and useful in improving character of interest, predicting response to selection and determining the relative importance of genetic effects (Larik *et al*., 2000; Khan *et al*., 2008; Laghari, *et al*, 2010). Response to selection shows the degree of gain obtained under a given selection pressure for the considered trait and predicts response to selection in diverse environments (Ahmed *et al*., 2007).

1.2 Statement of the problem

Genetic gain in plant breeding is an important tool in analysing the performance of breeding methods. Stem rust has gained much attention due to the drastic reduction it causes on global wheat production. The impact of infection on wheat worldwide are severe yield losses and reduction in grain quality. East Africa has been the origin of new physiological races for stem rust. Because of the evolution of new races, the major stem rust resistance genes previously deployed into wheat varieties planted by Kenyan farmers such as *Kenya Hawk,*

Kenya Wren, *Kenya Tae, Robin, Duma, Kwale, Chozi, Heroe, Njoro II, KS Mwamba* and *Mbuni* have succumbed to the *Ug99* and its variants due to the race-specific breeding strategy that has been used in the past. Therefore, in order to reduce impact of emerging new virulent races, CIMMYT scientists adopted a breeding method involving combining two or more minor stem rust resistance genes into a cultivar, called adult plant resistance (APR). The APR breeding method supposedly ensures that cultivars remain resistant over a long period (more than 5 years) of time compared to those cultivars bearing major genes. This has been made possible through shuttle breeding between Mexico and Kenya ensuring that cultivars selected are stable across a wide range of environmental conditions. Since CIMMYT scientists started deploying and considering minor genes in their selection for resistance to stem rust, its effectiveness and efficiency as to whether it is leading to development of resistant varieties is still not known as some varieties released bearing APR genes such as *Robin* have succumbed to the new races. Therefore, this study examined the versatility, dependence and effectiveness of using APR in development of stem rust resistant varieties of wheat.

1.3 Objectives

1.3.1 Broad objective

To contribute towards wheat production in Kenya through identification of resistant cultivars that farmers can grow to obtain better yields thereby, enhancing food security in Kenya.

1.3.2 Specific objectives

i) To assess the progress in breeding for adult plant resistance to stem rust in CIMMYT wheat lines.

ii) To evaluate CIMMYT wheat lines for seedling resistance to stem rust races *TTKSK*, *TTKST*, *TTTSK* and *TTKTK*.

iii) To evaluate CIMMYT wheat lines for adult plant resistance to stem rust races *TTKSK*, *TTKST*, *TTTSK* and *TTKTK*.

1.4 Hypotheses

i) There is no progress in using adult plant resistance to control stem rust in CIMMYT wheat lines.

ii) CIMMYT wheat lines lack seedling resistance to stem rust races *TTKSK*, *TTKST*, *TTTST* and *TTKTK*.

iii) CIMMYT wheat lines do not bear Adult plant resistance to stem rust races *TTKSK*, *TTKST*, *TTTSK* and *TTKTK*.

1.5 Justification

Wheat is the second most important food crop in the world after maize (*Zea mays*), and its production considered important for global food security (FAO, 2006). About 50 million hectares of wheat is cultivated worldwide annually, providing food to a population of about one billion people (Singh *et al*., 2011). In the past decades, wheat productivity has risen by 3.6 % per annum in developing countries (Dixon *et al*., 2009). This increase in yield was due to the increase in genetic potential, which is a result of international breeding efforts (FAO, 2006; Ortiz *et al*., 2008). However, between 1996 and 2005, CIMMYT found that the yield potential in spring wheat had slowed down to around 0.5 % per year (Fischer, 2007), and in Europe, its rate even stagnated (Brisson *et al*., 2010) due to the losses caused by stem rust.

Wheat consumption in Kenya stands at about 900,000 tonnes annually. This cannot be sustained by local production which has stagnated at about 330,000 tonnes. Since consumption is growing at an average annual rate of 4%, the import gap is similarly widening leading to an expenditure by government of about US$ 0.133 billion on wheat imports. A combination of changing diets and human population growth will result in increased demand for agricultural production of 60% to 100% between the years 2005 and 2050 (Alexandratos and Bruinsman, 2012). Increased demand for cereal products could even become high considering the substantial losses in wheat caused by stem rust globally (Oerke, 2006). According to Iqbal *et al*. (2010) about 80 to 90% of the global wheat cultivars have succumbed to the stem rust disease. Stem rust is capable of turning a healthy looking crop into a tangle of black stems and shrivelled grains at harvest time leading to yield losses exceeding 70% (Singh *et al*., 2008). Mitigating against this danger will require a refocus on strategies in the management of this menace (Bolton *et al*., 2008).

The most economical and environmentally friendly method to control rust is through the use of genetic resistance. Since rust pathogens are virulent to known resistance genes (Kolmer *et al*., 2009), there is need to identify, characterize and deploy new sources of resistance genes. Resistance to stem rust in wheat is conferred by one or more seedling genes or by genes conferring adult plant resistance. Previously resistant varieties have been rendered susceptible due to their narrow genetic base (race specific) (Beteselassie *et al*., 2007) leading to adoption of new breeding strategy called APR which is quantitative and offer a broad genetic base for resistance to stem rust and is supposedly more durable than seedling resistance. Therefore, there is need to deploy such genes into wheat varieties in order to achieve durable rust resistance.

CHAPTER TWO
LITERATURE REVIEW

2.1 History and genetics of wheat

Wheat (*Triticum aestivum* L.) is the universal cereal of old world agriculture and the foremost crop plant in the world, followed by rice (*Oryza sativa* L.) and maize (*Zea mays* L.) (Zohary and Hopf, 2000; Gustafson *et al*., 2009). Wheat belongs to the family Poaceae (grasses) which evolved 50-70 million years ago (Huang *et al*., 2002) and sub-family pooideae (Inda *et al*., 2008). The wheat genome comprises of three ploidy levels; diploid (2n=2*x*=14, AA); tetraploid (2n=2*x*=28, AABB); and hexaploid, (AABBDD) (Hancock, 2004; Pumphrey *et al*., 2009). Wild diploid wheat (*T. urartu*, 2n=2*x*=14, A^uA^u) hybridized with the B genome ancestor that is the closest relative of goat grass (*Aegilops speltoides*, 2n=4*x*=14, SS) 300,000-500,000 years before present (BP) (Dvorak and Akhunov, 2005) to produce wild emmer wheat (*T. dicoccoides*, 2n=4*x*=28, A^uA^uBB). Subconscious selection gradually created a cultivated emmer (*T. dicoccum*, 2n=4*x*=28, A^uA^uBB) that spontaneously hybridized with another goat grass (*Ae. tauschii*, 2n=4*x*=14, DD) around 9,000 BP to produce an early spelta (*T. spelta*, 2n=6*x*=42, A^uA^uBBDD) (Matsuoka and Nasuda, 2004; Giles and Brown, 2006; Dubcovsky and Dvorak, 2007; Haudry *et al*., 2007; Fu and Somers, 2009).

About 8,500 years before present (BP) natural mutation changed the ears of both emmer and spelta to an easily threshed type that later evolved into the free-threshing ears of durum (*T. durum*) and bread wheat. The free forms of wheat arose due to the dominant mutant gene at the *Q* locus located on chromosome 5A which modified the effects of recessive mutations at the *Tg* (tenacious glume) locus (Simons *et al*., 2006). Apparently, the sources of cultivated wheat ancestry are complicated by multiple factors including gene flow from wild cereals. Modern wheat cultivars usually refer to two species: the hexaploid bread wheat, (*T. aestivum* 2n=6*x*=42, A^uA^uBBDD), and the tetraploid, hard or durum-type wheat, (*T. durum* 2n=4*x*=28, A^uA^uBB) (Belderok *et al*., 2000). Earliest cultivated forms *einkorn* (diploid, AA) and *emmer* (tetraploid, AABB) wheat and their genetic relationships indicate that they originated from the South-Eastern part of Turkey (Dubcovsky and Dvorak, 2007). Cultivation spread to Near East by about 9,000 years ago when hexaploid bread wheat made its first appearance (Feldman, 2001).

2.2 Wheat production worldwide

Wheat is globally grown on about 218 million hectares producing about 750 million tonnes of grain annually (valued at US$ 179 billion) with 308 million tonnes produced by developing countries on 116 million hectares (FAO, 2017). The largest wheat producing countries are China, India, the United States and Russia, respectively. These countries account

for nearly 50% of the world wheat production. The remainder of the production is spread throughout the rest of wheat producing countries worldwide. In Kenya, wheat is grown on 160, 000 hectares, previously large-scale accounted for 75% of the area planted to wheat and 83% of the production but currently small scale farmers have taken up wheat production on smaller farms (Nyangito *et al*., 2002). The low production is due to limited access to mechanization, low market preferences, poor infrastructure together with increased urbanization and decreased public sector investment in wheat production (Maredia and Eicher, 1995; Reynolds and Tuberosa, 2008). In addition, the current climate changes and the natural resource degradation have led to limited water resources, biotic factors such as Russian wheat aphid (*Diuraphis noxia)*, Fusarium head blight (*Fusarium graminearum*), Septoria (*Septoria tritici*) and the cereal rusts further limits wheat production (Negassa *et al*., 2012). Wheat production cannot meet the demand, therefore it has to be increased at a rate of 2% per annum so as to meet this ever rising demand (Gupta *et al*., 2008).

2.3 The rusts of wheat

Leaf rust (*Puccinia triticina)*, yellow rust (*Puccinia striiformis*) and stem rust (*Puccinia graminis*) are the three most important rust diseases of wheat that occur worldwide. Of these three, leaf rust is the most common and widely distributed occurring in many regions more regularly than stem rust and stripe rust (Kolmer, 2005; 2013). In Kenya, most cultivars were resistant to it and therefore, its infection was not a problem for over 20 years. However, in the second decade of this century, it has infected wheat with severity of over 50% leading to yield losses of up to 40% in susceptible cultivars (Knot 1989; KARI, 2011).

Yellow rust has become a major threat every year as no commercial cultivar is resistant (Hovmoller *et al*., 2010; KARI, 1990-2012). The disease is found globally and is common in great wheat producing regions like China, US, Australia and the Middle East (Wellings, 2007; 2011). The major factors favouring yellow rust germination, infection, latent period, sporulation, spore survival and host resistance are moisture, temperature and wind (Chen, 2005). The pathogen is also able to mutate and multiply rapidly as well as move from one field to another through air borne dispersal mechanism, thereby making yellow rust an important wheat disease (Brown and Hovmoller, 2002).

Stem rust is an obligate biotroph with five spore stages and has a heterothallic mating design (Betesalassie *et al*., 2007). Wheat monoculture among the East African farmers has offered a green bridge for the rust spores leading to the increased stem rust distribution and frequency (Saari and Prescott, 1985; Singh *et al*., 2008). Stem rust infect primarily plant stems but can also be found on leaves, sheaths, glumes, awns and seed reducing a healthy wheat field

to black stubble of shrivelled kernels predisposing crops to extensive lodging and total crop loss (Wanyera *et al*., 2004; Vidal, 2009). It is primarily a disease on wheat, though it can also cause minor infections on certain cultivars of barley and rye.

2.4 Epidemiology of stem rust in wheat

The minimum, optimum, and maximum temperatures for urediniospore germination are 2, 15–24, and 30 ºC; and for sporulation 5, 30, and 40 ºC (Hogg *et al*., 1969; Roelfs *et al*., 1992), thus providing a vast range of favourable environmental conditions. Urediniospores initiate germination within 1–3 hours of contact with free moisture over a range of temperatures. In field conditions, 6–8 hours of dew period or free moisture from rains is required for the completion of infection process (Rowell, 1984). A stem rust pustule (uredenium) can produce 10,000 urediniospores per day (Mont, 1970). Stem rust is known to be able to spread over large distances (Kolmer, 2005). Urediniospores can be transported long distance by a single event as well as assisted dispersal, stepwise range expansion and extinction and recolonization (Singh *et al*., 2008). Stem rust spores have spread up to 8000 Km from the South of Africa all way to Australia by single event mode of dispersal (Brown and Hovmoller, 2002). Although these events are rare, the ability for spores to withstand a high range of environmental pressures make these large distance dispersal completely possible (Singh *et al*., 2008). The '*Puccinia* pathway' of North America, where spore are transferred by wind from south to north, exemplifies the extinction and recolonization mode, since the disease eventually ends once the wheat season is over (Schumman and Leonard, 2000). The current spread of the stem rust race *Ug99* is an example of stepwise range expansion because the strain first originated in Uganda in 1999 then migrated into the Middle East and eventually had its way into Asia (Singh *et al*., 2004).

2.5 Life cycle of stem rust fungus

The wheat stem rust fungus is a heteroecious obligate biotroph with a macrocyclic lifecycle featuring five distinct spore stages occurring during asexual reproduction on wheat or other Poaceae hosts, and during sexual reproduction on common barberry (*Berberis vulgaris* L.) or an alternate host Berberidaceae species (Singh *et al*., 2002; Kolmer *et al*., 2009). On barberry, *Puccinia graminis* occurs as pycnia and aecia and on wheat, as uredinia and telia (Voegele *et al*., 2009). The full stem rust lifecycle begins as an infected plant, with elongated blister-like pustules (uredinia) full of loose brownish-red urediniospores which form on the lower side of the leaf, but may occasionally penetrate the upper surface of the leaf (Singh *et al*., 2008). Teliospores are firmly attached to the plant tissue and are commonly left in the field on the crop residue to serve as specialized survival structures to survive the winter (Leonard

and Szabo, 2005; Kolmer *et al*., 2009). The nuclei of each teliospore contain a + mating type and a – mating type which are paired together in each nucleus and once dormant, the mating types fuse together to create a single diploid nucleus, containing two sets of chromosomes (Roelf *et al*., 1992). A single haploid nucleus is produced in a sugary nectar to function as male gametes, and monokaryotic hyphae are produced to function as the female gamete, each gamete is either a + or a – mating type to prevent self -fertilization, as the + mating type can only fuse with the – mating type (Ankister, 1999).

The teliospore then begins to germinate, and the four haploid nuclei migrate to one of four developing basidiospores, the four nuclei then divide to produce two haploid nuclei per basidiospore (Kolmer, 2013). When the basidiospores reach maturity, they are forcibly ejected and dispersed by air currents to infect the alternate host in which they penetrates the cuticle leading to formation of pycnia on the upper leaf surface. Nuclear division with paired + and – mating type nuclei causes the cells to change to a dikaryotic state to form an aecium (Leonard, 2005). When the aecium has matured, the aeciospores are released and wind dispersed to infect their cereal host after which the uredinial infections follow, which can then cycle continuously or develop into teliospores, thus completing the life cycle (Kolmer, 2013).

2.6 Symptoms of stem rust on wheat

The symptoms of wheat stem rust are not apparent until after incubation period. Usually the symptoms are noticeable 7 to 15 days after infection on wheat while on barberry, the infection starts to be noticeable a little earlier than on wheat, as it starts to be noticeable after 5 to 10 days (Schumman and Leonard, 2000). In wheat, rust infection mainly occurs on stems and leaf sheaths as brownish-red blister-like pustules (uredinia) and 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). Within two weeks after inoculation, a rust pustule appears at the point of inoculation. In heteroecious rusts, urediospores can reproduce themselves and re-infect wheat multiple times, the later developmental stage, teliospore, which is a black spore, is produced in telia to conclude the disease cycle of stem rust in wheat and to start a new life cycle (Leonard and Szabo, 2005). The pathogen also absorbs the nutrients from the plant tissues that would be used for grain development and hence significantly affects grain filling and grain quality (Xue *et al*., 2012)

2.7 Economic importance of stem rust in wheat

Stem rust is historically a major problem in all of Africa, the Middle East, all of Asia except Central Asia, Australia and New Zealand, Europe and the Americas (both North and South) (Saari and Prescott, 1985; Russel, 2012). Of the three rusts, stem rust is the most

destructive as it is capable of causing 100% yield loss since an average yield loss; leaf rust losses ranges from 5 to 16% and up to 84 % in epidemic years (CIMMYT, 1999; Bolton *et al*., 2008, Hodson, 2014). The scale of losses caused by stem rust depends on the environmental conditions that favour infection, susceptibility of cultivar and the time of disease onset in the growing season (Bigriwa *et al*., 2001; Pratt and Gordon, 2006). It causes poor seedling germination, slow growth, reduced height, foliar injury, reduced floret set, low forage quality, shrivelling of the kernel and reduced yield (Chen, 2005). Furthermore, it is capable of changing a healthy looking crop into a tangle of black stems and shrivelled grains at harvest time leading to yield losses (Singh *et al*., 2008). Yield losses are guaranteed to occur since the fungus intercepts nutrients' flow to the sink (head) and leads to very weak stems which lodge easily, leading to reduced wheat yields as harvesting becomes difficult (Xue *et al*., 2012).

2.8 Control of stem rust

Attempts have been made to minimize or control stem rust through fungicides use, eradication of alternate host, cultural practices and genetic control through the host. Management of wheat rust has mainly relied on deployment of resistant genes on adapted varieties and fungicide application (Roelf *et al*., 1992; Walter *et al*., 2012). Genetic control has advantages for environmental and economic reasons, particularly for farmers in the developing world, and because of the possibility that rust pathogens develop resistance to fungicides (Oliver, 2014).

2.8.1 Control of stem rust using fungicides

Fungicides have been widely investigated for stem rust control. With early disease detection and immediate application of fungicides economic levels of control can be achieved (Peterson, 2001). Fungicides are used in order to control the disease during the establishment and development of the wheat crop, increase productivity, reduce leaf and seed damage (McGrath, 2004). They reduce subsequent rust severity on plant parts that were slightly infected at the time of fungicide application, but they cannot protect plant parts that are already heavily infected because the plant tissues are already damaged (Beard *et al*., 2004). Fungicides are commonly applied in form of dust, granules, gas, and liquid but their effectiveness depends on susceptibility of variety, level of infection and stage of crop growth at application (Cook *et al*., 1999; Wanyera *et al*., 2016).

Fungicide control has been successfully used in Europe, United States, Brazil and Paraguay to control the rusts but its use is limited in developing countries due to enormous cost it adds to wheat production (Chen, 2005). It may also create health problems, adversely affect the environment, and result in the selection of fungicide resistant strains of the pathogen (Chen,

2007). Most of the fungicides currently used to control foliar diseases are not registered for the control of stem rust as the choice of an appropriate fungicide is difficult (Viljanen-Rollinson *et al*., 2006). Foliar fungicides can achieve economic control as long as they are applied at the right stage (Loughman *et al*., 2005). Application of fungicide for the management of diseases have led to some benefits including big grain size and good milling quality (Barlett *et al*., 2002).

Several commercial fungicides are being used for control of stem rust on wheat in Kenya which are categorized into systemic and specific fungicides. The systemic fungicides includes; Swing 250 EC (*epoxiconazole* + *carbendazim*) for use in both winter and spring wheat; Silvacur 375 EC (*tebuconazole* + *tridimenol*) which controls diseases by protective, curative and eradicative action; Folicur 250 EC (*tebuconazole)* which is a triazole fungicide and an effective and reliable solution against a wide spectrum of diseases in many crops by protective, curative and eradicative action; Orius 25 EW (*tebuconazole*) have protective, curative, eradicant action and inhibits ergosterol synthesis; Cotaf 5 EC (*hexaconazole*) is highly effective fungicide with protective action and controls wide range of diseases; AmistarXtra 280 SC (*azoxystrobin 200* g/L + *cyproconazole* 80 g/L) inhibits fungi by blocking electron transfer in mitochondrial hence inhibits respiration and Prosaro 250 EC (*prothioconazole* 125 g/L + *tebuconazole* 125 g/L) provides protective, curative and long lasting activity that offers activity for leaf and ear diseases in wheat. It efficiently stops all important steps of the fungal infection chain like appressoria and haustoria formation, mycelial growth as well as spore formation. Broad specrum fungicides includes; Artea 330 EC (*cyproconazole* 80 g/L + *propiconazole* 250 g/L) is a foliar fungicide the control of rust and leaf spots in wheat and barley; Stratego 250 EC (*trifloxystrobin* + *propiconazole*) controls diseases and works by interfering with respiration in plant pathogenic fungi, inhibition of spore germination, and by blocking fungal growth; Soprano C 250 EC (*epoxiconazole* 125 g/L + *carbendazim* 125 g/L) is of the triazole group and acts through inhibition of C14-demethylase in the sterol biosynthesis pathway and Nativo 300 SC (*trifloxystrobin* 100 g/L + *tebuconazole* 200 g/L) which controls fungal diseases (www.cropscience.bayer.co.za/en).

2.8.2 Control of stem rust through cultural practices

Cultural control refers to all aspects of plant husbandry which influence disease development, including use of resistant varieties and biological control which are the chief means of management in traditional farming systems (Thuston, 1990). Cultural practices involves crop rotation which helps to limit the genetic diversity and build-up of the pathogen population and also minimize the number of urediniospores produced , use of early maturing cultivars, timely planting, eradicating alternate host and Mulching (Bariana *et al*., 2007). The

environmental conditions that favour wheat and rust development are similar, therefore, avoiding excess nitrogen applications which leads to increase in susceptibility to diseases and frequent light application of irrigation water are generally helpful in controlling stem rust (Roelfs, 1985; Marcia *et al*., 2008). In areas where the disease over summers, destruction of volunteer wheat and other susceptible grasses several weeks before planting also reduces inoculum level and delays initial infection (Knott, 1989; Mehta, 2014).

2.8.3 Genetic resistance to stem rust

Genetic resistance is the most economical and environmentally friendly method to reduce damage caused by stem rust disease. The impact of improvements in genetic resistance of wheat to rusts has generated a large proportion of the return on global economic investment in international wheat research (Reynolds and Borlaug, 2006). Over 70 stem rust resistance (*Sr*) genes have been identified and characterized against the different races of stem rust (McIntosh *et al*., 2003; Rahmatov *et al*., 2016). Twenty of these stem rust resistance genes were transferred into the *Triticum aestivum* from the wild relatives of wheat by introgression of wheat alien species chromosome translocations through genetic engineering (Klindworth *et al*., 2012).

2.8.3.1 Seedling resistance to stem rust

Seedling resistance is a type of resistance conferred by major genes and functions against specific rust races but not all (Steffenson *et al*., 2007; Babiker *et al*., 2009). Seedling resistance genes are expressed through hypersensitive responses; and the rapid death of the infected cells thereby restricting the spread of the pathogen to other parts of the plant (Jin *et al*., 2007; Lowe *et al*., 2011). Seedling resistance genes whose effects are easily phenotyped in greenhouse tests on seedlings and/ or adult plants are the most studied (McIntosh *et al*., 1995; 2003). These include; *Sr1A:1R, Sr13, Sr22, Sr25, Sr26, Sr27, , Sr32, Sr33, Sr35, Sr37, Sr40, Sr42, SrTmp* and *SrWeb* (Jin *et al*., 2007; Olivera *et al*., 2012; Zhang *et al*., 2012; Singh *et al*., 2013). In general, seedling resistance is race specific and therefore short lived due to frequent changes in virulence of the pathogen population, therefore wheat breeders need to continuously identify and incorporate new resistance genes into the existing commercial varieties (Crute and Pink, 1996; Lin *et al*., 2000). Though race-specific, these genes could be pyramided into wheat cultivars to broaden the base of resistance and improve its stability (Leornard and Szabo, 2005).

2.8.3.2 Adult plant resistance (APR) to stem rust

Adult or slow rusting resistance genes are considered more durable than seedling resistance and their expression by the carrier host plant marked by long latent period, few and small uredinia and low spore production (Bjarko and Line, 1988; Singh, 2012). These genes

are effective against a range of stem rust races with each gene contributing small and equal effects on the phenotype (Bariana and McIntosh, 1995; Stuthman *et al*., 2007). The pyramiding of four to five APR genes can confer near immunity against stem rust disease but may be difficult to accomplish due to large population sizes required to select transgressive segregants (Knott, 1982; Singh, 2012). Therefore, it is important to understand the mode of inheritance and number of genes conferring slow rusting as this helps breeders in deciding the right time to start selection and choosing the optimum population sizes to be grown at various segregating stages of population (Das *et al*., 2006).

For instance, the *Sr2* gene confers slow rusting in wheat and is linked with pseudo-black chaff (PBC) phenotype. However, excessive expression of PBC is considered to be an undesirable trait as it reduces yield and leads to the elimination of lines in breeding programs (McNeil *et al*., 2008). This gene has been effective against wheat stem rust fungus since 1920, constitutes non-hypersensitive, partial reaction and has varying disease severities with regard to differences in genetic and environmental backgrounds (Ayliffe *et al*., 2008; McNeil *et al*., 2008). Recent characterization in Kenya with *Ug99* of various mapping populations involving crosses of APR wheat with a susceptible parent indicates that, inheritance of complex APR is similar to that for leaf and stripe rusts (Singh and Trethowan, 2007).

2.9 Genetic gain in wheat breeding

The broad aim of plant breeding is to improve genetically the performance of cultivars of species in the most efficient manner possible. The response to selection resulting from significant genetic variation and high heritability constitute genetic gain (Falconer and Mackay, 1996; Shukla *et al*., 2006). There has been a steady increase in productivity since the green revolution associated with improvements in yield potential, resistance to disease, and adaptation to abiotic stresses as well as good agronomic practices (Evenson and Gollin, 2003; Reynolds and Borlaug, 2006). For example, a study to determine gains in selection for low nitrogen tolerance in maize (*Zea mays*) resulted in gains of 2.3% and 1.9% under low and high nitrogen, respectively (Omoigui *et al*., 2005). Also, gains in yield of up to 33% have been realized in barley (Alojzije *et al*., 2006) while in potato (*Solanum tuberosum*), genetic gains for heat tolerance in three cycles of recurrent selection was 37.8% (Flavio *et al*., 2010). In black bean (*Phaseolus vulgaris*), gains per year in seed yield, tolerance to lodging and 100-seed weight of 1.1%, 1.7% and 0.65%, respectively, have been achieved (Luis *et al*., 2014). In wheat, gains in yield of up to 0.53% have been realized (Leonardo *et al*., 2017).

Heritability estimates together with expected genetic gain, are more useful than the heritability values alone in predicting the effects of selecting the best genotype (Najeeb *et al*., 2009). Genetic gain (per cycle) (G_c) also called response to selection was expressed by Lush (1945) as;

$$G_c = h^2 D \quad (1)$$

Where h^2 is heritability in the narrow sense and *D* is the selection differential. Genetic gain per year (G_y) is obtained by dividing the genetic gain per cycle by the number of years (y) required to complete a cycle of selection: $G_y = G_c / y$. Narrow-sense heritability is the proportion of the total variation attributed to additive genetic variance in the population

$$h^2 = \frac{\sigma_A^2}{\sigma_{ph}^2} \quad (2)$$

Where σ_A^2 is the additive genetic variance and σ_{ph}^2 is the phenotypic variance. The selection differential is the difference between the mean of the genotype selected from a population and the overall mean of the population from which they were selected. The selection differential can be expressed as

$$D = k\sigma_{ph} \quad (3)$$

Where k the selection differential is expressed in standard units and σ_{ph} is the square root of the phenotypic variance.

Heritability can also be determined using offspring regressions. The resemblance between offspring and a parent gives $\frac{1}{2}$ of heritability. In some sense the simplest design is the parent-offspring regression. The regression of offspring phenotype (z_{oi}) given the phenotypic value of one of its parents (z_{pi}).

$$z_{oi} = \alpha + bz_{pi} + e_i \quad (4)$$

The slope b (the regression coefficient) can also be written as $b_{y|x}$ to signify that the slope is for the regression of y on x, i.e., the denominator in b is the variance of x.

$$z_{oi} = \alpha + b_{o|p}z_{pi} + e_i \quad (5)$$

The above equation implies that the predicted value by for y given we know x is

$$z_{oi} = \mu + b_{o|p}(z_{pi} - \mu) + e_i \quad (6)$$

The alternative formulation follows since the regression passes through the mean of both variables (offspring and parental phenotypes). The expected regression slope $b_{o|p}$ is

$$E(b_{o/p}) = \frac{\sigma(z_o z_p)}{\sigma^2(z_p)} = \frac{\sigma_A^2}{2} + \frac{E_{o,}E_p}{\sigma_z^2} = \frac{h^2}{2} \text{ (Fikret, 2011).} \quad (7)$$

Expected genetic gain per cycle of selection under different intrapopulation schemes with noninbred parents can be calculated as follows: Recurrent Phenotypic selection: without gridding into sub-blocks $\frac{kc\sigma_A^2}{\sqrt{\sigma_u^2 + \sigma^2 + \sigma_{AE}^2 + \sigma_{DE}^2 + \sigma_A^2 + \sigma_D^2}}$ (8)

With gridding into subblocks $\frac{kc\sigma_A^2}{\sqrt{\sigma_u^2 + \sigma_{AE}^2 + \sigma_{DE}^2 + \sigma_A^2 + \sigma_D^2}}$ (9)

Modified ear to row $\frac{kc1/4\sigma_A^2}{\sqrt{\frac{\sigma_e^2}{rt} + \frac{1/4\sigma_{AE}^2}{t} + 1/4\sigma_A^2}}$ (10)

Half-sib $\frac{kc1/4\sigma_A^2}{\sqrt{\frac{\sigma_e^2}{rt} + \frac{1/4\sigma_{AE}^2}{t} + 1/4\sigma_A^2}}$ (11)

Full-sib $\frac{kc1/2\sigma_A^2}{\sqrt{\frac{\sigma_e^2}{rt} + \frac{(1/2\sigma_{AE}^2 + 1/4\sigma_{DE}^2)}{t} + 1/2\sigma_A^2 + 1/4\sigma_D^2}}$ (12)

Selfed progeny, $S_{0:1}$ lines $\frac{kc\sigma_A^{2\prime}}{\sqrt{\frac{\sigma_e^2}{rt} + \frac{(1/2\sigma_{AE}^{2\prime} + 1/4\sigma_{DE}^2)}{t} + \sigma_A^{2\prime} + 1/4\sigma_D^2}}$ (13)

Expected genetic gain per cycle of selection for population cross under different interpopulation selection schemes with noninbred parents: Reciprocal half-sib selection

$$\frac{kc1/4\sigma_{A(1)}^2}{\sqrt{\frac{\sigma_{e(1)}^2}{rt} + \frac{1/4\sigma_{AE(1)}^2}{t} + 1/4\sigma_{A(1)}^2}} + \frac{kc1/4\sigma_{A(2)}^2}{\sqrt{\frac{\sigma_{e(2)}^2}{rt} + \frac{1/4\sigma_{AE(2)}^2}{t} + 1/4\sigma_{A(2)}^2}} \quad (14)$$

Reciprocal full-sib selection $\frac{kc1/2\sigma_A^2}{\sqrt{\frac{\sigma_e^2}{rt} + \frac{(1/2\sigma_{AE}^{2\prime} + 1/4\sigma_{DE}^{2\prime})}{t} + \sigma_A^{2\prime} + 1/4\sigma_D^{2\prime}}}$ (15)

Where σ_u^2 is the within plot environmental variance, σ_{AE}^2 and σ_{DE}^2 are the additive by environmental and dominance by environmental interactions, σ_A^2 and σ_D^2 are the additive and dominance variance, *k* is the standardized selection differential, *n* is the number of plants per plot, *r* is the number of replications per environment, *t* is the number of environments. , $\sigma_A^{2\prime}$ is additive genetic variance plus a component that is mainly a function of degree of dominance

(1) refers to components in population 1 and (2) refers to components in population 2 (Empig *et al*., 1972; Sprague and Eberhart, 1977). The average annual genetic progress (GP_a), in percentage, can also be calculated for each characteristic by dividing the regression slope (b_1) by the intercept (b_o) of the regression according to the following equation adapted from Matos *et al*. (2007)

$$GP_a = (\frac{b_1}{b_o} \times 100) / 2 \quad (16)$$

CHAPTER THREE

PROGRESS IN USING ADULT PLANT RESISTANCE IN BREEDING FOR STEM RUST RESISTANCE IN CIMMYT STEM RUST RESISTANCE SCREENING NURSERIES (SRRSN).

Abstract

Stem rust caused by *Puccinia graminis* f.sp. *tritici* Erikss. and Henning is the most devastating disease of both bread wheat (*Triticum aestivum L.*) and durum wheat (*Triticum durum Desf.*) worldwide. Deployment of Adult plant resistance (APR) genes is one of the strategies used by CIMMYT scientists to provide resistance against rust diseases. A field study over two seasons (2015 and 2016) was carried out at the Kenya Agricultural and Livestock Research Organization (KALRO), Njoro to assess the progress made in using APR in breeding for stem rust resistance to the *Ug99* race group using wheat lines from CIMMYT Stem Rust Resistance Screening Nurseries (SRRSNs). Seven hundred and forty-four lines randomly selected from five SRRSN (1st, 3rd, 5th, 7th and 9th) were planted in an *Alpha lattice* design and observations recorded for stem rust severity. Progress in using APR was assessed using severity data from the nurseries between years 2005 and 2016. The proportion of lines which showed severities of ≤30% were higher across all the nurseries in all years of evaluation compared to those with severities of ≥35%. The number of lines which exhibited severities of ≤30% increased in the first, third and fifth nurseries in the first three years of evaluation but later reduced in 2015. In 2016, these proportions increased again. In the seventh and ninth nurseries, the proportions declined in 2015 but later increased in 2016 from the initial proportion. The number of lines which revealed severities of ≥35% kept on reducing and increasing with time from the first to the ninth nurseries but these proportions were still lower than those for the lines which showed severities of ≤30%. The effect due to season was highly significant (P≤0.001) for Area Under Disease Progress Curve (AUDPC) and a thousand kernel weight and significant (P≤0.05) for hectolitre weight. While effect due to line was significant (P≤0.001) for AUDPC, hectolitre weight and a thousand kernel weight. There was evidence of effectiveness of APR as over 75% of the lines showed a severity of ≤30% and this was observed across all the five nurseries.

3.1 Introduction

Stem rust caused by *Puccinia graminis* f.sp. *tritici* is a major disease of common or bread wheat *(Triticum aestivum L.*) which is the second most important cereal in the world after rice (*Oryza sativa*). With the world population projected to reach 9.3 billion people by 2050, it is imperative to concurrently increase food production including that of wheat production

(United Nations, 2011). One such method to ensure the health of the wheat crop in the world is to protect it from diseases such as stem rust. The losses caused by this disease are heavy and have been documented in several countries in the past such as India, USA, Australia and Canada (Kolmer, 2001; Park, 2007). The original race of this pathogen, *TTKSK* (*Ug99*) which originated from Uganda in 1999 overcame the *Sr31* (Stem rust resistance gene) gene and has since evolved into more virulent races such as *TTKST (*virulent to *Sr24*)*, TTKTK* (virulent to *SrTmp*) and *TTTSK* (virulent to *Sr36*) in Kenya. Its rapid movement from Africa, that is, from eastern Africa to Arabian Peninsula and with a possibility of affecting wheat production in Indian Subcontinent (Singh *et al*., 2006; Jin *et al*., 2008; 2009) has made breeding for resistance important globally to mitigate the threat of this devastating disease.

One of the effective methods of controlling wheat stem rust is to deploy *Sr* genes that are effective against the *Ug99* race group (*TTKSK* and its variants). Such an effort is being undertaken at the International Maize and Wheat Improvement Center (CIMMYT) and other regional wheat breeding programs (Singh *et al*., 2008). To date, more than 70 *Sr* genes have been characterized in wheat (McIntosh *et al*., 2013, 2014; Rahmatov *et al*., 2016), approximately 34 of which remain effective against races of the *Ug99* lineage (Singh *et al*., 2015). Of these, 18 were derived from the wheat progenitors and related species (Singh *et al*., 2006; Xu *et al*., 2008). However, only a few of the 70 genes confer APR (quantitative resistance), these include the *Sr2*, *Sr*55, *Sr*56, *Sr*57, and *Sr*58 (Knott, 1968; Lagudah *et al*., 2006; Singh *et al*., 2013; Herrera-Foessel *et al*., 2014; Bansal *et al*., 2014; Kolmer *et al*., 2015). Fungicides are also effective in controlling rust diseases, however, resource poor farmers cannot afford to spray fungicides either due to high cost or unavailability of the chemicals. Even for farmers in the developed world, the disease can be devastating if fungicides are not applied frequently, in a timely manner, or at high rates (Chen, 2005).

Quantitative disease resistance is more durable but more difficult to evaluate because it is expressed in mature plants depending on inoculum load and sequential infection (Rutkoski *et al*., 2011; Hickey *et al*., 2012). Slow rusting durable resistance genes confer resistance to a broad range of stem rust races as it is conditioned by several genes each having small effects on the phenotype (Bariana and McIntosh, 1995; Stuthman *et al*., 2007). Furthermore, being quantitatively inherited, APR is associated with the absence of a hypersensitive response to the pathogen (Hare and McIntosh, 1979; Knott, 1982). Deployment of combinations of resistance genes should improve the durability of resistance in commercial cultivars by reducing the possibility of corresponding simultaneous mutation events in the pathogen (Liu *et al*., 2011; Singh, 2012). Pyramiding four to five APR genes into a cultivar can confer near immunity

against diseases but this may be difficult to achieve due to large population sizes required to select desirable transgressive segregants (Singh *et al*., 2005; Singh, 2012). Combining multiple seedling (also known as all stage resistance) genes, alone or with APR genes has also been proposed and utilized to obtain durable resistance against the disease (Ayliffe *et al*., 2008; Mago *et al*., 2011; Evanega *et al*., 2014).

Measuring genetic gain in a trait is essential to ascertain if a breeding strategy is correct and effective in any breeding program. Two ways to estimate the genetic gain are often used in evaluating the efficiency of breeding programs: (i) conducting experiments with old and modern cultivars (Matus *et al*., 2012; Cormier *et al*., 2013, Bilgin *et al*., 2015), and (ii) using data from multi-environment trials (Cargnin *et al*., 2008; Oury *et al*., 2012, Crespo-Herrera *et al*., 2017). Genetic gain results from changes in the allele constitution that improves a variety. Over the past years, successful improvements in wheat through extensive breeding programs have led to narrowing of genetic diversity. This concerns breeders as the potential of genetic gain becomes more limited with a smaller genetic pool from which to choose beneficial genes (Feuillet *et al*., 2008). The objective of this study was to determine the progress made in using APR in controlling stem rust in CIMMYT lines.

3.2 Materials and Methods

3.2.1 Experimental Site

This study was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO), Njoro (0^{o} 20' S, 35^{o} 56' E) for two seasons (2015 and 2016) which is at an elevation of 2,185 meters above sea level and experiences mean minimum and maximum temperatures of 9.54±0.11 ^{o}C (night) and 22.83±0.14 ^{o}C (day). The area receives 1005.00±51.53 mm of rain annually (Kenya Metereological Station Identification Number 9031021) (Jaetzold and Schmidt, 2006). However, the prevailing environmental conditions of this area also favours epidemics of stem ruts on Kenyan wheat varieties.

3.2.2 Genotypes

A total of 744 wheat lines from five (1st, 3rd, 5th, 7th and 9th) International Wheat Stem Rust Resistance Screening Nurseries were evaluated alongside four checks used in the screening facility. The check varieties were: *Robin* (high yielding variety but susceptible to stem rust race TTKTK/TTKTT), *Canadian Cunningham Kennedy* (Cacuke) (highly susceptible to stem rust), *Kingbird* (APR to Ug99 race group) and *Sunbird* (carrying *SrND643* gene). The 1st nursery comprised of 103 lines which were selected in 2006. The 3rd nursery consisted of 110 lines which were selected in 2008. The 5th nursery consisted of 135 lines which

were selected in 2010. The 7th nursery consisted of 150 lines which were selected in 2012 and the 9th nursery was made up of 246 lines which were selected in 2014.

3.2.3 Experimental Procedure

Land preparation was accomplished as suitable for sowing wheat. Seed of each line was uniformly hand spread in a double row measuring 0.7 m × 0.2 m with alleyway of 0.3 m wide between them. An A*lpha lattice* design with two replicates was used. During sowing, diammonium phosphate (DAP) was applied at the rate of 150 kg ha $^{-1}$ to provide an equivalent of 27 kg N. ha $^{-1}$ and 30.08 kg P. ha^{-1}. A mixture of susceptible cultivars (spreader) were planted perpendicular to all the plots and around the plots. These susceptible cultivars were then inoculated with stem rust spores when the plants were at stem elongation stage (GS 30) (Zadok's *et al*., 1974) by injecting them with the inoculum using a syringe. The inoculation was done in the evening (when conditions were favourable for spore germination) to create an artificial disease epidemic and ensure uniform inoculum dissemination. In absence of rainfall, the plants were irrigated. During the testing season the prevalent races were *TTKSK* (with virulence to *Sr31)*, *TTKST* (with virulence to *Sr24*), *TTTSK* (with virulence to *Sr36*), *TTKTK* (with virulence to *SrTmp*) and *TTKTT* (with virulence to *Sr24* and *SrTmp*)

When the crop had emerged, Hussar Evolution (*Fenoxaprop-pethyl* 64 g ha^{-1} + *Idosulfuron methyl sodium* 8 g. ha^{-1} + *Mefenpyr-diethyl* 24 g. ha^{-1}) was applied at the rate of 1.0 l ha^{-1} as post-emergence herbicide for the control of both grass and broad-leaved weeds. At tillering growth stage (GS 20-29) (Zadok's *et al*., 1974), Buctril MC (B*romoxynil ectanoate* 281 g. ha $^{-1}$ *and MCPA Ethyl Hexyl Ester* 281 g. ha^{-1}) herbicide was applied at the rate of 1.25 l ha^{-1} to control broad-leaved weeds. The plants were top dressed with Calcium Ammonium Nitrate (CAN) at stem elongation stage (GS 30) (Zadok's *et al*.,1974) at the rate of 100 kg ha^{-1} to supply 26 kg ha $^{-1}$ of N. Thunder OD 145 *(Imidachloprid* 30 g ha^{-1} +*Beta-cyfluthrin* 13 g ha^{-1}) a systemic insecticide was applied at tillering (20-29) growth stage at the rate of 0.3 l ha^{-1} to control the Russian wheat aphid (RWA) and other cereal aphid vectors that transmit the barley yellow dwarf virus (BYDV).

3.2.4 Data Collection

Wheat lines were evaluated for stem rust severity on a scale of 0% (immune) to 100% (completely susceptible) depending on the extent of the area affected by the disease, according to modified Cobb scale (Peterson *et al*., 1948). Evaluation was done 5 times at an interval of 7 days beginning at the time when disease was first observed up to plant maturity (GS 70-89) (Zadoks *et al*., 1974). Data was also made on days to: heading, flowering and maturity. The days to heading was determined when heads of 50% of the plants in a plot had emerged and

days to flowering when 50% of the plants in a plot had flowered. At physiological maturity, measurements were made on five randomly selected plants per entry for plant height, measured from the base at ground level to the tip of plant excluding awns. Spike length was measured from the first node where the first spikelet emerges to the spike tip, biomass was measured by weighing whole plants in a plot excluding the roots. A thousand kernel weight and hectolitre weight were determined after sun drying.

3.2.5 Data analyses

Area under disease progress curve (AUDPC) was used to estimate disease severity. It was computed using the formula by Wilcoxson *et al*. (1975) and AUDPC CIMMYT programme.

$$AUDPC = \sum_{i}^{n-1}\left[\left(\frac{y_i + y_{i+1}}{2}\right)\left(t_{i+1} - t_i\right)\right] \quad (1)$$

Where; t_i is the time in days of each reading, y_i is the percentage of affected part of the plant at each reading, n is the number of readings, $t\ (i+1)$ is the second assessment date of two consecutive assessment, $y\ (i+1)$ is the disease severity on assessment date $t\ (i+1)$. The data was analysed using SAS (SAS, 2012) by applying the statistical equation below:

$$Y_{ijklmn} = \mu + S_i + R_{j(i)} + B_{k(j)} + N_l + NS_{il} + L_{m(l)} + \varepsilon_{ijklmn} \quad (2)$$

Y_{ijklmn}= Observation of experimental units, μ= Overall mean, S_i =Effect due to i^{th} season, $R_{j(i)}$ effect due to j^{th} replicate within i^{th} season, $B_{k(j)}$=effect due k^{th} block within j^{th} replicate, N_l=effect due to l^{th} nursery, NS_{il} = effect due to interaction between i^{th} season and l^{th} nursery, $L_{m(l)}$=effect due to m^{th} line within l^{th} nursery, ε_{ijklmn} =random error component.

Mean comparisons was done basing on Tukey's procedure at 5% probability to separate the different wheat lines using the formula:

$$w = q_{\alpha,(p,f)}\sqrt{\frac{MSE}{r}} \quad (3)$$

Where: w is the critical difference and MSE is the mean square error (Gomez and Gomez, 1984).

Correlation analysis was then conducted to determine the association between AUDPC and agronomic traits using the formula below:

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{(n\sum x^2 - (\sum x)^2) \times (n\sum y^2 - (\sum y)^2)}} \quad (4)$$

Where *r* is the correlation coefficient, $x = (x_i - \overline{x})$, $y = (y_i - \overline{y})$ with a value ranging from -1 to +1 (Herrero *et al*., 2011)

To determine genetic progress for stem rust resistance, the disease severity scores from 2005 to 2016 data were used with stem rust severities grouped into two: 0-30 and 35-100. The number of lines falling into each group was counted in each nursery. The proportion of lines in each group was then calculated from the counts and the progress determined by comparing the proportion of the groups across the years.

3.3 Results

3.3.1 Analysis of variance for AUDPC, Agronomic traits and Phenotypic traits.

The combined analysis of variance results for the two seasons showed significant ($P \leq 0.001$) differences between 2015 (off season) and 2016 (main season) for area under disease progress curve (AUDPC), biomass, plant height and number of days to physiological maturity. The effect due to season was also significant ($P \leq 0.01$) for a thousand kernel weight, spike length and grain filling period. Further, season effect was significant ($P \leq 0.05$) for hectolitre weight, number of days to heading and number of days to flowering. There were significant ($P \leq 0.001$) effects among the lines evaluated for AUDPC, hectolitre weight, kernel weight, biomass, spike length, plant height, number of days to heading, number of days to flowering, number of days to physiological maturity and grain filling period (Table 3.1).

3.3.2 Genetic progress in the use of adult plant resistance

The wheat lines in the first SRRSN were first evaluated in 2005 and the proportion of those that showed severities of ≤30% was high (86.41%). This proportion increased progressively in the subsequent years until 2015 when it declined to 52.43%. However, in 2016, the proportion again increased. A similar pattern was observed in the third SRRSN where the proportion of lines which revealed severities of ≤30% was high when the lines were first evaluated in 2005 and it remained high until in 2008 when the number reduced drastically but later increased in the years 2015 and 2016. In the fifth SRRSN, the number of lines which showed severities of ≤30% was high when the first evaluation was done in 2009. This number declined in 2015, rising again in 2016. Similar trends were observed for the proportion of lines which revealed severities of ≤30% in the seventh and ninth SRRSN. The number of lines which displayed severities of ≤30% increased in each test year in the first, third and fifth SRRSN but reduced in 2015. In 2016, the numbers increased albeit marginally. In the seventh and ninth SRRSN, the proportions reduced in 2015 but increased in 2016. As for the number of lines which showed severities of ≥35%, the pattern was in contrast to the increases noted above, that

is, they reduced and increased with time from the first to the ninth SRRSN but these proportions were still lower than those for the lines which showed severities of ≤30% (Figure 3.1). The proportion of lines which revealed severities of ≤30% was high in all the SRRSN while proportion of lines that showed severities of ≥35% was low in all the SRRSN (Figure 3.2).

3.3.3 AUDPC, Agronomic traits and Phenotypic traits of wheat

Between the two seasons and for all the nurseries, the mean AUDPC was significantly higher in 2015 (238.89) than that for 2016 (131.92). Thus there was a 44.77% disease severity decline in 2016. At the individual nurseries level, stem rust severity was similarly higher in 2015 than in 2016 as shown by the AUDPCs. Mean hectolitre weight, kernel weight, biomass, plant height and spike length for the combined nurseries in 2016 season were higher than those for 2015 season. Individual nurseries also depicted similar trends where season 2016 had higher means for grain yield, hectolitre weight, kernel weight, biomass, plant height, spike length and harvest index than mean grain yield, hectolitre weight, kernel weight, biomass, plant height and spike length for 2015 season. Plants in 2016 season took longer days to heading, flowering and maturity translating to a longer grain filling period than those in 2015 season. For individual nurseries, mean days to heading, flowering, maturity and grain filling for 2016 season were also longer than those for 2015 season (Table 3.2).

3.3.4 Correlation analysis

There were significant (r=-0.44***, r=-0.45*** and r=-0.43***) negative correlations between AUDPC and kernel weight, hectolitre weight, and biomass respectively in 2015. However, significant (r=0.78***, r=0.41***) positive correlations were observed between kernel weight and hectolitre weight and biomass. A positive highly significant (r=0.43***) correlation also occurred between hectolitre weight and biomass. Similarly, there were significant (r=-0.29***, r=-0.36*** and r=-0.53***) negative correlations between AUDPC and kernel weight, hectolitre weight, and biomass in 2016. Significant (r=0.69***, r=0.44***) positive correlations were observed between kernel weight and hectolitre weight and biomass. A positive significant (r=0.43***) correlation also occurred between hectolitre weight and biomass in 2016 (Table 3.3)

3.4 Discussion

The results of combined analysis of variance for the two seasons showed difference in performance between off season (2015) and main season (2016) for various traits. This showed that the environment under which wheat is grown creates a tremendous impact on the growth, development and yielding ability of wheat. Every wheat cultivar has its own requirements of

temperature and light for growth, flowering and finally the production of grains (Haider, 2007; Aslani and Mehrvar, 2012) and in different environmental conditions, same genotype performs differently (Duncan *et al*., 2015). The differences between the lines for all the traits measured could be as a result of the various number of genes found in those lines and variation among the wheat lines tested. In addition, the difference in performance among the lines tested could be attributed to by the complex interaction between the lines and many environmental factors (Friedrich *et al*., 2016).

The gains in the performance of adult plant resistance computed from 2005 to 2016 were variable across the nurseries. The proportion of lines which showed low disease severities (0-30%) increased over time while those with high severities (≥35%) reduced with time except in 2008 in the third SSRSN and in 2015 in the remaining SRRSNs when the proportions of lines with severities of ≤30% started reducing. This is suspected to have been caused by the emergence of further new and more virulent races of the *Ug99* group which overcame the existing *Sr* genes then. Since its first discovery, 13 races within the *Ug99* group have been identified across several countries in Africa and Middle East (Mondal *et al*., 2016). The newly evolved races present in wheat fields in Kenya include; *TTKST* (with virulence to *Sr24*), *TTTSK* (with virulence to *Sr36*), *TTKTK* (with virulence to *SrTmp*) and *TTKTT* (with virulence to *Sr24* and *SrTmp*). Resistance often breaks down due to what Qamar *et al*. (2007) describe as the "arms race" between the fungus and the host plant. This arises when virulent stem rust races increase in frequency hence strong selection pressure is wielded upon the pathogen population leading to emergence of new dominant races that end up overcoming the *Sr* genes in the wheat as was witnessed in 1999 with the emergence of the race *Ug99* (Wanyera *et al*., 2006). Also, resistance genes last an average of five to six years before they succumb to new races (Kilpatrick, 1975; Wellings and McIntosh, 1990; Jeffrey *et al*., 2014), and therefore, the results observed of the number of lines with low severities fit into this cycle.

The increase in the number of lines which showed severities of ≤30% from one nursery to the next could have been due to introduction of new genes in the subsequent nurseries. The first SRRSN had resistance lines that were both race specific, APR and combination of both in different genetic backgrounds. Some of the race specific genes present in these lines included *Sr25, Sr24+Sr36, Sr33, SrTmp, SrSynt, SrSha7, Sr Cdbrd and SrUnknown.* These race specific genes however conferred intermediate resistance to the stem rust but when they were combined with APR backgrounds, they displayed good levels of field resistance. Sources of APR which

Table 3.1 Mean squares from combined analysis of variance of CIMMYT wheat lines from five SRRSN for AUDPC for stem rust, agronomic and phonological traits grown in Njoro, Kenya in 2015 and 2016.

Source of variation	df	Expected mean squares	Area under disease progress curve	Hectolitre weight	Kernel weight	Biomass	Plant height
Replicate	1	σ^2_ε +76 σ^2_L+14960 σ^2_B+284240 σ^2_R	303798.32	5658.05	864.00	11620.95	7105.02
Blocks within Replicates	36	σ^2_ε +8σ^2_L+14960 σ^2_B	12324.65	111.09	22.00	91.97	92.88
Nursery	4	σ^2_ε +152 σ^2_L+2992 σ^2_B+56848 σ^2_R+56848 σ^2_{NS}+56848 σ^2_S+113696 σ^2_N	106879.71	3943.09	1866.71	3583.47	1899.77
Season	1	σ^2_ε +76 σ^2_L+7480σ^2_B+142120 σ^2_R+56848 σ^2_{NS}+284280 σ^2_S	8098709.35***	19456.64*	33900.91**	381634.52***	580806.71***
Nursery × Season	4	σ^2_ε +76σ^2_L+1496 σ^2_B+28424σ^2_R+56848 σ^2_{NS}	26838.29	2343.61	282.49	1652.39	1053.67
Lines/Nursery	743	σ^2_ε +152σ^2_L	28267.86***	165.52***	70.65***	115.24***	119.60***
Error	2202	σ^2_ε	3234.14	43.64	22.03	33.16	66.06
CV			30.66	14.16	23.48	20.85	9.44
R^2			0.82	0.67	0.70	0.88	0.84

Table 3.1 *Cont*.

Source of variation	df	Expected mean squares	Spike length	Days to heading	Days to flowering	Days to maturity	Grain filling period
Replicate	1	σ^2_ε+76 σ^2_L+14960 σ^2_B+284240 σ^2_R	0.38	915.93	468.57	338.65	140.71
Blocks within Replicates	36	σ^2_ε+8σ^2_L+14960 σ^2_B	2.29	45.12	53.23	31.50	55.24
Nursery	4	σ^2_ε+152 σ^2_L+2992 σ^2_B+56848 σ^2_R+56848 σ^2_{NS}+56848 σ^2_S+113696 σ^2_N	15.87	1196.62	1000.75	49.94	1114.52
Season	1	σ^2_ε+76 σ^2_L+7480σ^2_B+142120 σ^2_R+56848 σ^2_{NS}+284280 σ^2_S	895.03**	28363.26*	22213.43*	146107.92***	45721.86**
Nursery × Season	4	σ^2_ε+76σ^2_L+1496 σ^2_B+28424σ^2_R+56848 σ^2_{NS}	12.58	693.75	708.81	44.36	958.31
Lines/Nursery	743	σ^2_ε+152σ^2_L	3.43***	68.63***	56.89***	33.49***	55.23***
Error	2202	σ^2_ε	1.19	29.39	27.72	22.12	4.13
CV			11.34	7.89	7.29	4.20	14.17
R^2			0.60	0.62	0.59	0.79	0.58

*, **, *** significance at P≤0.05, P≤0.01 and P≤0.001, respectively.
Test H=Nursery E=Season, Test H=Season E=Nursery×Season, Test H=Replicates E= Blocks within Replicates, Test H=Blocks within Replicates E= Lines within Nurseries' Test H= Lines within Nursery= E=Random error component.

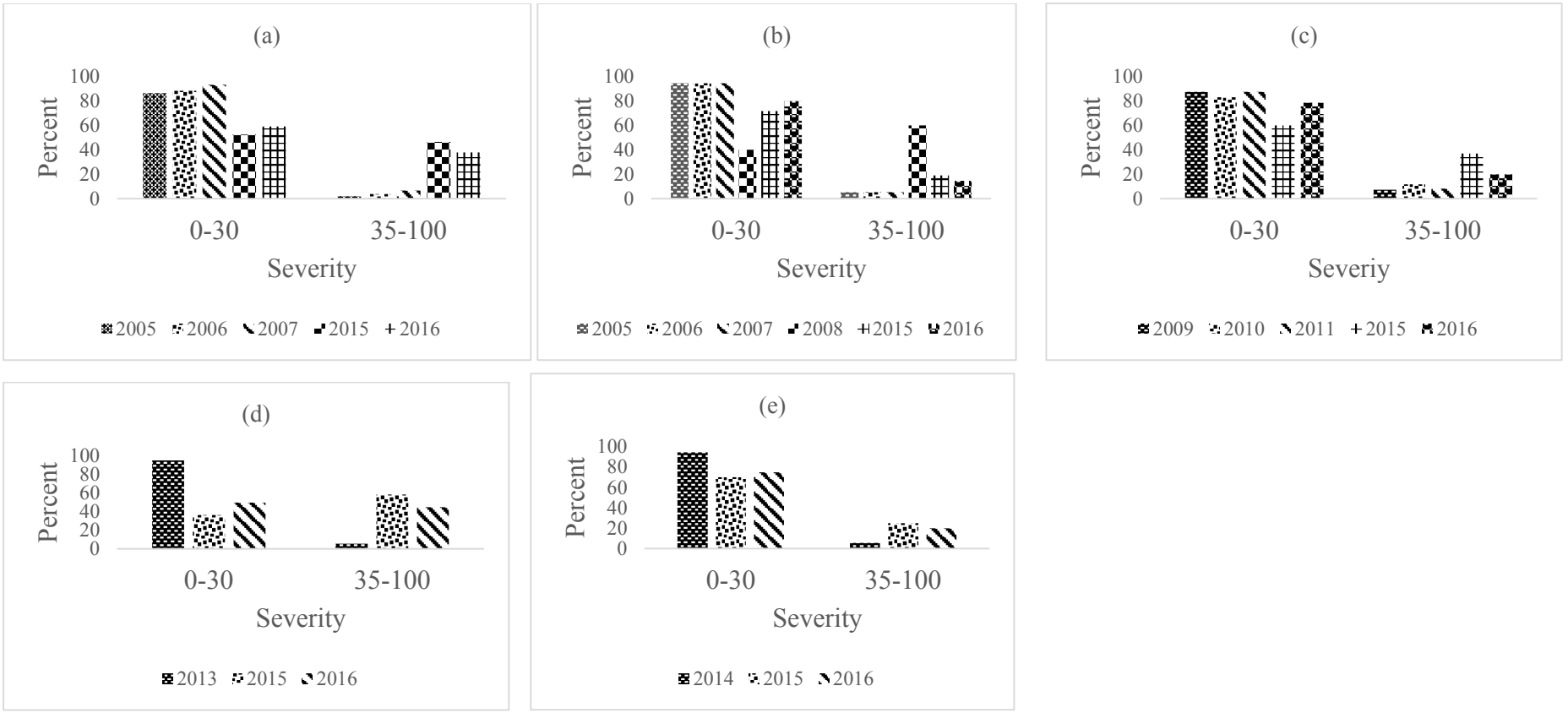


Figure 3.1 Proportion of CIMMYT wheat lines with disease severities from 2005 to 2016. (a) First SRRSN, (b) Third SRRSN, (c) Fifth SRRSN, (d) Seventh SRRSN, (e) Ninth SRRRSN.

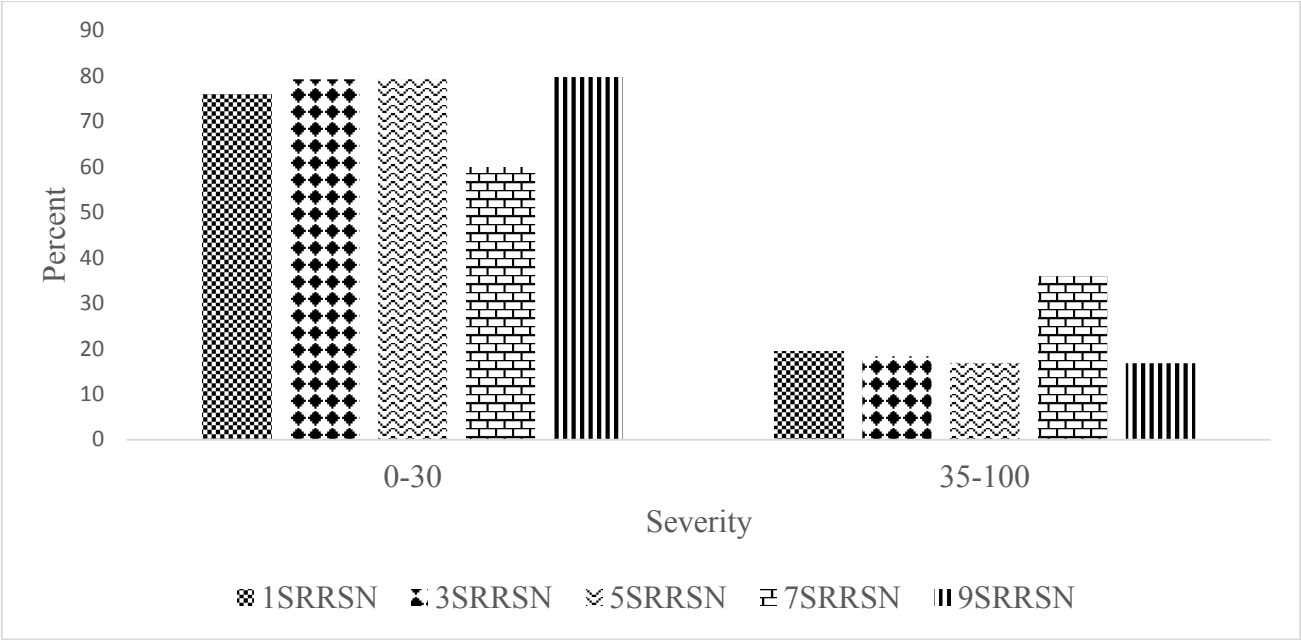


Figure 3.2 Proportion of CIMMYT wheat lines with disease severities from the first, third, fifth, seventh and ninth SRRRSNs from 2005 to 2016.

Table 3.2 Means of combined and individual nursery during different seasons for AUDPC for stem rust, Agronomic and Phonological traits.

Nurseries	Season	Area under disease progress curve	Hectolitre weight (Hl Kg^{-1})	Kernel weight	Biomass	Plant height	Spike length	Time to heading	Time to Flowering	Time to maturity	Grain filling period
				g		cm		days			
All nurseries(Combined) (N=748)	2015	238.89 a	44.51 b	16.37 b	215.19 b	71.33 b	9.04 b	65.53 b	69.14 b	104.55 b	39.33 b
	2016	131.92 b	48.82 a	23.61 a	558.63 a	100.95 a	10.26 a	72.20 a	75.32 a	119.16 a	46.96 a
	MSD	5.73	0.47	0.34	8.27	0.58	0.08	0.39	0.38	0.34	0.44
1st Stem Rust Resistance Screening Nursery (N=103)	2015	249.19 a	40.48 b	14.47 a	225.68 b	72.79 b	9.11 b	67.44 b	70.64 b	103.44 b	36.00 b
	2016	132.04 b	46.63 a	18.88 b	470.45 a	97.37 a	9.97 a	73.35 a	75.86 a	118.81 a	45.46 a
	MSD	16.73	1.39	0.89	21.96	1.67	0.23	1.55	1.38	1.07	1.59
3rd Stem Rust Resistance Screening Nursery (N=110)	2015	212.45 a	38.48 b	13.41 b	168.55 b	71.09 b	9.31 b	69.91 b	73.60 b	105.68 b	35.77 b
	2016	112.59 b	47.23 a	22.11 a	499.22 a	101.81 a	10.33 a	74.30 a	77.41 a	120.25 a	45.95 a
	MSD	13.99	1.32	0.96	23.19	1.19	0.22	1.01	1.05	0.89	1.10

Cont. Table 3.2

Nurseries	Season	Area under disease progress curve	Hectolitre weight (Hl Kg^{-1})	Kernel weight	Biomass	Plant height	Spike length	Time to heading	Time to Flowering	Time to maturity	Grain filling period
5th Stem Rust Resistance Screening Nursery (N=135)	2015	269.73 a	43.04 b	16.53 b	193.32 b	70.15 a	9.09 b	64.01 a	68.15 a	104.12 b	40.11 b
	2016	144.02 b	49.41 a	23.59 a	544.12 a	102.32 b	10.47 a	71.55 b	75.34 b	118.11 a	46.56 a
	MSD	13.09	1.07	0.76	18.85	1.71	0.22	0.98	0.75	0.77	0.99
7th Stem Rust Resistance Screening Nursery (N=150)	2015	260.75 a	41.02 b	15.07 b	196.04 b	71.13 b	9.29 b	66.82 b	70.77 b	104.10 b	37.28 b
	2016	150.76 b	47.06 a	22.58 a	560.49 a	99.33 a	10.28 a	71.99 a	74.74 a	119.26 a	47.27 a
	MSD	13.44	1.09	0.76	18.12	1.30	0.18	0.79	0.89	0.82	0.89
9th Stem Rust Resistance Screening Nursery (N=246)	2015	216.49 a	51.69 a	19.16 b	254.71 b	71.59 b	8.71 b	61.95 b	66.10 b	105.01 b	43.07 b
	2016	122.52 b	51.17 a	26.83 a	627.81 a	102.27 a	10.22 a	71.28 a	74.52 a	119.33 a	48.05 a
	MSD	9.93	0.77	0.59	14.66	0.93	0.11	0.51	0.55	0.53	0.52

Table 3.3 Correlation analysis for AUDPC, hectolitre weight, kernel weight, biomass for 2015 and 2016 seasons.

	AUDPC	Kernel weight	Hectolitre weight	Biomass
Season one (2015)				
AUDPC		-0.44***	-0.45***	-0.43***
Kernel weight			0.78***	0.41***
Hectolitre weight				0.43***
Biomass				
Season Two (2016)				
AUDPC		-0.29***	-0.36***	-0.53***
Kernel weight			0.69***	0.44***
Hectolitre weight				0.43***
Biomass				

*, **, *** significance at P≤0.05, P≤0.01 and P≤0.001, respectively.

include *Pavon*, *Juchi*, *Kiritati*, *Huirvis* and Kingbird were used to combine resistance from other sources to enhance the levels of resistance through additive interactions. Several of the lines were found to be effective against the race *TTKSK* in the year 2005 and 2006 however race changes with virulence to *Sr24* led to some of the lines becoming susceptible to *TTKST* (*Ug99+Sr24* Virulence) as early as 2006. Isolate within the *Ug99* lineage with virulence to *Sr36* was identified in Kenya in 2014. Virulence for another effective gene *SrTmp* was detected in Kenya in 2014 (*TTKTK*, *TTKTT*) along with Digelu race *TKTTF* introduced from Ethiopia which resulted in several of the first SRRSN becoming susceptible in 2015 and 2016 seasons.

The third SRRSN nursery had race specific genes *Sr25, Sr24+Sr36, Sr33, SrTmp, SrYananc, SrSynt, SrSha7, SrND643, SrHUW234, SrUnknown*. Even through higher frequencies of race specific genes were effective from 2005-2008, the evolution of *TTKST*, *TTKTK* and *TTKTT* along with Digelu races in the time period had resulted in some of these genes becoming moderately susceptible to susceptible to the *Ug99* race group leading to the reduction in the number of lines with low disease severity. However lines that combined good levels of APR such as *Pavon*, *Kingbird*, *Huirvis* and *Kiritati* exhibited good levels of field resistance.

The fifth SRRSN had race specific genes *Sr25, Sr26, SrND643, SrHuw234, SrSha7, SrTmp, SrTnmu, SrYanac* genes with some uncharacterized genes in different genetic backgrounds which were effective against the *Ug99* race group present in 2009, 2010 and 2011. Lines carrying *Sr31* and *Sr24* were almost negligible in the crossing block by selecting parents that were resistant to the two races of the *Ug99* lineage. Genes *SrND643, SrHuw234* showed reduced effectiveness in 2015 and 2016 largely due to the races *TTKTK*, *TTKTT* and *TKTTF* (Digelu race) which also defeated one of the popular variety "Robin" in Kenya carrying stem rust gene *SrTmp*. Virulence for *SrTmp* was identified for both the *Ug99* lineage and Digelu race which compounded vulnerability of several breeding materials in 2015 and 2016.

Seventh SRRSN and ninth SRRSN had *Ug99* effective race specific resistant genes that were predominant in the crosses including *Sr22, Sr25, shortened Sr26, Srtmp, SrHUW234, SrND643, SrYanac, SrBavis* and some uncharacterized genes. APR sources include *Danphe*, improved *Danphe* crosses, *Kingbird*, *Kiritati*, *Huirivis* and several APR+ Moderately effective race specific resistance genes showed enhanced levels of resistance. In order to enhance the diversity of resistance several old tall Kenyan varieties (*Kenya Swara, kenya Fahari, Kenya Kudu, Kenya Nyangumi*) which displayed good levels of APR were crossed to several high yielding lines at CIMMYT and breeding efforts were successful in combining additive effect

APR in high yielding backgrounds including variety *Borlaug 100* and one such variety is named *Kasuku* which is under further testing under NPT trails by KEPHIS. Some of the CIMMYT APR lines such as *Kingbird*, *Kiritati*, *Juchi*, *Pavon* and *Paruala* which were developed in 2005 are still effective towards stem rust and are conferring moderate to high levels of field resistance against the *Ug99* race group and Digelu race group confirming long term durability can be achieved by deploying race nonspecific APR genes.

The lines tested reacted differently to stem rust in the two seasons. This could be due to the variations in weather conditions in the two seasons which influenced the interaction between stem rust pathogen and wheat (Hellen *et al*., 2016). Due to variation in infection rates in different lines, it may be concluded that, the ones which exhibited low infection rates had effective APR genes therefore were able to resist disease infection and establishment while those that revealed high infection rates had ineffective APR genes or lacked APR genes.

The hectolitre and kernel weights obtained in the 2016 season were higher than of 2015 and this confirms that wheat indeed performs better in the main (2016) than off (2015) season. This is because stem rust infection in 2015 season was much higher than in 2016 season. At physiological level, the higher infections on plants meant they expended more energy in defense mechanisms rather than in growth and grain formation (Smedeguard-Petersen and Tolstrup, 1985). Moreover, the tissue damage caused by hypersensitive reactions also contributes to kernels reduction (Khanna *et al*., 2005). Variation in hectolitre weight and thousand kernel weight could also have been attributed to by the variation in environmental conditions between the two seasons as earlier suggested. This therefore demonstrated that, the environmental conditions could affect the grain physical characteristics and hence kernels thereby impacting on end use quality. Environmental conditions has a significant impact on thousand kernel weight and hectolitre weight of various wheat genotypes (Lopes *et al*., 2012; Li *et al*., 2013). In addition, water deficit and elevated temperatures above average during grain filling period experienced during 2015 season could have led to reduction in thousand kernel weight and hectolitre weight as similar findings were demonstrated by Erekul and Kohn, (2006) in winter wheat. In 2016 season, lines grew taller as compared to 2015 season, in addition plants in 2016 season had longer spikes as compared to those in off season. This could have been due to the favourable environmental conditions in the main season, also, plant height and spike length depends on lines as different lines had different plant height and spike length.

Wheat lines which were grown in the 2015 season took few days to reach physiological maturity as compared to those in the 2016 season due to the limited amount of rainfall received in 2015. Duration of the crop cycle is affected by water deficit which accelerates senescence. This is due to early expression of the genes associated with remobilization of proteins which are redirected from leaves to the reproductive organs (Pic *et al*., 2002). The reduction in the duration of the crop cycle is an adaptive mechanism, since it allows the plant to complete its life cycle earlier while there is still water in the soil and redirects assimilates to the reproductive organs. Therefore reducing the total intercepted light and biomass accumulation as it was observed in the results where biomass in off season was less than that in main season. It may also affect seed weight as it was found in this experiment that yield in 2015 season was less than that in 2016 season.

The AUDPC was negatively correlated to grain yield and yield components, this reveals that, an increase in AUDPC leads to reduction in yield, kernel weight, hectolitre weight and biomass. Since grain yield loss is strongly correlated with AUDPC, high levels of partial resistance are needed to prevent significant grain yield loss (Ochoa and Parlevliet, 2007). In addition, significant correlation exists between mean disease severity and percentage loss for a thousand kernel weight and grain yield per plant (El-Shamy *et al*., 2011).

3.5 Conclusions

The basis of this field experiment was to determine if there was any progress made in the use of APR as a breeding strategy to manage wheat stem rust. From the results, it was noted that there was progressive yearly rise in the number of lines with low stem rust severity. However, in 2015, this number declined slightly due to what is suspected to be the emergence of new and more virulent races such as *TTKTK*, *TTKST* and *TTTSK* which could have overwhelmed the existing resistance, also because some of the genes had lasted more than five years. It is evident therefore that APR is effective in slowing infectivity of the existing races leading to more resistant lines progressively. Therefore, there is progress in resistance to stem rust in using the APR breeding strategy in the management of stem rust in wheat. The results further showed that stem rust significantly reduces grain yield as was observed between the two seasons where disease pressure was varied/high. It was further observed that those lines with effective APR genes consistently yielded better than those without. Thus, these lines can be advanced and eventually released as APR bearing cultivars or used subsequently for breeding as sources of effective APR genes to the traditional susceptible wheat cultivars.

CHAPTER FOUR

SEEDLING AND ADULT PLANT RESISTANCE OF CIMMYT WHEAT (*Triticum aestivum L.*) LINES TO STEM RUST (*Puccinia graminis* f. sp. *tritici*) RACE *Ug99* AND ITS VARIANTS

Abstract

Stem rust race *Ug99* and its variants are virulent to a large number of resistant genes present in widely grown wheat (*Triticum aestivum L.)* cultivars. This study was conducted to evaluate (i) seedling reaction to four stem rust races *TTKSK, TTKST, TTKTK* and *TTTSK* (ii) adult plant reaction to the four races and (iii) the rate of development of the stem rust races on CIMMYT wheat lines. The evaluation was conducted in the greenhouse with the adult plant resistance experiment laid in a randomized complete block design (RCBD). Out of the 39 lines evaluated, only *SRG21*, *SRG34* and *SRG39* showed a reaction of 3 to race *TTKST*, *SRG22* exhibited a reaction of 3 to race *TTKTK*, *SRG25*, *SRG32*, *SRG36* and *SRG37* displayed a reaction of 3 to race *TTKSK* and *SRG27* and *SRG39* showed a reaction of 3 to race *TTTSK* the rest revealed infection types of between 0 and 2. In evaluation of lines for adult plant reaction to stem rust race *TTKST*, only four lines (0.13%) exhibited disease severity of ≤5% while 99.87% (35 lines) of the lines exhibited a severity of ≥10%. In contrary, 17 (43.59%) lines showed a severity of ≤5% while 56.41% showed a severity of ≥10% to races *TTKTK* and *TTKSK*. 46.15% of the lines (18 line) demonstrated a severity of ≤5% while 53.85% of the lines (21 lines) demonstrated a severity of ≥10% to race *TTTSK.* All lines revealed a moderately susceptible to susceptible (MSS) response to race *TTKST* and *TTKTK* except for lines *SRG13* which was moderately susceptible (MS) to race *TTKST* and *SRG27* which was MS to race *TTKTK*. For race *TTKSK*, 82.05% of the lines exhibited a MSS response, 5% exhibited a MS response, 7% exhibited a moderately resistant/ moderately susceptible (M) response and 2.56% exhibited a susceptible (S) response while for race *TTTSK*, 84.61% showed a MSS response, 10.25% revealed moderately resistant (MR) and 5.12% exhibited MS responses. The rates of increase in spore sizes for all the lines inoculated with the four races were different with same lines inoculated with different races showing different rates of increase. Line *SRG35* had the highest rate of increase in spore size of 5.72 mm/day with race *TTTKSK* but the same line had the lowest rate in spore size increase of 0.60 mm/day with race *TTKTK.* Lines *SRG7*, *SRG13*, *SRG24* and *SRG35* showed low final disease severity, low infection types and low AUDPC with all the four races therefore can be released as new varieties.

4.1 Introduction

Stem rust race *Ug99* and its variants are major constraint to wheat (*Triticum aestivum L.)* production across wheat growing regions in Africa as they are capable of causing yield losses of 80 to 100% in susceptible cultivars (Singh *et al*., 2011). The wheat growing regions of Eastern Africa are proven to be one of the major origins of new stem rust races of wheat (Singh *et al.*, 2006). For the last five decades, the devastating effects of stem rust races were reduced by deploying resistant genes derived from bread wheat (AABBDD 2n=6x=42) and secondary gene pool of wild relatives (McIntosh *et al*., 1995; Mago *et al*., 2011; Haile *et al*., 2013). However, widely deployed resistance genes *Sr24*, *Sr31*, and *Sr36* have been rendered ineffective to the stem rust by the emergence of the highly virulent race *TTKSK* and its variants (Pretorius *et al*., 2000; Jin *et al*., 2008; 2009). Currently, at least eleven variants of *TTKSK* have been described that are virulent to *Sr9h, Sr13, Sr1RS^{Amigo}* and *SrTmp* (Jin *et al*., 2008; Rouse *et al*., 2014; Patpour *et al*., 2016).

In most wheat breeding programs, the best and preferred strategy is to develop cultivars with durable resistance genes (Kolmer 1996; Singh *et al*., 2005). Two categories of resistance genes have been widely recognized in wheat breeding for rust resistance; Seedling resistance which is conferred monogenically and adult-plant resistance (APR) which is conferred polygenically (Chen, 2005). Several qualitative *Sr* genes are race specific and have been mapped on specific chromosomes (Jin *et al*., 2007; Singh *et al*., 2011). Qualitative genes are phenotyped as present or absent by observing the characteristic low or high infection types displayed by them (Jin *et al*., 2007). They are usually characterized by a hypersensitive reaction upon stem rust infection, and usually confers a high level of resistance that is effective in all stages of plant development (Park *et al*., 2011). A major risk associated with the utilization of qualitative resistance genes is their ability to become susceptible when they are deployed alone in wheat cultivars. This has been demonstrated by *Sr24*, *Sr36*, and resistance in cultivar (*cv.*) 'Matlabas' becoming susceptible to race *TTKSK* (Pretorius *et al*., 2012; Kolmer and Acevedo, 2016).

The repeated appearance of new and more virulent races of the pathogen population through a single-step mutation and/or sexual recombination events brought on by single gene deployment in wheat led to the alternative strategy of combining or "pyramiding" multiple major resistance genes into cultivars (Dangl and Jones, 2001; Pink, 2002). This strategy has been effective in controlling stem rust since the 1950s in the northern Great Plains of North America and Australia (Line and Chen, 1995; Leonard and Szabo, 2005; Park, 2007).

Polygenically inherited resistance is often based on multiple minor genes and slows down pathogen infection and colonization in adult wheat plants (Gustafson and Shaner, 1982; Kolmer 1996; Collins *et al*., 2007). APR is typically identified by phenotyping wheat plants at the seedling stage in the greenhouse, then subsequently evaluating adult plants in the field (Ellis *et al*., 2014). However, the accuracy of phenotyping in the field can be compromised by the effects of environmental factors such as weather patterns, inoculum pressure, sequential infection, differences in plant maturity and the presence of other diseases that influence the expression of APR genes upon infection (Hickey *et al*., 2012). The APR type of resistance can be more durable than single gene resistance due to the race non-specificity of the resistance genes involved (Singh *et al*., 2000; 2005). So far, about five APR genes have been characterized and catalogued in wheat (Krattinger *et al*., 2009; Herrera-Foessel *et al*., 2011; 2012; Bansal *et al*., 2014). These genes are *Sr2*, *Sr55* (*Lr67/Yr46/ Pm46*), *Sr56*, *Sr57* (*Lr34*/*Yr18*/*Pm38*), and *Sr58* (*Lr46/ Yr29/Pm39*) confers resistance against stem rust that are polygenically inherited in wheat (William *et al*., 2006; Lillemo *et al*., 2008; Krattinger *et al*., 2009; Herrera-Foessel *et al*., 2014; Kolmer *et al*., 2015).

The genes *Sr2, 9h, 13, 14, 15, 21, 22, 24, 25, 26, 27, 28, 29, 32, 33, 35, 36, 37, 39, 40, 42, 43, 44, 45, 46, 47, 50, 51, 52, 53, 55, 57, 58, Huw234, ND643, Yae, SrTA10171, SrTA1662, SrTA10187, SrTmp, and Sr1RS*^{Amigo} are effective to at least one pathotype within the *Ug99* race group (Marais and Marais, 1994; Mago *et al*., 2004; Jin *et al*., 2008; Pumphrey *et al*., 2012; Mujeeb-Kazi *et al*., 2013; Rouse *et al*., 2014; Kielsmeier-Cook *et al*., 2015; Singh *et al*., 2015). However, *Sr9h, Sr21, Sr24, Sr36* and *SrTmp* have failed to confer resistance to individual *Ug99* races, while effective to others (Patpour *et al*., 2016). Moreover, not all genes aforementioned can be introgressed into plants due to inadequate protection levels in adult plants, occurrence of virulence in other *Pgt* races, or undesirable linkage drag (Singh *et al*., 2015). The objectives of this study were therefore to evaluate seedling and adult plant reaction to four stem rust races *TTKSK, TTKST, TTKTK* and *TTTSK* and the rate of development of the stem rust races on CIMMYT wheat lines.

4.2 Materials and Methods

4.2.1 Stem rust sample collection and purification.

Single spores used for race purification were collected from *cvs. Cacuke*, *Kwale*, *Kingbird* and *Robin* planted at KALRO, Njoro trap nursery. These *cvs.* were used because they are highly susceptible to the stem rust races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK*. The fresh single spores were collected into gelatine capsules from the leaves and stems of the infected plants. The purification was done in the greenhouse where the rust races *TTKST*, *TTKSK*,

TTTSK and *TTKTK* were purified on *cvs. Cacuke (Sr24)*, *Kwale (Sr31)*, *Kingbird (Sr36)* and *Robin (SrTmp)*, respectively. The *cvs.* were planted in plastic pots of 6 cm-diameter and height of 6 cm. The pots were filled with approximately 127 cm^3 of vermiculite, levelled and 10 seeds of each *cv.* planted in 15 pots at an approximate depth of 1 cm. The pots were watered to field capacity and placed in greenhouse growth chamber. The inoculum was then prepared when the plants had reached GS 12 by suspending single spores collected from each *cv.* from the trap nursery in distilled water at a concentration of 4×10^{10} spore per ml. Seedlings were sprayed with the inoculum using a hand sprayer as a fine mist at a distance of 30 cm from the sprayer to the plants. They were then placed in an incubation chamber with the incubator maintained at 16-18 ^{o}C temperature for 24 hours for sporulation to take place. Pots were then moved to greenhouse bench where conditions were regulated at 12 hours (h) photoperiod, at temperature of 18 to 25 ^{o}C and relative humidity of 60 to 70%. Light was provided by fluorescent tube at a distance of 1.1 m above the plants and disease infection was monitored. A second set of *cvs. Cacuke*, *Kwale*, *Kingbird* and *Robin* was planted in plastic pots mentioned earlier for the increase of the spores. Single pustules were collected from the first set 14 days post inoculation, inoculum prepared from them and inoculated onto the second set. Pure fresh spores were then collected from the second set, suspended in distilled water and the solution mixed well which was used to inoculate the experimental lines.

4.2.2. Evaluation of wheat lines for seedling resistance

Thirty five wheat lines selected from lines used in field experiment (Coded as stem rust gain (*SRG* 1-35)) together with 4 checks (*SRG36, SRG37, SRG38* and *SRG39*) were planted in 6 cm-diameter plastic pots in a greenhouse. Each pot was filled with approximately 127 cm^3 of vermiculite, levelled and 5 seeds from each line planted at an approximate depth of 1 cm per pot. The seeds were then watered to field capacity and pots placed in the growth chamber of the greenhouse. Inoculation with races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK* was done by hand spraying when plants were at GS 12 in the inoculation chamber using the inoculum prepared following the procedure explained in section 4.3.1. The inoculated plants were then placed in an incubation chamber under natural light at 16-18 ^{o}C for 24 h after which they were moved to a greenhouse bench with a 12 h photoperiod, at temperature of 18 to 25 ^{o}C and relative humidity of 60 to 70% for disease evaluation after 14 days post inoculation. Seedlings were evaluated based on a 0-4 scale of infection types adopted from Stakman *et al*. (1962) where 0-2 is low infection type (IT), therefore resistant and 3-4 is high IT, therefore susceptible.

4.2.3 Evaluation of wheat lines for adult plant resistance

The same lines used in the seedling resistance experiment were planted in 13 cm-diameter plastic pots and placed in a greenhouse. Each pot was filled with about 1145 cm^3 of soil levelled and 5 seeds of each line planted into each pot at an approximate depth of 1 cm in a randomized complete block design (RCBD) with two replicates. Diammonium phosphate (DAP) was applied at the rate of 57 mg pot^{-1} at sowing to provide an equivalent of 10 mg N pot^{-1} and 11 mg P pot^{-1}. Calcium Ammonium Nitrate (CAN) was applied at stem elongation stage (GS 30) (Zadoks *et al*., 1974) at the rate of 38 mg pot^{-1} to supply a booster of 9.8 mg N pot^{-1}. The plants were then inoculated with races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK* at booting stage GS 41-47 with inoculum prepared using the procedure explained in section 4.2.1. The inoculation was done by injecting each and every plant using a syringe in all the pots in the evening when conditions were favourable for spore germination to create an artificial disease epidemic and ensure uniform inoculum dissemination.

4.2.4 Data collection

Wheat lines were evaluated for severity on a scale of 0% (immune) to 100% (completely susceptible) depending on the area affected by stem rust, following a modified Cobb scale (Peterson *et al*., 1948). Infection responses were based on the size of stem rust pustules and amount of associated chlorosis and necrosis. Infection response categories: Resistant (R), moderately resistant (MR), intermediate (M), moderately susceptible (MS) and susceptible (S) according to Roelfs *et al*., 1992. Overlapping infection response categories were noted when two different infection responses occurred on a single stem (MR-MS ratings indicating MR pustules on the same stem as MS pustules). The predominant category was listed first such that MR-MS differs from MS-MR. Evaluation was done at 7 days intervals beginning at the time when disease was first observed up to plant maturity (GS 70-89) (Zadoks *et al*., 1974). From two marked plants per line, spore area was estimated. The length and width of random uredinia per stem was measured and the same spore was traced until the day uredinium appearance stabilized as shown in Figure 4.1.

Figure 4.1 Marked spore/uredinium of stem rust on line *SRG12* measured until it stabilized.

Biomass, thousand kernel weight and yield were also measured. Harvest index was then computed by dividing the yield by total biomass.

4.2.5 Data analyses for adult plant reactions in the greenhouse

Since the spores were assumed to be spherical, Uredinium size was calcutated according to the formula by Lee and Shaner (1985)

$$Uredinium\ size = Length(mm) \times Width(mm) \times \prod /4 \quad (1)$$

Size of stem rust spores of lines inoculated with the four races were then compared. Four sister lines (sets A and B) were selected and regression analysis conducted to show the rate of progress in the spore area in the four races. Area under the disease progress curve for adult plant was computed using the formula by Wilcoxson *et al*. (1975) and AUDPC CIMMYT programme (CIMMYT, 2008).

$$AUDPC = \sum_{i}^{n-1} \left[\left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i) \right] \quad (2)$$

Where; t_i is the time in days of each reading, y_i is the percentage of affected part of the plant at each reading, n is the number of readings, $t\ (i+1)$ is the second assessment date of two consecutive assessment, $y\ (i+1)$ is the disease severity on assessment date $t\ (i+1)$.
Analysis of variance was done using SAS by applying the statistical equations below:

$$Y_{ijk} = \mu + R_i + G_j + B_k + GB_{jk} + \varepsilon_{ijkl} \quad (3)$$

Where Y_{ijkl} is the observation of experimental units, R_i is the effect due to i^{th} replicate, G_j is the

effect due to line in the j^{th} line, B_k is the effect due to k^{th} race, GB_{jk} effect due to interaction between j^{th} line and k^{th} race and ε_{ijkl} is the random error effect.

To get the effect of number of days after planting, the following model was used:

$$Y_{ijklm} = \mu + R_i + G_j + B_k + GB_{jk} + S_l + GBS_{jkl} + \varepsilon_{ijklm} \quad (4)$$

Where Y_{ijklm} is the observation of experimental units, R_i is the effect due to i^{th} replicate, G_j is the effect due to j^{th} line in the i^{th} replicate, B_k is the effect due to k^{th} race in the j^{th} line, GB_{jk} effect due to interaction between j^{th} line and k^{th} race, S_l is the effect due to l^{th} number of days after planting, GBS_{jkl} effect due to interaction among j^{th} line, k^{th} race and l^{th} number of days after planting and ε_{ijklm} is the random error effect.

Mean comparisons was done based on LSD procedure at 5% probability to separate the different wheat genotypes using the formula:

$$LSD = t_{\alpha/2}\sqrt{\frac{2MSE}{r}} \quad (5)$$

Where t is the error degree of freedom, r is the number of replicate, MSE is the mean square error (Gomez and Gomez, 1984).

Standard error (SE) for the means was computed using the formula: $SE = \frac{\sigma}{\sqrt{n}}$ (6)

Where σ is the standard deviation and n is the sample size

4.3 Results

4.3.1Analysis of variance for AUDPC, spore/uredinium area, yield and yield components

There were significant (P≤0.001) effects due to line, race and line×race for area under disease progress curve, yield, kernel weight and harvest index. In addition, line and race effects were significant (P≤0.01 and P≤0.001, respectively) for biomass (Table 4.1). Effects due to line, race, line×race and line×race×stage were significant (P≤0.001) for length of the spore, width of the spore and area of the spore (Table 4.2).

4.3.2 Effects of stem rust races *TTKSK*, *TTTSK*, *TTKTK* and *TTKST*

Among the races evaluated, *TTTSK* exhibited the highest mean AUDPC of 153.64 while *TTKSK* exhibited the lowest mean AUDPC of 121.31. Sine *TTTSK* is a variant of *TTKSK*, there was an increase of 26% AUDPC. In an attempt to determine the effect of the four races on yield, kernel weight and biomass, Lines which were inoculated with race *TTKSK* showed the highest mean yield of 1.13 g/plant while those that were inoculated with race *TTKTK*

displayed the lowest mean yield of 0.46 g/plant. Highest mean kernel weight and biomass of 11.25 g/plant and 4.52 g/plant, respectively were observed on plants which were inoculated with race *TTTSK* while lowest mean kernel weight and biomass of 4.47 g/plant and 2.85 g/plant, respectively were observed on plants which were inoculated with race *TTTSK*. Highest harvest index of 0.29 was detected on plants inoculated with race *TTKSK* but its spore area of 3.54 mm^2 was the smallest while *TTKST* exhibited the lowest harvest index of 0.16 but its spore area of 4.29 mm^2 was the biggest (Table 4.3). Check cultivar *SRG39* -revealed the highest mean AUDPC of 518.33 which was significantly higher than that for line *SRG33* of 195.77. This revealed a difference of 61.28% between the worst performed check and the worst performed line. Line *SRG3* displayed the lowest mean AUDPC of 65.58 which was 67.31% lower than that for *SRG33*. Largest spore area of 7.25 mm^2 was observed on line *SRG25*, this was 6.76% higher than that for the check which displayed the largest area (*SRG37* 6.76 mm^2) while smallest spore area was observed on line *SRG7* of 0.88 mm^2 (Table 4.4).

The lines showed varying levels of resistance to the four stem rust races with most lines showing more than one infection types to the same race and same line exhibiting different infection types with different races at seedling stage.

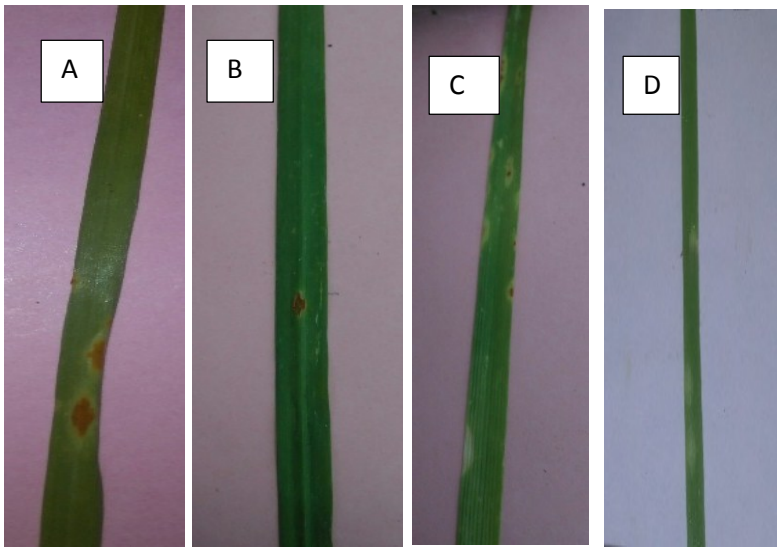


Figure 4.2 Leaves of line *SRG12* showing different infection types of 3, 2, 1 and ; (fleck) with races (A) *TTKTK* (IT 3), (B) *TTKSK* (IT 2), *(C) TTTSK* (IT 1) and (D) *TTKST* (fleck), respectively.

Table 4.1 Mean squares from combined analysis of variance of thirty five wheat (*Triticum aestivum*) lines from five CIMMYT SRRSN and four checks for AUDPC for stem rust, yield, thousand kernel weight, biomass and harvest index.

Source of variation	df	Expected mean squares	Area Under Disease Progress Curve	Yield	Kernel Weight	Biomass	Harvest index
Replicate	1	$\sigma_\varepsilon^2 + 312\sigma_\varepsilon^2$	26972.79	3.64	256.37	27.40	9.11
Line	38	$\sigma_\varepsilon^2 + 8\sigma_{LB}^2 + 16\sigma_L^2$	40648.04***	1.28***	147.82***	10.62**	0.72***
Race	3	$\sigma_\varepsilon^2 + 156\sigma_B^2$	16633.54***	95.23***	9416.07***	445.49***	9.11***
Line×Race	114	$\sigma_\varepsilon^2 + 8\sigma_{LB}^2$	6796.19***	1.00***	118.68***	5.99	0.42***
Residual	155	σ_ε^2	1218.49	0.23	22.29	5.14	0.15
CV			24.59	20.98	19.47	21.49	23.24
R^2			0.93	0.92	0.93	0.75	0.84

*, **, *** significance at P≤0.05, P≤0.01 and P≤0.001, respectively.

Test H=Line E=Line×Race, Test H=Line×Race E=Random error component, Test H=Race E= E=Random error component.

Table 4.2 Mean squares from combined analysis of variance of thirty five wheat (*Triticum aestivum*) lines from five CIMMYT SRRSN and four checks for spore/uredinium length, width and area.

Source of variation	df	Expected mean squares	Length of spore	Width of spore	Area of spore ($\times 10^6$)
Replicate	1	$\sigma_\varepsilon^2 + 1560\sigma_R^2$	4308.67	329.54	5.23
Line	38	$\sigma_\varepsilon^2 + 80\sigma_L^2$	103096.68***	499.37***	97.60***
Race	3	$\sigma_\varepsilon^2 + 780\sigma_B^2$	64198.80***	1762.85***	39.87***
Line×Race	114	$\sigma_\varepsilon^2 + 20\sigma_{LB}^2$	57725.43***	357.76***	41.90***
Stage	4	$\sigma_\varepsilon^2 + 624\sigma_S^2$	2363239.66***	25096.13	1949.63***
Line×Race×Stage	620	$\sigma_\varepsilon^2 + 4\sigma_{LBS}^2$	5277.85***	64.31***	6.72***
Residual	779	σ_ε^2	1624.27	25.56	0.36
CV			26.86	20.99	15.60
R^2			0.95	0.91	0.98

*, **, *** significance at P≤0.05, P≤0.01 and P≤0.001, respectively.

Random error component was used as error term for all the variables

Also, line *SRG12* displayed more than one infection type of 2 and 1 on the same leaf with race *TTKSK* as shown in Figure 4.3.

Figure 4.3 Line *SRG12* showing more than one infection types on the same leaf

The seedling infection types (IT) ranged from 0 to 2 except for a few lines which showed susceptible response (IT 3). Out of the 39 lines evaluated, only *SRG21, SRG34* and *SRG39* showed a susceptible reaction (IT 3) to race *TTKST* while *SRG22* exhibited a susceptible reaction (IT 3) to race *TTKTK*, *SRG25, SRG32, SRG36* and *SRG37* displayed a susceptible reaction (IT 3) to race *TTKSK* and *SRG27* while *SRG39* revealed a susceptible reaction (IT 3) to race *TTTSK* the rest showed infection types of between 0 and 2 (Table 4.5).

In evaluation of lines for adult plant reaction to stem rust race *TTKST*, only four lines exhibited disease severity of ≤5% while 35 lines exhibited a severity of ≥10%. In contrary, 17 lines showed a severity of ≤5% while 22 lines showed a severity of ≥10% to races *TTKTK* and *TTKSK*. Eighteen lines displayed a severity of ≤5% while 21 lines displayed a severity of ≥10% to race *TTTSK*. All lines revealed a MSS response to race *TTKST* and *TTKTK* except for lines *SRG13* which was moderately susceptible (MS) to race *TTKST* and *SRG27* which was MS to race *TTKTK*. For race *TTKSK*, 82.05% of the lines exhibited a MSS response, 5% exhibited a MS response, 7% exhibited a moderately resistant/ moderately susceptible (M) response and 2.56% exhibited a susceptible (S) response while for race *TTTSK*, 84.61% displayed a MSS response, 10.25% showed moderately resistant (MR) and 5.12% showed MS responses (Table 4.5).

Among the lines inoculated with race *TTKST*, *SRG39, SRG16, SRG27, SRG28* and *SRG12* displayed increase in spore sizes at the rates of 0.61 mm/day, 1.31 mm/day, 1.54 mm/day, 7.48 mm/day and 1.58 mm/day respectively. Spore sizes of lines *SRG28* and *SRG39* did not increase from the 92nd day to 99th day and 85th to 92nd day, respectively. Line *SRG39*

spore size later increased after 92nd day to 99th day. All lines showed faster increase in spore sizes than that for the check (*SRG39*) (Figure 4.4a). Lines *SRG36, SRG16, SRG27, SRG28* and *SRG12* inoculated with race *TTKTK* showed increase in size of spores at the rates of 6.22 mm/day, 1.31 mm/day, 2.20 mm/day, 2.32 mm/day and 2.13 mm/day, respectively (Figure 4.4b). The rate of increase in spore sizes of lines *SRG39*, *SRG12*, *SRG28*, *SRG27* and *SRG16* with race *TTKSK* were 0.61 mm/day, 3.17 mm/day, 1.54 mm/day, 4.69 mm/day and 1.40 mm/day, respectively. The spore size of line *SRG39* stopped increasing between days 85 and 92 but again increased after 92 days (Figure 4.4c). Lines *SRG37, SRG16, SRG27, SRG28* and *SRG12* exhibited increase in spore sizes at the rates of 1.14 mm/day, 3.14 mm/day, 2.90 mm/day, 9.82 mm/day and 4.94 mm/day, respectively when they were inoculated with race *TTTSK* (Figure 4.4d).

Among the lines inoculated with race *TTKST*, *SRG29, SRG34, SRG35* and *SRG30* showed continuous increase in spore sizes at the rates of 7.46 mm/day, 1.98 mm/day, 1.86 mm/day and 3.02 mm/day, respectively. In contrary, line *SRG39* showed increase in spore size at a rate of 0.61 mm/day but between days 85 and 92, the spore size remained constant but later increased after 92 days (Figure 4.5a). Lines *SRG29, SRG34, SRG30, SRG35* and *SRG36* exhibited increase in spore sizes at the rates of 1.12 mm/day, 0.90 mm/day, 0.74 mm/day, 0.60 mm/day and 6.22 mm/day, respectively with race *TTKTK* but spore size of line *SRG35* stopped increasing after 92 days (Figure 4.5b). For race *TTKSK,* lines *SRG29, SRG34, SRG30, SRG35* and *SRG39* showed increase in spore sizes at the rates of 0.38 mm/day, 2.80 mm/day, 4.34 mm/ day, 1.33 mm/day and 0.61 mm/day, respectively but spore size of line *SRG39* stopped increasing between days 85 and 92 but later increased after 92 days (Figure 4.5c). Lines *SRG29, SRG34, SRG30, SRG35* and *SRG37* exhibited increase in spore sizes at the rates of 1.43 mm/day, 1.50 mm/day, 4.94 mm/day, 5.72 mm/day and 1.50 mm/day, respectively (Figure 4.5d). The rates of increase in spore sizes for all the lines inoculated with the four races were different with same lines inoculated with different races showing different rates of increase. For example, line *SRG35* had the highest rate of increase in spore size of 5.72 mm/day with race *TTTSK* but the same line had the lowest rate in spore size increase of 0.60 mm/day with race *TTKTK*.

4.3.3 Agronomic traits of wheat

Highest mean biomass of 4.08 g/plant was displayed by line *SRG2*, this was 9.93% lower than that for *SRG36* (check) which showed a mean biomass of 4.58 g/plant while line *SRG23* exhibited the least mean biomass of 2.90 g/plant. Line *SRG13* showed the highest mean

Table 4.3 Mean comparisons for four stem rust races for AUDPC for stem rust, yield, thousand kernel weight, biomass, harvest index and spore area on CIMMYT wheat lines.

Races	Area Under Disease Progress Curve	Yield	Kernel Weight	Biomass	Harvest index	Area
		g/plant				(mm^2)
TTTSK	153.64 a	1.12 a	11.25 a	4.52 a	0.25 b	3.78 b
TTKSK	121.31 c	1.13 a	11.23 a	3.79 b	0.29 a	3.54 c
TTKTK	142.14 b	0.52 c	5.39 b	2.89 c	0.18 c	3.77 b
TTKST	150.73 ab	0.46 b	4.47 c	2.85 c	0.16 a	4.29 a
$LSD_{0.05}$	11.04	0.05	0.49	0.24	0.04	0.08

Means bearing same letters within the same column are not significantly different according to LSD (Least significant difference).

Table 4.4 Mean comparisons for CIMMYT wheat lines for Area Under Disease Progress Curve for stem rust, yield, thousand kernel weight, biomass, harvest index and spore/ Uredinium area.

Code	AUDPC	Biomass	Kernel weight	Yield	Harvest index	Spore Area
		g/plant				(mm^2)
SRG1	108.8 k-p	3.84 a-f	10.69 a	1.09 a	0.28 c-g	5.87 d
SRG2	136.83 f-j	4.08 a-c	4.19 n	0.95 a-e	0.23 j-k	3.32 m
SRG3	65.58 q	3.38 a-c	8.37 d-h	0.74 h-l	0.22 j-l	1.01 t
SRG4	126.02h-i	3.18 a-d	7.72 f-g	0.74 h-l	0.23 d-h	4.01 h-j
SRG5	149.08 e-i	3.09 g-i	6.19 j-m	0.71 h-l	0.23 e-h	3.34 m
SRG6	149.77 d-i	3.11 f-i	8.09 d-i	0.77 f-j	0.25 c-g	4.16 g-i
SRG7	93.39 l-q	3.39 c-i	7.83 e-i	0.72 h-l	0.21 c-g	0.89 t
SRG8	117.27 i-m	3.58 c-i	8.06 d-i	0.83 c-l	0.23 j-k	2.77 p-q
SRG9	89.02 l-q	3.11 f-i	10.39 a-c	0.97 a-d	0.31 a-b	2.44 r
SRG10	77.95 o-q	3.51 c-i	9.49 b-c	0.86 b-h	0.25 a-c	2.02 s
SRG11	128.95 g-h	3.44 c-i	8.43 d-h	0.76 f-k	0.22 h-j	3.88 j-l
SRG12	138.02 f-j	3.38 c-i	7.11 h-l	0.76 f-k	0.22 h-j	4.38 f-g
SRG13	118.39 i-m	3.99 a-d	11.12 a	0.99 a-c	0.25 a-c	3.97 h-k
SRG14	112.95 j-n	3.62 c-i	8.59 d-h	0.92 b-g	0.25 a-c	3.12 m-n
SRG15	152.52 d-h	3.48 c-i	8.58 d-h	0.86 b-g	0.25 a-c	4.23 f-h
SRG16	138.08 f-j	3.46 c-i	7.78 f-i	0.76 f-k	0.22 j-l	2.56 q-r
SRG17	142.70 e-j	3.15 f-i	7.90 e-i	0.79 e-j	0.25 a-c	4.99 e
SRG18	184.08 b-d	3.32 d-i	9.06 c-g	0.93 a-f	0.28 a-c	5.71 d
SRG19	136.14 f-j	3.57 c-i	9.20 b-f	0.95 a-e	0.27 a-c	6.46 c
SRG20	161.95 c-g	3.67 c-h	8.02 e-i	0.81 d-j	0.22 j-l	4.46 f
SRG21	79.95 n-q	3.34 c-i	8.40 d-h	0.84 c-i	0.25 c-g	2.73 p-q
SRG22	150.02 d-i	3.41 c-i	8.60 d-h	0.83 c-i	0.24 j-k	2.08 s
SRG23	162.58 c-f	2.90 i	5.76 l-n	0.58 l-m	0.20 j-k	3.13 m-n
SRG24	122.95 i-m	3.04 h-i	7.49 h-j	0.71 h-l	0.23 j-l	3.20 m-n
SRG25	170.20 b-f	3.71 c-h	8.07 d-i	0.79 e-i	0.21 c-g	7.25 a
SRG26	129.64 g-k	3.63 c-i	9.19 b-f	0.92 b-f	0.25 a-c	3.95 i-k

Code	AUDPC	Biomass	Kernel weight	Yield	Harvest index	Spore Area
		g/plant				(mm^2)
SRG27	88.77 m-q	3.66 c-h	8.63 d-h	0.87 b-h	0.24 j-k	3.04 n-o
SRG28	152.33 d-h	3.15 f-i	9.50 b-c	0.93 b-f	0.29 a	5.21 e
SRG29	95.52 k-q	3.95 a-e	9.38 b-e	0.94 a-e	0.24 j-k	3.73 k-l
SRG30	95.33 k-q	3.74 b-h	7.41 h-k	0.81 d-l	0.22 j-l	2.59 p-r
SRG31	110.02 j-o	2.98 i	4.27 m	0.59 l-m	0.19 i-l	1.14 t
SRG32	75.02 p-q	3.21 e-i	7.51 h-k	0.69 i-l	0.21 c-g	2.82 o-p
SRG33	195.77 b-c	3.15 f-i	8.07 d-i	0.72 h-l	0.23 j-l	5.86 d
SRG34	108.89 k-p	4.02 a-d	9.19 b-c	1.02 a	0.25 a-c	4.01 h-j
SRG35	125.64 h-i	3.52 c-i	7.60 g-j	0.79 e-j	0.22 j-l	4.31 f-g
SRG36	202.58 b	4.47 a-b	6.74 i-l	0.65 j-l	0.15 l-m	4.15 h-j
SRG37	180.77 b-e	4.53 a	6.58 i-m	0.60 k-m	0.13 m	6.76 b
SRG38	152.52 d-h	3.98 a-d	5.99 k-m	0.76 g-k	0.19 j-l	3.69 l
SRG39	518.33 a	3.31 d-i	5.07 m-n	0.44 m	0.13 m	6.64 b-c
$LSD_{0.05}$	34.48	0.75	1.55	0.10	0.12	0.26

Means bearing same letters are not significantly different. LSD: Least significant difference.

Table 4.5 Infection types and responses of CIMMYT wheat lines to four stem rust races evaluated in the greenhouse at seedling and adult plant stages.

Code	*TTKSK*			*TTKST*			*TTTSK*			*TTKTK*		
	Final rust severity (%)	AUDPC	Seedling IT	Final rust severity (%)	AUDPC	Seedling IT	Final rust severity (%)	AUDPC	Seedling IT	Final rust severity (%)	AUDPC	Seedling IT
SRG1	25 MSS	270	0 1	10 MSS	128	; 1	30 MSS	163	1 ;	5 MSS	110	2
SRG2	20 MSS	128	1 ;	20 MSS	238	0 1	15 MSS	178	2 1	15 MSS	128	1 ;
SRG3	10 MSS	36	1 0	15 MSS	80	; 1	5 MS	22	0 ;	15 MSS	110	; 0
SRG4	5 MSS	22	0 ;	20 MSS	115	0 ;	5MS	22	0 ;	10 MSS	220	; 0
SRG5	5 MSS	110	0 ;	25 MSS	180	0 ;	10 MSS	128	; 1	15 MSS	248	;
SRG6	10 MSS	142	2 1	25 MSS	180	0 1	20 MSS	160	; 0	5 MSS	110	;
SRG7	5 MS	22	0 1	5 MSS	92	0 ;	5 MSS	110	; 0	5 MSS	110	0
SRG8	10 MSS	62	; 1	10 MSS	128	0 ;	10 MSS	110	; 0	10 MSS	110	0
SRG9	5 MSS	110	0 ;	15 MSS	54	0 ;	10 MSS	110	; 0	10 MSS	54	;
SRG10	5 MSS	110	0 ;	20 MSS	54	0 ;	5 MSS	22	0 ;	10 MSS	62	0
SRG11	10 MSS	110	1 ;	30 MSS	288	0 ;	5 MSS	110	2	5 MSS	36	; 1
SRG12	10 MSS	143	1 ; 2	10 MSS	128	0 ; 1	5 MSS	110	1	15 MSS	178	2 3
SRG13	5 MSS	110	0 ;	5 MS	36	0 ;	15 MSS	112	0 ;	5 MSS	110	1 ;
SRG14	15 MSS	110	2 2+	15 MSS	128	2 1	5 MSS	110	2	10 MSS	110	1 ;

Code	*TTKSK* Final rust severity (%)	AUDPC	Seedling IT	*TTKST* Final rust severity (%)	AUDPC	Seedling IT	*TTTSK* Final rust severity (%)	AUDPC	Seedling IT	*TTKTK* Final rust severity (%)	AUDPC	Seedling IT
SRG15	5 MR	110	1 ;	20 MSS	160	1 ;	5 MR	110	0 ;	10 MSS	160	; 0
SRG16	20 MSS	160	0 ;	25 MSS	195	0 ;	5 MSS	133	1	5 MSS	110	; 1
SRG17	5 S	110	2 1	10 MSS	128	1 0	5 MSS	128	1	10 MSS	110	2 1
SRG18	20 MSS	270	1 ;	15 MSS	160	2 1	15 MSS	128	2	10 MSS	128	2 1
SRG19	10 MSS	92	; 1	15 MSS	210	1 ;	15 MSS	183	;	5 MSS	110	1 ;
SRG20	15 MSS	160	; 1	15 MSS	145	; 1	10 MSS	128	1 0	5 MSS	92	1 ;
SRG21	5 MSS	36	0 1	15 MSS	80	2 3	5 MS	22	1 ;	5 MSS	36	2 ;
SRG22	10 MSS	110	0 1	30 MSS	195	2 1	5 MSS	110	1	20 MSS	248	2 3
SRG23	15 MSS	160	; 1	10 MSS	80	2 1	15 MSS	270	;	5 MSS	110	2 1
SRG25	5 MSS	110	3 2	20 MSS	265	; 1	15 MSS	198	1 ;	10 MSS	110	2 1
SRG26	5 MSS	110	; 1	30 MSS	283	; 1	20 MSS	178	2 ;	10 MSS	62	; 0
SRG27	10 MSS	62	1 ;	15 MSS	160	0	5 MSS	110	3	5 MS	36	; 0
SRG28	10 MSS	110	2	10 MSS	160	0 ;	10 MSS	128	0 ;	15 MSS	178	1 ;
SRG29	5 M	22	0 ;	10 MSS	110	2 1	5 MSS	22	2	5 MSS	110	1 ;
SRG30	5 M	110	2 1	10 MSS	80	2 1	5 MSS	92	0	10 MSS	110	0 ;
SRG31	10 M	62	0 1	10 MSS	160	; 0	10 MSS	92	0 ;	5 MSS	110	; 0
SRG32	5 MS	22	3	10 MSS	92	0 ;	10 MSS	62	0;	5 MSS	62	; 1

Code	*TTKSK* Final rust severity (%)	AUDPC	Seedling IT	*TTKST* Final rust severity (%)	AUDPC	Seedling IT	*TTTSK* Final rust severity (%)	AUDPC	Seedling IT	*TTKTK* Final rust severity (%)	AUDPC	Seedling IT
SRG33	5 MSS	110	1 0	20 MSS	178	1 0	5 MSS	110	1;	10 MSS	198	1 2
SRG34	5 MSS	110	0	10 MSS	62	3 2+	5 MSS	92	2 1	5 MSS	110	; 1
SRG35	5 MSS	110	0 ;	5 MSS	92	2 1	10 MSS	128	1 0	10 MSS	110	1 ;
SRG36	15 MSS	160	3	15 MSS	128	1 0	30 MSS	315	1 ;	15 MSS	215	2 1
SRG37	10 MSS	110	3 2	10 MSS	220	1 ;	10 MSS	220	1 ;	5 MSS	92	2 2+
SRG38	10 MSS	110	0	10 MSS	62	1 ;	20 MSS	220	0	5 MSS	110	0 1
SRG39	30 MSS	253	0	30 MSS	518	2 3	50 MSS	713	3	20 MR	270	1 2

MR= moderately resistant, M=moderately resistant/Moderately susceptible; MS= moderately susceptible; S= Susceptible; MSS= moderately susceptible to susceptible.

IT infection type

AUDPC Area under disease progress curve

N/B For seedling infection type, the predominant infection types were listed first.

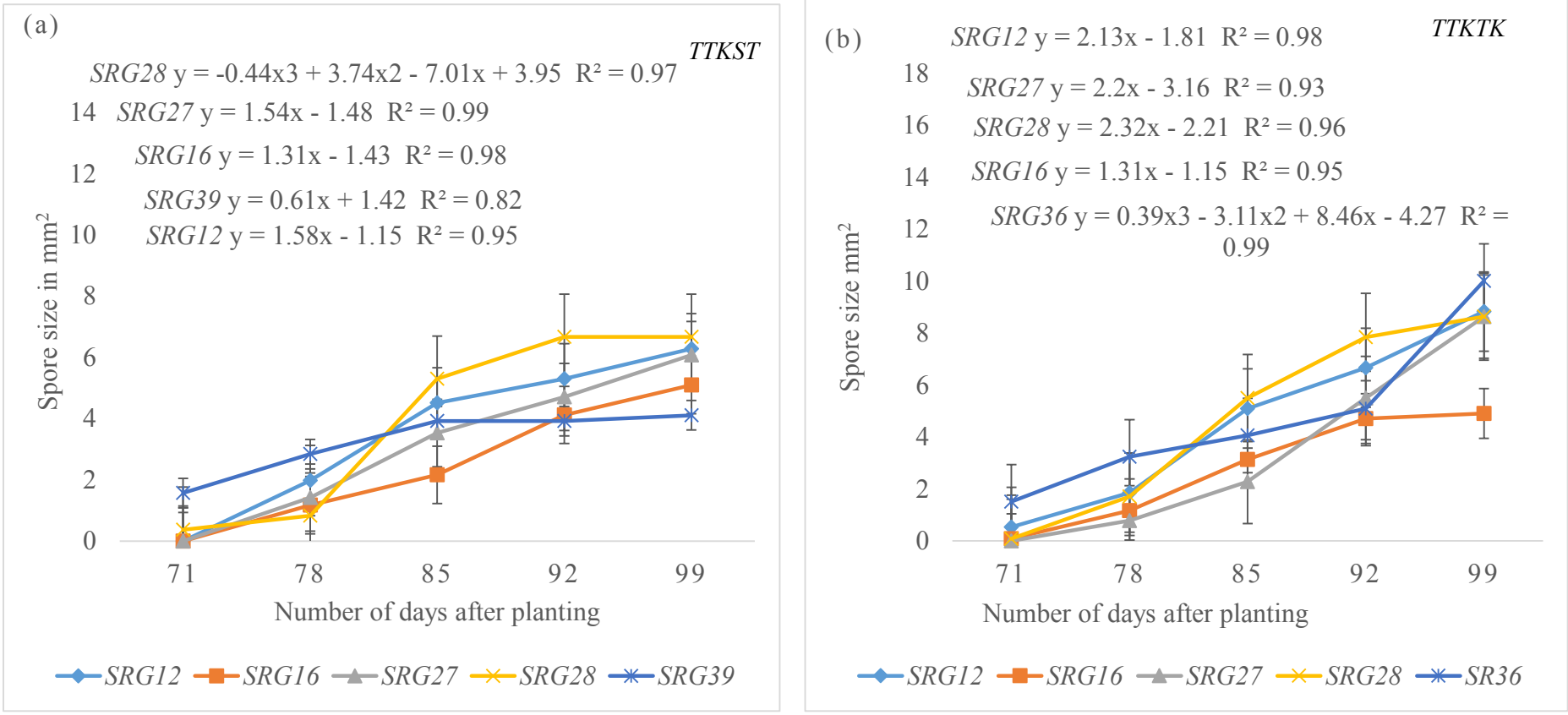
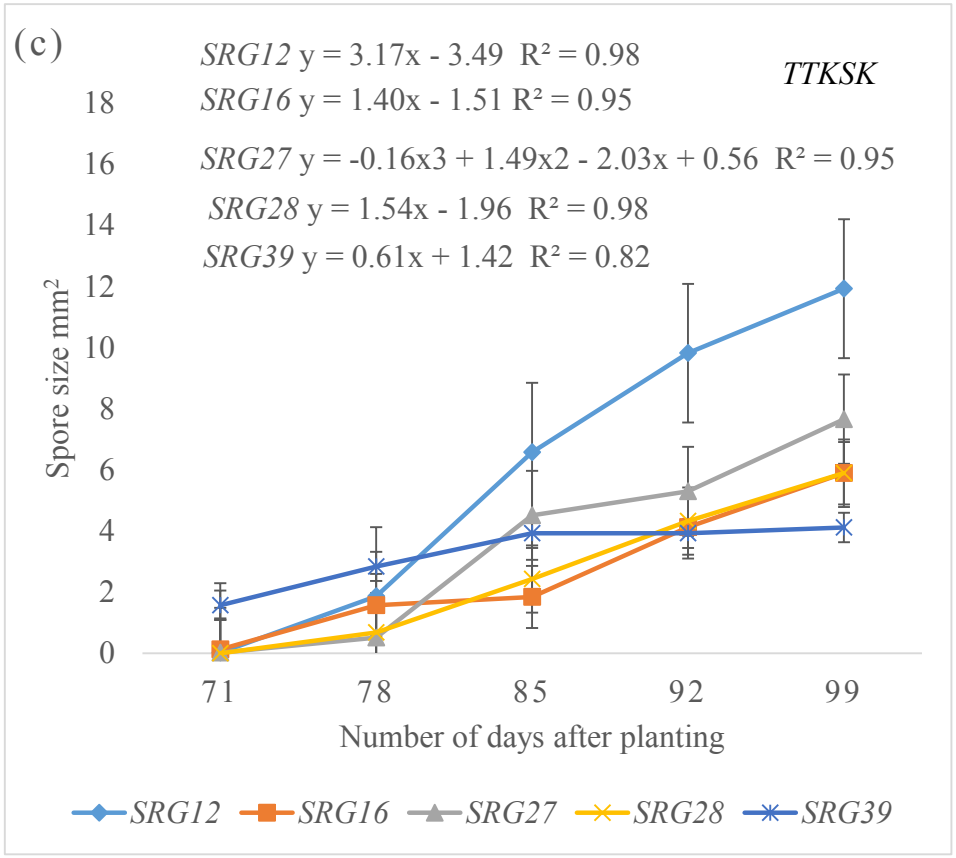
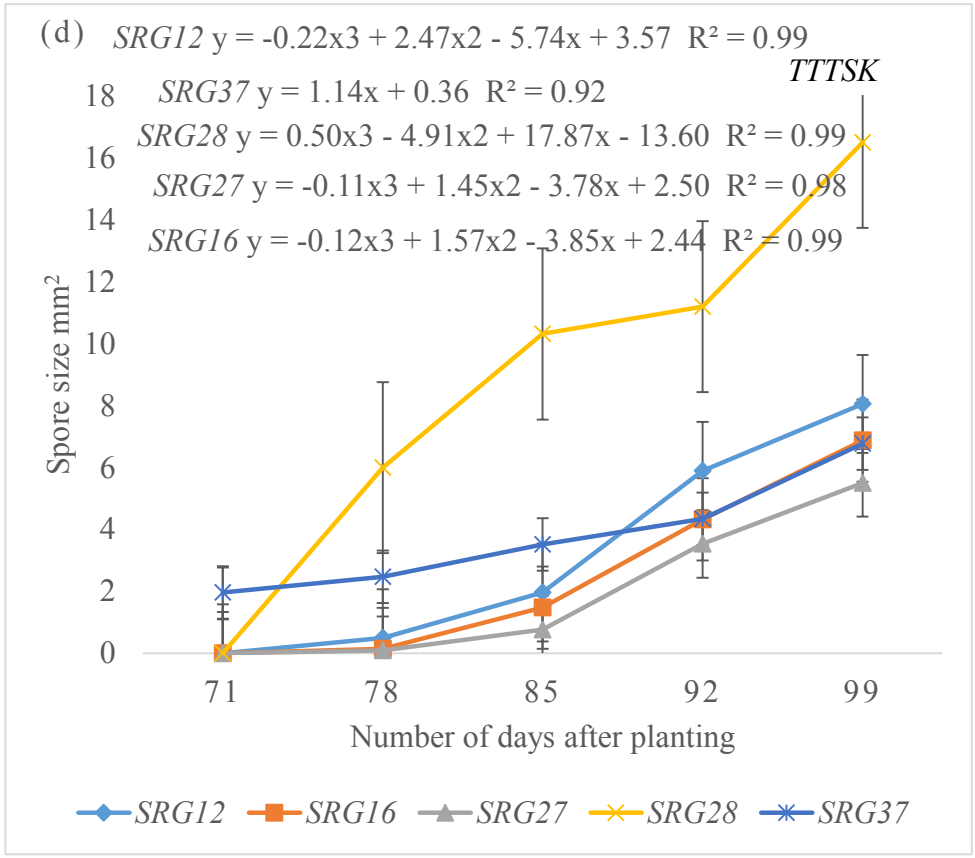


Figure 4.4 Spore sizes of CIMMYT wheat lines (set A) inoculated with four different races obtained at different number of days after planting. (a) Lines inoculated with race *TTKST*, (b) Lines inoculated with race *TTKTK*, (c) Lines inoculated with race *TTKSK*, (d) Lines inoculated with race *TTTSK*.

Cont. Figure 4.4





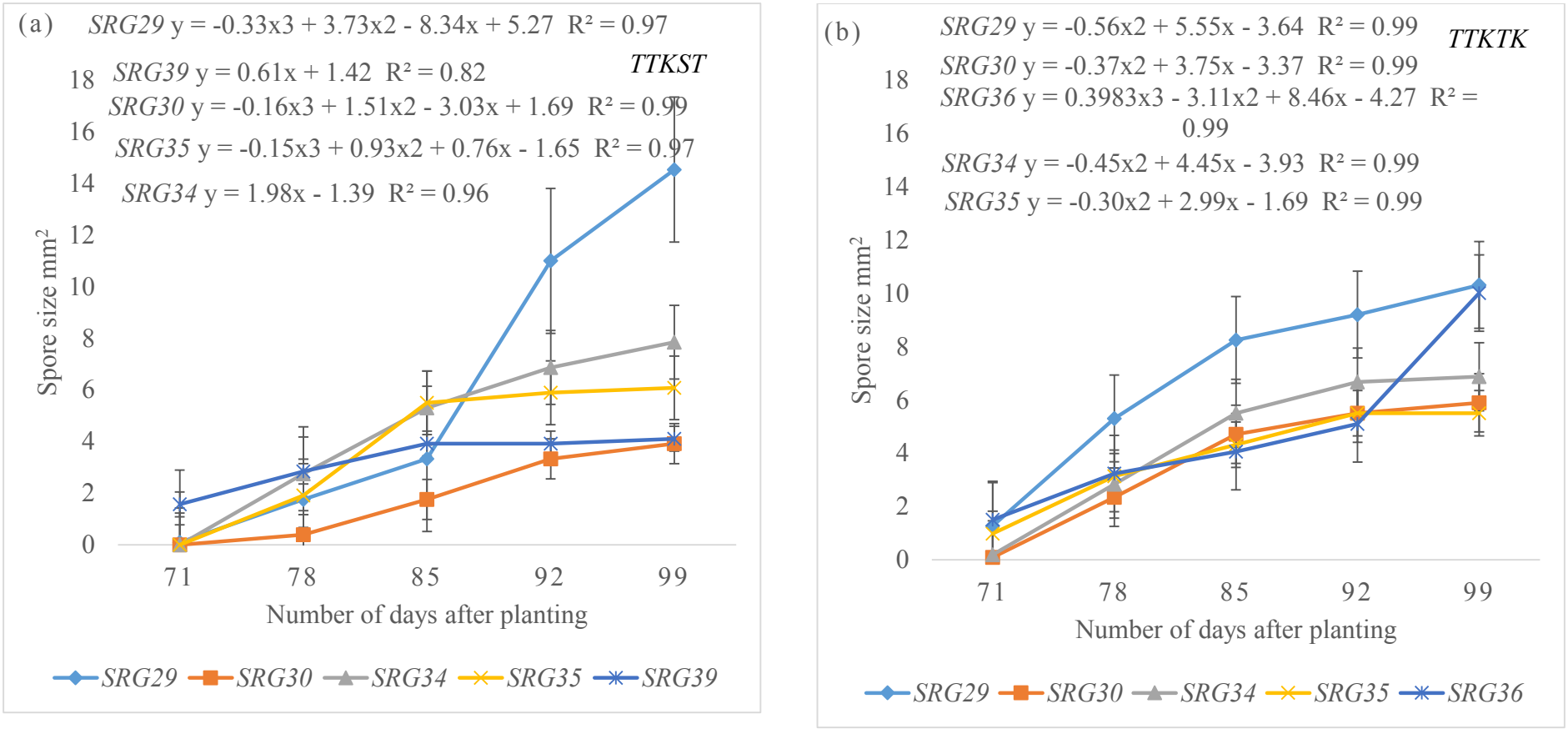
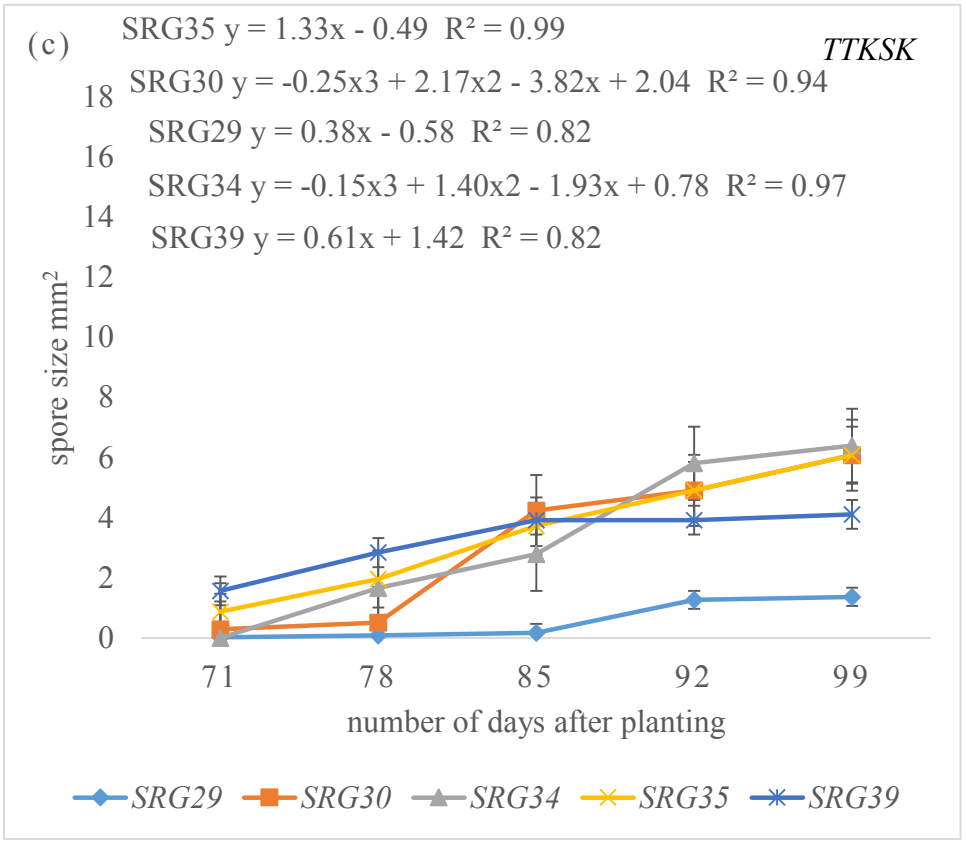
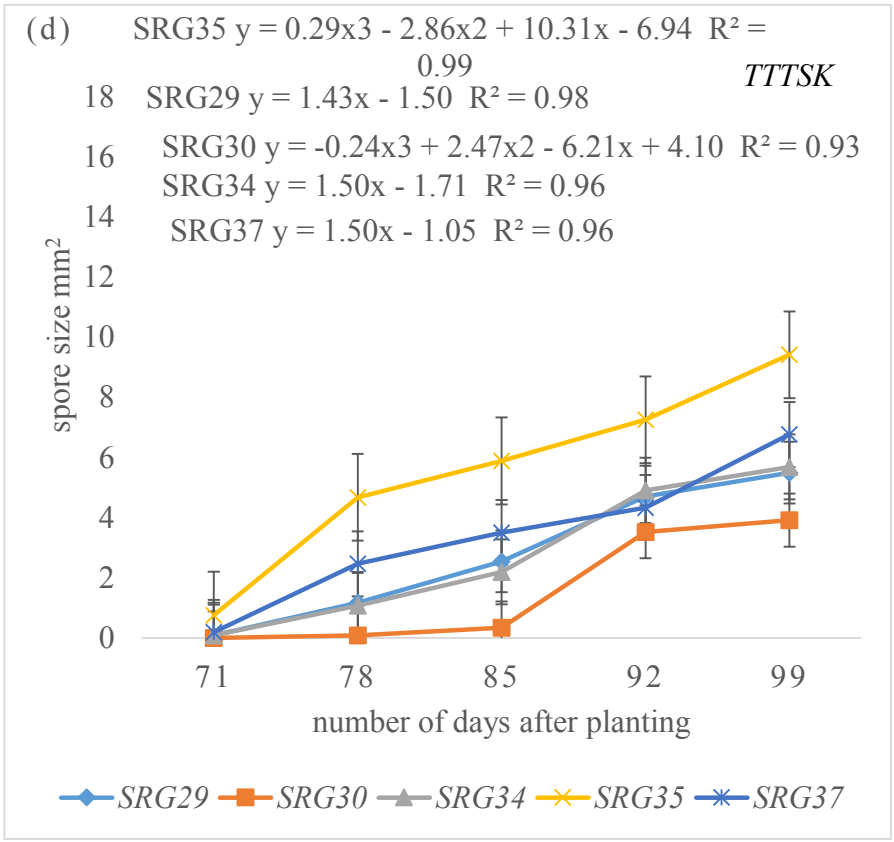


Figure 4.5 Spore sizes of CIMMYT wheat lines (set B) inoculated with four different races obtained at different number of days after planting. (a) Lines inoculated with race *TTKST*, (b) Lines inoculated with race *TTKTK*, (c) Lines inoculated with race *TTKSK*, (d) Lines inoculated with race *TTTS*

Cont. Figure 4.5





kernel weight of 11.12 g/plant while the least mean 1000 kernel weight of 4.19 g/plant was observed on line *SRG2*. Highest mean yield of 1.09 g/plant was demonstrated by line *SRG1*, this was significantly higher than that of *SRG38* (check) of 0.76 g/plant. Lowest mean yield of 0.59 g/plant was observed on line *SRG31* but this was still higher than the mean yield of the least performed check (*SRG39*) of 0.44 g/plant. Line *SRG28* showed the highest harvest index of 0.29 while least harvest index of 0.19 was exhibited by line *SRG31*. (Table 4.4).

4.4 Discussion

Significant effect due to line suggest that the lines had genetic variations for the traits measured. Significant effect due to race suggests that the four races differed in their virulence as shown by their different effects on the parameters measured. Significant effects due to line×race indicates that there were variations in response of the lines to the races used in this experiment. Since the new races identified after race *TTKSK* showed higher mean AUDPC and lower yields than race *TTKSK*, this indicates that the new races are more virulent than the original race and are capable of reducing yield significantly as they exhibited lower mean yields than the mean yield for race *TTKSK*. This was also shown by the smaller spore sizes observed on lines inoculated with race *TTKSK* compared to those inoculated with the other three races. This further demonstrated that, as much as the *Sr*31 gene was broken down, the varieties having it still bear some level of resistance to stem rust, thus preventing reduction in yields and increase in the spore size with time.

The lines which showed higher AUDPC displayed lower mean yield, indicating that AUDPC is negatively correlated with yield since AUDPC shows the level of infection. The results showed that line *SRG39* which showed the highest AUDPC had the lowest mean yield, therefore, AUDPC is directly related with the yield loss (Subba Rao *et al*., 2008). Varieties which revealed low AUDPC may have good level of adult plant resistance (Wang *et al*., 2005). In this study, lines showed good level of APR to race *TTKSK* since the mean AUDPC was low.

Results of the reaction of wheat lines to the four races showed that the tested lines vary in their degrees of resistance to stem rust races, the reason could probably be due to the difference in virulence among the stem rust races, differences in the number of resistance genes present and mode of gene action. Low infection types at seedling stage could have been due to the lines having resistance conferred by one single major gene that was broken down at adult plant stage (Mwando *et al*., 2011). Those that revealed high infection types do not have effective seedling resistance genes against the races, these lines may possess race non-specific resistance (Sawhney, 1995) and may provide durable resistance when their field assessment

results confirm their slow rusting character. Some of the lines showed moderately resistance reactions at adult plant stage. The lines which showed some resistance at adult stage may contain a major gene that remained resistant at seedling and at adult stage or they may have minor genes that are working together to reduce the disease (Roelfs, 1992). Also, those that exhibited low values of slow rusting at adult plant stage could have durable resistance (Singh *et al*., 2005). Performance among sister lines suggested that they all had effective APR genes since they all showed a severity of ≤30% with the four races. The variation between sister lines *SRG31* and *SRG* 32 suggested that they both have a gene for seedling resistance to all the four races except for SRG 32 which lacks seedling resistance gene to race *TTTSK*. This difference may be attributed to the recombination of genes during development of the recombinant inbred lines.

In this study, most of the lines showed a final severity of ≤30%, Safavi and Afshari, (2012) proposed that wheat lines with final rust severity values of 1-30%, 31-50% and 51-70% were regarded as possessing high, moderate, and low levels of slow rusting resistance, respectively. Therefore the lines in this study had high levels of slow rusting. Lines with a low final disease severity under high disease pressure may possess more additive genes (Singh *et al*., 2005). Final severity has been used previously as a parameter to assess slow rusting behaviour of wheat lines (Ali *et al*., 2009; Shah *et al*., 2010; Tabassum, 2011; Safavi and Afshari, 2012). The steady increase in spore sizes in some of the lines tested could have been due to lack of effective resistant genes in those lines. While those that their spore sizes remained constant had some resistant genes which lead to necrosis and chlorosis of the infected areas, consequently the death of the spores, therefore preventing the progress or development of the spore.

The difference in biomass among the lines tested might be as a result of genetic makeup of parental material of these lines, because all the lines were provided with the same environmental and management conditions. Similar results were observed by Dahleen *et al.* (1991) who found varied quantities of total biomass for varieties developed in the diversified regions. Moreover, Yagbasanlar and Ozkan, (1995) also found similar results regarding the biomass in different wheat varieties. High grain yield and kernel weight observed in some lines might also be associated with parental genetic makeup of these lines, since under similar environmental and crop management conditions, the grain yield diferred significantly. Grain yield of wheat varieties is mostly associated with the environmental conditions (Porfiri *et al.,* 2001). Similar responses have been observed by Wamatu and Thomas, (2002) in pigeon pea.

The low yield and low kernel weight observed in some lines could be because the plants respond to inoculation with energy demanding physiological processes, probably defense reactions, using stored host energy that otherwise would go to growth and seed production. In addition, a reduction in photosynthetic leaf area due to hypersensitive flecking also can cause yield reductions (Samborski and Peturson, 1960; Khanna *et al*., 2005). In a study by Sayre *et al*. (1998), grain yield losses due to leaf rust in bread wheat were associated with reductions in kernel weight, kernels per square meter, spikes per square meter, and grain-filling rate. The relationship between reduction in kernel weight and yield losses caused by rust has been found by other authors (Salaza *et al*., 1993; Singh and Huerta, 1994). In addition, the kernel weight, which is a function of size and weight/ density of individual kernels, has been shown to be affected by rust (Chester, 1946; Griffey *et al*., 1994).

4.5 Conclusions

Evaluation of wheat lines for their resistances is very important in integrated stem rust management. Most of the wheat lines tested do not have adequate resistance to the dominant races (*TTKSK*, *TTKST*, *TTKTK* and *TTTSK*) at seedling and adult plant stages. However, lines *SRG7, SRG13, SRG24* and *SRG35* showed low final disease severity (both in the field and greenhouse), low infection types and low AUDPC with the four races. These lines are recommended as sources of stem rust resistance genes for the study area. Regular assessment and evaluation of wheat lines against stem rust races is vital for virulence and/or avirulence information in Kenya.

CHAPTER FIVE
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION

5.1 General Discussion

The study showed that the number of lines which exhibited severity of ≤30% was high in all the nurseries across all years of evaluation, though this number reduced slightly in some years of evaluation but later increased. The proportion of lines that showed severity of ≥35% was low in all the nurseries across all years of evaluation. These observations suggest to a variety of factors affecting APR in the field such as pathogen variation, temperature fluctuations and inoculum density. The fact that the number of lines which exhibited severity of ≤30% reduced slightly but later increased is a pointer to the evolution of new and more virulent races able to overcome the existing *Sr* genes as races vary in pathogenicity, aggressiveness and virulence (Stakman and Piemseisal, 1917; Anna *et al*., 2016). This therefore shows that, the APR genes present in the lines advanced to 2016 season confer resistance to several races as suggested. Moreover, given that most lines revealed severity of ≤30% confirms a known fact that APR genes do not confer full resistance but allow for some level of infection.

The results further demonstrated that wheat better performed in the main season than in off season. Both grain yield and its components of the tested lines were significantly affected during 2015 off season. The difference in yields between the two seasons could be attributed to either difference in environmental conditions or due to differences in stem rust infection. Environmental conditions such as temperature and moisture considerably affect disease expressions and consequently yield. Several researchers have reported stem rust reducing grain yields of wheat cultivars (Singh *et al*., 2008).

Some of the lines showed resistant responses in the field with less visible stem rust infections. Some of them with moderately resistant to moderately susceptible response while some showed low stem rust severities. When these genotypes were assessed in the greenhouse for adult plant resistance, they showed low AUDPC implying presence of some level of partial resistance. The rate of disease progression was slow among these lines. This type of responses could be attributed to a combined effect of all the resistance factors during disease progression (Ali *et al*., 2008). These lines could be good sources of partial or slow rusting resistance to stem rust conditioned by additive gene action (Kaur and Bariana, 2010).

The lines tested exhibited different responses at seedling and adult stage to the four different races used. These races varied in their ability to infect different wheat lines. This could be due to the difference in genetic makeup of these lines. These new races have reduced the number of major rust resistance genes that are available for use (Kolmer, 2005). For example, *Ug99* carries virulence to gene *Sr31*, which was known for its durability. Stem rust resistance in wheat cultivars with *Sr31* remained effective for more than thirty years (Wanyera *et al*., 2006). This race has evolved even further, accumulating additional virulence to important *Sr* genes, notably *Sr24* and *Sr36* among others (Jin *et al*., 2008; 2009). Wheat lines that exhibited susceptible (3) reaction may possess race non-specific resistance (Sawhney, 1995) and these lines may provide durable resistance when their field assessment results confirm their slow rusting character. Hence, candidates for source of slow rusting resistance were those lines that exhibited susceptible (3) reaction types.

Slow rusting characteristics of cultivars have been described and estimated by means of disease severity at a certain crop development stage, the area under disease progress curve or the measurement of the apparent infection rates and coefficients of infection values (Pathan and Park, 2007). The present study found considerable variation in the final rust severities of the lines tested that could be attributed to differences in the number of resistance genes present and mode of gene action.

5.2 Conclusions

i) The study showed that there was increase in the number of resistant lines developed through the use of APR strategy in the first years of evaluation across all the nurseries.

ii) The study further showed that, the lines tested had effective seedling resistance genes for the four tested races.

iii) The lines which showed IT 3 at seedling stage have APR genes and therefore can be used as sources of new APR genes. Also, the lines which revealed low rates in increase of spore size might be having effective slow rusting genes, therefore can be used as sources of new resistant genes.

5.3 Recommendations

i) The continuous evolution of new races led to the reduction in number of lines which showed low severity in 2015, therefore, there is need for continuous evaluation of wheat genotypes in order to identify new resistant genes which can be incorporated into wheat varieties.

ii) The lines which showed low IT and low disease severity can be crossed with local varieties in order to introgress those effective genes into the local varieties.

iii) Also, those lines which showed high IT can be used as sources of slow rusting genes.

iv) Lines which revealed low rates of spore development could be subjected to molecular work in order to identify the genes responsible and can be used as sources of APR genes.

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APPENDICES

Appendix 1 SAS procedure for field experiment data

```
Data gain;
Input nurseries $ season rep blocks lines audpc hwt kwt
biomass ph spklth dth dtf     dtm;
gfp=(dtm-dtf);
cards;
;
Proc glm;
Class nurseries season rep blocks lines;
Model audpc hwt kwt biomass ph spklth dth dtf dtm
gfp=nurseries season     nurseries*season rep blocks(rep)
lines(nurseries)/ss4;
Test H= season E= nurseries*season;
Test H=nurseries E=season;
Test H=nurseries*season e=rep;
Test H=rep E=blocks(rep);
Test H=blocks(rep) E=lines(nurseries);
Means     season/tukey E=nurseries*season;
Random nurseries season nurseries*season rep
blocks(rep)lines(nurseries)*season;
Run;
Proc sort; by nurseries;
Proc glm; by nurseries;
Class season rep blocks  lines;
Model audpc hwt kwt biomass ph spklth dth dtf dtm gfp=season
rep blocks(rep) lines lines*season/ss4;
Means season/tukey;
Run;
```

Appendix 2 SAS procedures for APR experiment data

```
Data apr;
Input lines race rep bmss audpc hwt kwt yield;
Hi=(yield/bmss);
Cards;
;
Proc glm;
Class lines race rep;
Model yield bmss audpc hwt kwt Hi=lines race rep  lines*race/
ss4;
Means lines race lines*race/lsd;
Run;

Data area;
Input lines race rep stage l w area;
Cards;
;
Proc glm;
Class lines race rep stage;
Model l w area=lines race lines*race rep stage
lines*race*stage/ss4;
Means lines race lines*race stage lines*race*stage/lsd;
Run;
```

Appendix 3 Categories of infection severities to stem rust for CIMMYT wheat lines in five Stem Rust Resistance Screening nurseries from 2005 to 2016.

Nursery	Year	% disease severity (0-30)		% disease severity (35-100)	
		No. of lines	%	No. of lines	%
1st SRRSN (N=103)	2005	89	86.41	2	1.94
	2006	91	88.34	4	3.88
	2007	96	93.20	7	6.79
	2015	54	52.43	48	46.60
	2016	61	59.22	39	37.86
	MEAN	78.20	75.92	20.00	19.42
3rd SRRSN (N=110)	2005	104	94.54	6	5.45
	2006	104	94.54	6	5.45
	2007	104	94.54	6	5.45
	2008	66	60.00	44	40.00
	2015	79	71.81	21	19.09
	2016	88	80.00	16	14.54
	MEAN	87.17	79.24	20.17	18.33
5th SRRSN (N=135)	2009	118	87.41	10	7.41
	2010	112	82.96	16	11.85
	2011	118	87.41	11	8.15
	2015	81	60.00	50	37.04
	2016	106	78.52	27	20.00
	MEAN	107.00	79.26	22.8	16.89
7th SRRSN (N=150)	2013	142	94.60	8	5.30
	2015	64	42.67	57	38.00
	2016	74	49.30	67	44.60
	MEAN	90.00	59.96	54.00	35.97
9th SRRSN (N=246)	2014	236	95.93	14	5.69
	2015	175	71.14	62	25.20
	2016	187	76.02	50	20.32
	MEAN	199.33	81.03	42.00	17.07

Appendix 4 pedigrees of lines evaluated in seedling and adult plant experiments in the greenhouse

Code	Pedigree
SRG1	KRICHAUFF/2*PASTOR
SRG2	KRICHAUFF/2*PASTOR
SRG3	SERI*3//RL6010/4*YR/3/PASTOR
SRG4	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92
SRG5	PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/CIRCUS
SRG6	PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/CIRCUS
SRG7	THB/KEA//PF85487/3/MILAN
SRG8	THB/KEA//PF85487/3/RIVADENEIRA 4
SRG9	WHEAR/VIVITSI//WHEAR
SRG10	WHEAR/TUKURU//WHEAR
SRG11	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/CIRCUS
SRG12	PFAU/SERI.1B//AMAD/3/VARIS
SRG13	PBW343*2/KUKUNA//KIRITATI
SRG14	INQALAB 91*2/KUKUNA//KIRITATI
SRG15	ND643/2*WBLL1
SRG16	PFAU/SERI.1B//AMAD/3/VARIS
SRG17	TRCH/SRTU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
SRG18	TRCH/SRTU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
SRG19	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI
SRG20	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI
SRG21	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2*2/KUKUNA
SRG22	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2*2/KUKUNA
SRG23	KACHU/KIRITATI
SRG24	KACHU/KIRITATI
SRG25	KIRITATI//ATTILA*2/PASTOR/3/AKURI
SRG26	KIRITATI//ATTILA*2/PASTOR/3/AKURI ALTAR 84/AE.SQUARROSA

Cont. Appendix 4

Code	Pedigree
SRG27	(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1BALTAR 84/AE.SQUARROSA
SRG28	(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B
SRG29	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/BECARD
SRG30	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/NELOKI
SRG31	MUNAL #1*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07
SRG32	MUNAL #1*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07
SRG33	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KHVAKI/5/PBW343*2/KUKUNA/6/TRCH/SRTU//KACHU
SRG34	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/4/EGA BONNIE ROCK
SRG35	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/4/GLADIUS
SRG36	BABAX/LR42//BABAX*2/3/TUKURU
SRG37	TAM200/TUI/76//CAR422/ANA/5/BOBWHITE/CROW/BUC/PAVON76/3/YR/4/TAP
SRG38	R1122(KSRRVI)ND643//2*WBLL1
SRG39	

Appendix 5 Abstract of a paper published from the second and third objectives

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 12, No. 4, p. 53-63, 2018

RESEARCH PAPER OPEN ACCESS

Evaluation of CIMMYT wheat (*Triticum aestivum* L.) lines for seedling and adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) race *UG99* and its variants

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Article published on April 27, 2018

Key words: Stem rust, Seedling resistance, Adult plant resistance, Races

Abstract

Stem rust races *Ug99* and its variants are virulent to a large number of resistant genes present in the widely grown wheat (*Triticum aestivum* L.) cultivars. This study was conducted to evaluate seedling and adult plant reaction to four stem rust races *TTKSK, TTKST, TTKTK* and *TTTSK* in CIMMYT wheat lines. The evaluation was conducted in the greenhouse with the adult plant resistance experiment conducted in a randomized complete block design (RCBD). Out of the 39 lines evaluated, only *SRG21*, *SRG34* and *SRG39* showed a reaction of 3 to race *TTKST*, *SRG22* exhibited a reaction of 3 to race *TTKTK*, *SRG25*, *SRG32*, *SRG36* and *SRG37* displayed a reaction of 3 to race *TTKSK* and *SRG27* and *SRG39* showed a reaction of 3 to race *TTTSK* the rest revealed infection types of between 0 and 2. In the evaluation of lines for adult plant reaction to stem rust race *TTKST*, only 0.13% of the lines exhibited disease severity of ≤5% while 99.87% of the lines exhibited a severity of ≥10%. In contrast, 43.59% lines showed a severity of ≤5% while 56.41% showed a severity of ≥10% to races *TTKTK* and *TTKSK*. 46.15% of the lines demonstrated a severity of ≤5% while 53.85% of the lines demonstrated a severity of ≥10% to race *TTTSK*. Lines *SRG7*, *SRG13*, *SRG24* and *SRG35* showed low final disease severity, low infection types and low AUDPC with all the four races.