EVALUATION OF ADVANCED WHEAT (*Triticum aestivum* L.) LINES FOR STEM RUST (*Puccinia graminis* f. sp. *tritici*) RESISTANCE AND YIELD IN KENYA

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DECLARATION AND APPROVAL

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This thesis is my original work and has not been submitted for examination in any other institution.

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DEDICATION

To my mother Rachel and siblings; Evans, Collins, and Mercy for their endless love and moral support.

ABSTRACT

Stem rust (*Puccinia graminis* f. sp. *tritici*) is one of the most important diseases of wheat (*Triticum aestivum* L.) and is known to cause major wheat yield losses in Kenya as well as other wheat growing countries of Africa and Asia. The present study aimed at; i) determining seedling, adult plant resistance and yield of 64 advanced wheat breeding lines, and ii) estimating the kind of gene action in inheritance of adult plant resistance to stem rust and yield components in a 6 × 6 diallel cross of Kenyan wheat. The seedling resistance of the lines was evaluated in a greenhouse at the Kenya Agricultural and Livestock Research Organization, Njoro under artificial inoculation, while the adult plant resistance and yield were assessed across three different locations in Kenya, i.e. Njoro, Timau and Mau Narok. The seedling infection type (IT) was determined following a procedure based on a "0" to "4" scale. The Area Under Disease Progress Stairs (AUDPS), the Coefficient of Infection (CI) based on the field disease reactions and yield performances across sites were also used to compare the genotypes. Among the screened genotypes, 39% exhibited resistance (IT's of ";", "1", "2" or combinations), 2% were intermediates and the rest (59%) showed susceptible reaction. There was a significant ($P < 0.05$) genotype, location and genotype x location interaction for AUDPS, CI and yield. Grain yield across sites was linearly and inversely related to CI and AUDPS with correlation values of; -0.87 for CI and -0.69 for AUDPS. Considering the adult plant disease response and yield potential, KSL 42 and KSL 3 consistently ranked among the top performers. These genotypes can therefore be utilized in Kenyan wheat breeding programs for improvement of yield and stem rust resistance with emphasis on adult plant resistance. Results of genetic analysis revealed that both general combining ability (GCA) and specific combining ability (SCA) effects were significant ($P < 0.05$) for all traits studied. However, the GCA effects were predominant. Additionally, the covariance/ variance (Wr/Vr) graph revealed partial dominance for stem rust infection, the number of days to heading and the number of productive tillers. Over-dominance was displayed for grain yield and plant height. Inclusion of parents KSL 13 and KSL 42 as well as crosses KSL 34/KSL 52, *NjBw II*/KSL 42, *Kwale*/KSL 13, KSL 34/KSL 42 in a breeding program would produce desired segregants. These could therefore be exploited successfully in enhancement of stem rust disease resistance as well as yield in areas prone to stem rust infection. The desirable alleles in these different sources can be accumulated by gamete selection.

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LIST OF ABBREVIATIONS

A.S.L	Above the Seal Level
AMMI	Additive Main Effect and Multiplicative Interaction
APR	Adult Plant Resistance
AUDPS	Area Under Disease Progress Stairs
BGRI	Borlaug Global Rust Initiative
CIMMYT	International Centre for Maize and Wheat Improvement. With a Spanish acronym, Centro Internacional de Mejoramiento de Mayz y Trigo (CIMMYT)
DRRW	Durable Rust Resistance in Wheat
FAO	Food and Agriculture Organization
FAS	Food and Agriculture Service
IT	Infection Type
KALRO	Kenya Agricultural and Livestock Research Organization
KSL	Kenya Selection
PBC	Pseudo-Black Chaff
SRRSN	Stem Rust Resistance Screening Nursery
Ug99	Uganda 1999
USDA	United States Department of Agriculture

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Wheat (*Triticum aestivum* L.) the second most important food crop in the world. A recent report by FAO (2012) estimates the world wheat production to have risen to a record 700 million tons in the year 2011 from 553.92 million tons in 2003/2004, 607 million in 2007 and 655.7 million tons in 2010 (FAO, 2012). In Kenya, wheat is second to maize in importance with an annual production of 0.2 million tons in 2009, 0.25 million tons in 2010, 0.2 million tons in 2011 and 0.25 million tons in 2012. Production cannot meet the demand which has been growing at 5% per annum in recent years to 0.9 million tons in 2012 (FAO, 2012). This implies that Kenya imports about 0.65 million tons of wheat annually. However, wheat production can be increased by addressing the current constraints that face the farmers, especially small-scale who have limited resources and lack access to production technologies. In spite of the relatively low wheat production, Kenyans consume 183 kilocalories per day in form of bread, *chapatis* and other confectionery products (USDA, 2012).

Apart from abiotic constrains mainly drought and low soil fertility, there are also biotic constrains including disease, weed and insect attacks. Important diseases include; rusts smuts, bunts, leaf blight, powdery mildew, and head scab (Priyamvada *et al.*, 2011). Biotic factors can destroy between 31% and 42% of all crops annually (Park, 2007). The most important of all the diseases are those caused by the fungal pathogens, and a few caused by viruses and bacteria (McIntosh *et al.*, 1998). Rust diseases cause huge losses (Priyamvada *et al.*, 2011). Leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) cause 60% loss of yield while stem rust (*Puccinia graminis* f. sp. *tritici*) can cause up to 100% loss in case of an epidemic, or when a susceptible cultivar is grown (Park, 2007). Stem rust is the most limiting factor to wheat production (Singh *et al.*, 2004; Pretorius *et al.*, 2007), because of its wide distribution, its capacity to mutate through migration, mutation and recombination to new races that can attack the previously resistant cultivars. It can move long distances and develop rapidly under optimal environmental conditions (Priyamvada *et al.*, 2011). Breeding for host plant resistance is one of the most viable and sustainable control measures (Singh *et al.*, 2004). Knowledge on availability, diversity, and type of genetic resistance is important in efforts to fight the stem rust. This study therefore seeks to evaluate stem rust resistance at seedling and

adult plant stage; assess yield potential and estimate genetic mechanism associated with inheritance of the disease resistance and yield related traits in advanced wheat breeding lines selected from a stem rust screening nursery, KARI-Njoro.

1.2 Statement of the Problem

Stem rust is historically the most damaging rust disease of wheat. It continues to be a major problem in wheat production and a threat to food security in Kenya and other wheat producing countries of Africa and Asia. The rust fungus is always evolving to new races, often through mutation in presence of susceptible variety and favorable conditions. The latest race of stem rust, popularly known as *Ug99* was identified in 1999 from wheat fields in Uganda. A mutant version of *Ug99* identified in Kenya in 2006, has led to susceptibility of commercial wheat cultivars causing serious damage. With favorable conditions, stem rust is most severe in susceptible varieties and when it begins on the crop before flowering resulting in lower kernel weight and decreased numbers of kernel. This translates up to 100% yield losses. Genetic resistance remains the most viable and sustainable control measure. However, this is complicated by the constant mutation and occurrence of new variants. Therefore, there is a need for durable and multiple disease resistance conditioned by minor genes having additive effects.

1.3 Objectives

1.3.1 General Objective

To contribute to improved wheat production through development of stem rust resistant and high yielding varieties of wheat.

1.3.2 Specific Objectives

i. To determine seedling resistance of stem rust of wheat genotypes under greenhouse conditions.
ii. To determine adult plant resistance to stem rust of wheat genotypes under field conditions.
iii. To determine the yield performance of wheat genotypes across different locations.
iv. To estimate the kind of gene action associated with adult plant resistance to stem rust and yield related traits.

1.4 Null Hypotheses

i. There is no seedling resistance to stem rust in evaluated wheat genotypes under greenhouse conditions.
ii. There is no adult plant resistance to stem rust in the evaluated wheat genotypes.
iii. There is no variation in yield performance of the evaluated wheat genotypes across different locations.
iv. There is no additive and dominant gene action in the control of adult plant resistance to stem rust and yield related traits in evaluated wheat genotypes.

1.5 Justification

Stem rust has historically caused multiple epidemics and associated crop failures but has been controlled to some extent for the past 50 years by utilization of host resistance. As a result, there was a major shift in priority and resources from stem rust research and breeding. This was not until 1998/1999 when a virulent race of the pathogen was detected in Uganda. Commonly called *Ug99*, the new race and its variants has managed to overcome major resistant genes used to combat the disease and has led to susceptibility in 90% of the world's commercial wheat varieties. Major yield losses have been experienced along these migration paths, and this has clearly compromised food security and livelihood. For instance, in 2007, a major wheat yield loss was experience in Narok, Kenya and fungicides had to be used heavily to protect wheat in late-planted areas.

Although stem rust can be controlled to a greater extent through the use of fungicides, resource-poor farmers in developing countries who cannot afford high cost of fungicides would still suffer. Growing genetically resistant varieties remains the best and environmentally friendly strategy for commercial farmers everywhere. Various national and international research programs are currently engaged in developing high yielding and rust resistant varieties based on durable resistance especially to *Ug99*. It is important to cross-check the disease resistance as well as yield potential of the breeding lines at every stage to ensure that novel sources of resistance to the emerging strains of the pathogen as well as good yield potential are identified, gathered and utilized. Development of durably resistant varieties will reduce the cost of production and frequency of serious epidemics; this will clearly enhance wheat production in Kenya and other wheat dependent countries.

1.6 Reference

Food and Agriculture Organization (FAO). (2012). Crop prospects and food situation. Retrieved from www.fao.org/giews/ (verified 26 July 2012).

McIntosh, R. A., Hart, G. E., Devos K. M., Gale M. D., & Rogers, W. J. (1998). Catalogue of gene symbols for wheat. In A. E. Slinkard (Ed.), *Proceedings of the 9th International Wheat Genetics Symposium 2 to 7 August 1998* (pp.1-235). Saskatoon, Canada.

Park, R. F. (2007). Stem rust of wheat in Australia. *Australian Journal of Agricultural Resource, 58*, 558-566.

Pretorius, Z. A., Pakendorf, K. W., Marais, G. F., Prins, R., & Komen, J. S. (2007). Challenges for sustainable control of cereal rust diseases in South Africa. *Australian Journal of Agricultural Research, 58, 593*-601.

Priyamvada, A., Saharan, M. S., & Ratan, T. (2011). Durable resistance in wheat. *International Journal of Genetics and Molecular Biology, 3*, 104-114.

Singh, R. P., William, H. M., Huerta-Espino, J., & Rosewarne, G. (2004). Wheat rust in Asia: Meeting the challenges with old and new technologies. In *Proceedings of the 4th International Crop Science Congress* (pp. 1-13). Brisbane, Australia.

United States Department of Agriculture (USDA) (2012). Wheat production in Kenya by year. Retrieved from http://www.indexmundi.com/agriculture (verified 31 March 2012).

CHAPTER TWO
LITERATURE REVIEW

2.1 Wheat

2.1.1 Genetics and Botany of Wheat

Einkorn wheat (*T. monococcum*) is a diploid wheat species (genomes AA, 2n=2x=14) while other species are polyploids, having more than two sets of diploid chromosomes (Morris and Sears, 1967). There are two species of wheat that are tetraploid; the Emmer wheats (*T. turgidum* L.) with the genome constitution AABB and Timopheevi wheats (*T. timopheevi* Zhuk) which have genomic constitution AAGG (Lilienfeld and Kihara, 1934). The common wheat (*T. aestivum*) is a complex allohexaploid species (genomes AABBDD, 2n=2x=42) derived from a hybrid between cultivated Emmer (*T. turgidum* AABB, 2n=2x=28) and the grass species, *Ae. tauschii* (genomes DD, 2n=2x=14) (Sears, 1965; Shewry, 2009). It is a self-pollinator with a fibrous root system, stalks or tillers that have five or six internodes and a spike with a central axis called a rachis; each rachis gives rise to a spikelet consisting of a pair of outer glumes that enclose three to four florets (Symko, 1999).

2.1.2 History and Evolution of Wheat

All *triticum* species originate from the 'Fertile Crescent' of the Near East, which covers the eastern Mediterranean, South eastern Turkey, northern Iraq and western Iran, and its neighboring regions of the Transcaucasus, and northern Iran (Lev-Yadun *et al.*, 2000). Archaeological records indicate that emmer wheat was domesticated in the Fertile Crescent region 10,000 years ago (Nesbitt and Samuel 1996; Feldman and Kislev, 2007). Due to changes in agro ecological conditions of cultivation in the eastern Mediterranean region, the domesticated emmer wheat may have undergone considerable varietal changes resulting in the contemporary cultivar known as durum wheat (*T. turgidum* subsp. *durum*) (Feldman and Kislev, 2007). The cultivated durum wheat then migrated north-eastward together with the spread of agriculture across and beyond the Fertile Crescent region. This resulted in hybridization of *T. turgidum* and the grass species *Aegilops tauschii* as the female and male ancestors, respectively leading to formation of allopolypoid species *T. aestivum* (Kihara, 1944; McFadden and Sears, 1944). This marked the beginning of a recurring process of alloploidization which has since produced the

allohexaploid common wheat which was recorded as early as 8,600 to 7,800 years ago in south-eastern Turkey (Hillman, 1978; Nesbitt, 2001).

2.1.3 Wheat Production

Bread wheat is the second most important cereal in Kenya after maize (GOK, 1997). The current average annual production is about 0.3 million tons which is far short of Kenya's domestic demand of about 0.9 tons (FAO, 2012). The deficit is met by wheat imports from wheat sufficient countries.

Wheat is grown as a rain-fed crop between 1500m to 3000m above sea level in areas such as; Eldoret, Londiani, Molo, Narok and Nakuru. They have deep and fertile soils and the yield is very high compared with other parts of Kenya. Wheat growing regions of Kenya also extend from Trans Nzoia through Elgeyo, to Uasin Gishu and Marakwet Districts though the yield in these areas is usually low because the soils are not very fertile.

2.1.4 Importance of Wheat

Wheat provides more nourishment for more people than any other food. In the developed countries, it provides 40% to 60 % of the calories in the diet (http://www.allaboutwheat.info/production.html, accessed on 5th Feb 2013). In Kenya, it is mainly used as a human food and livestock feed (USDA-FAS, 2011). It is nutritious, easily processed into various types of food such as bread, pan breads *chapatis*, and for home-use baking (USDA-FAS, 2011). Bran from flour milling is used in livestock feed and the germ is valuable addition to feed concentrate while the grains can be fed to livestock whole or coarsely ground. The wheat plant is also used as a pasture feed before stem elongation and this practice permits plant regeneration and grain harvest. Wheat straw is used as a source of fibre (ACPFG, 2008). Wheat gluten and starch have several industrial uses, and due to its ability to be elastic, gluten has been used for preparations of adhesive, coatings, polymers etc. while starch has been used to replace some cosmetics and pharmaceutical products (http://www.kswheat.com/consumerspageid261, accessed on 5th Feb 2013).

2.2 Stem Rust

The stem rust caused by *Puccinia graminis* f. sp. *tritici* is an important disease of wheat in many wheat-growing regions of the world (Pretorius *et al.*, 2000; 2007; Jin *et al.*, 2008a). The pathogen also infects rye (*Secale cereale*) and barley (*Hordeum vulgare*), although the main host

is wheat (Jin *et al.*, 2008a; 2008b). The fungus has severely devastated wheat crop historically, becoming the most feared disease in the wheat growing regions globally (Singh *et al.*, 2011). It causes a lot of damage as it attacks not only the leaf blade, but also the leaf sheath, stem and spike (head) of the plant. The fungus feeds on the sugars produced by the host plant thus reducing the yields and can cause death of the plant under severe infection (Schuman *et al.*, 2000).The rust fungi are highly specialized pathogens and they constantly evolve to new races through migration, mutation, and recombination among the existing genotypes.

2.2.1 History of the Stem Rust

The pathogen dates back to the biblical times, and is known to be recurring to date (Singh *et al.*, 2006; 2008). However, epidemics were detected as early as 150 years ago in the near and far East Europe and the America. In the years 1903 and 1905, and 1950 to 1954, the rust epidemics caused major famines in Asia and massive grain loss in North America (IFPRI, 2009). There are reports of stem rust epidemics on traditional tall wheat varieties documented in: Indian subcontinent, Europe, North America, Latin America and Australia in the 18^{th}, 19^{th} and 20^{th} centuries (IFPRI, 2009). There are also reports of epidemics on Modern Semi dwarf wheats experienced in regions such as India from 1970 to 1973 and Ethiopia from 1993 to 1994 (IFPRI, 2009).

2.2.2 Symptoms of the Stem Rust

The stem rust life cycle is very complex; however, some of the obvious structures that can be seen with a naked eye are the black rust uredinial and brown telial stages (IFPRI, 2009). According to Schuman *et al.* (2000), the rusts appear first as reddish pustules on the stems or leaves of the plant but prior to pustule formation, "flecks" may appear. Before the spore masses break through the epidermis, the infection sites feel rough to the touch; as they break through, the surface tissues take on a ragged and torn appearance (www.wheatdoctor.cimmyt.org/). Pustules containing masses of urediospores may also occur on both sides of the leaves, on the stems, on the spikes and awns. With light infections, the pustules usually appear separate and scattered, but with heavy infections, they may coalesce (Schuman *et al.*, 2000). When rusts infect a previously healthy crop as late as a month before harvesting, it turns it into a tangled mass of black stem, which produces little, or no grain (www.wheatdoctor.cimmyt.org/).

2.2.3 Epidemiology and Virulence of Stem Rust

Wheat rust utilize live host for its development (Roelfs and Martel, 1984). There are several modes of dispersal documented. The first mode is the single event which is long distance dispersal under natural airborne conditions and it results in the pathogen colonizing new regions e.g., the wheat stem rust introduction into Australia from South Africa in 1969 (Brown and Hovmøller, 2002). The second mode is through an assisted long-distance dispersal e.g., on travellers' clothes or infected plant material is also important element in colonization of new areas by pathogens (Singh *et al.,* 2006). The third major mode of dispersal is step-wise range expansion, and is the most common mode of dispersal. It occurs in short distances, within country or region (Singh *et al.,* 2006).

The rust has been controlled to some extent for the past 50 years by utilization of host plant resistance. But the pathogen is constantly mutating for instance the *Ug99* pathotype of stem rust which was discovered in 1999 and has managed to overcome the most effective resistant genes (Jin *et al.,* 2007; Singh *et al.,* 2011). Following the detection of the *Ug99* in Uganda, the fungus is shown to have migrated to Rift valley province of Kenya by the end of the same year and later in 2001 to Eastern Kenya. In 2003, the race was detected in Ethiopia and available evidence suggests that *Ug99* was already established in the eastern African highlands in the same year, and have been spreading (Singh *et al.,* 2006). In the recent past, the pathogen has been swiftly migrating across countries and continents and in January 2006, it was confirmed that the pathogen, which previously occupied the eastern Africa, was infecting wheat in Yemen in the Arabian Peninsula; and Iran in 2007 (Singh *et al.,* 2009).

2.2.4 Life Cycle of Stem Rust

The fungus is an obligate parasite; it is heteroecious and has five spore stages and two hosts (Leonard and Szabo, 2005). The disease cycle of the rust pathogen starts when the susceptible wheat crop gets exposed to the stem rust spores, urenidiospores, which are the primary inoculum either from the volunteer plants, the alternate host Barberry (*Berberis vulgaris* L.) or the late-maturing wheat plants still in the field (Schumann and Leonard, 2000). The urediniospores germinate one to three days after infection under optimum temperatures of 30^{o}C and a dew period of six to eight hours, followed by production of germ tube and apressorium then subsequent penetration at the same temperature conditions and at least 10,000 lux of light for penetration (Roelfs *et al.,* 1992). As the host plant matures, the urediniospores produce

dicaryotik (N+N) teliospores as karyogamy occurs and teliospores become diploid (2N) (Leonard and Szabo, 2005). With rains and favorable temperatures, the teliospores germinate and undergo meiosis producing diploid basidiospores borne on structures called basidia (Leonard and Szabo, 2005). Basidiospores then infect the alternate hosts such as common barberry, germinate, and produce a haploid mycelium, which colonizes the leaf tissue, which form pycnia inside the leaf (Leonard and Szabo, 2005). The pycnia produce receptive hyphae and pycniospores of a single mating type that serve as the female and male gametes (Schumann & Leonard, 2000). Mating of the male and female pycniospores results in the formation of aeciospores that are dicaryotic (N+N) and produced in aecia on the lower surface of the leaf 7 to 10 days post fertilization (Roelfs *et al.*, 1992). Aeciospores are then hydroscopically released from aecia and are airborne to infect wheat meters or even kilometers away resulting in production of dicaryotic (N+N) uredinia with urediniospores under optimum temperatures of 30^{o}C and a dew period of six to eight hours. This completes the life cycle (Leonard and Szabo, 2005).

2.3 Stem Rust Resistance in Wheat

2.3.1 Adult Plant Resistance to Stem Rust

Adult plant resistance (APR) also known as durable resistance is a mechanism of resistance based on minor genes (van Ginkel and Rajaram, 1993) that can be used over a large area, for a long time, and especially when the host is exposed to a wide spectrum of the pathogen and yet remains resistant (Johnson, 1981). The APR genes offer a primary means of durable resistance and can also confer levels of resistance that approach immunity when combined with major genes (Sign *et al.*, 2000). According to Ayliffe *et al.* (2008), this type of resistance not only offers durable resistance but is also a broad-spectrum resistance being effective against all pathogen isolates and not just those that contain a specific effector gene. The most powerful tool to test adult plant resistance is to grow a cultivar for a long period in an environment favoring the disease. However, it can also be tested by either growing the cultivar/s in many locations or testing with many races of a pathogen from an existing population (Johnson, 1981). Evaluation of the APR in the field is often done using the modified cobb scale where the percentage of the possible tissue covered by rust ranging from 5% to 100% is determined (Peterson *et al.*, 1948). This type of resistance has also been assessed through Area Under Disease Progress Curve (AUDPC), infection rate and final disease severity as reported in Ali *et al.* (2007). The host

response to infection has been classified into four categories; R= resistant, MR= moderately resistant, MS= moderately susceptible, S= susceptible, and overlapping responses between two categories will be denoted using a dash between the categories as reported by Roelfs *et al.* (1992).

2.3.2 Seedling Resistance to Stem Rust

Seedling resistance also known as major gene or race specific resistance confers effective level of resistance to a specific physiological race of the pathogen. This implies that the host-pathogen relationship mediated by major genes in the cereals typifies Flor's gene-for-gene hypothesis; "for every gene (avirulent gene) that confers pathogenicity in a pathogen, there is always a corresponding gene (resistant gene), that confers resistance in the host" (Flor, 1971). Even though this type of resistance can be completely effective against some races of the pathogen, it can be vulnerable to at least one other race of the same pathogen and so the defense afforded by such major gene/s is not reliable since the pathogen is always evolving to new physiologic races (Lowe *et al.*, 2011). This type of resistance is often lost very rapidly due to the constant evolution of virulence by the pathogen and so the alternate way to safeguard crop production is to search for partial resistance based on minor genes (Singh *et al.*, 2004). Seedling type of resistance has been assessed through a procedure proposed by Stakman *et al.* (1962) based on "0" to "4" scale where "0" is no disease and the genotype is resistant while "4" shows the highly susceptible genotype.

2.4 Breeding for Stem Rust Resistance

For several decades in recent years, the problem of wheat stem rust declined due to the use of genetic resistance. This was realized especially through the identification and mapping of fifty resistance genes in wheat (McIntosh *et al.*, 1998). By the year 2011, more than 60 *Sr* resistant genes, both effective and ineffective were listed; all but *Sr2* are race-specific (Singh *et al.*, 2011). Even though existence of several unidentified race non-specific APR genes have been reported (Bhavani *et al.*, 2011; Njau *et al.*, 2013) only *Sr2* is catalogued (Sign *et al.*, 2006). It is known to confer slow rust resistance (Sunderwirth and Roelfs, 1980) or inadequate resistance under high disease pressure (Singh *et al*., 2011). It is located in the short arm of chromosome 3B from a cultivar Hope, a cross between Marquis and Yaslov emmer (McIntosh and Brown, 1997). The *Sr2* gene has been genetically linked to head and stem melanism referred to as pseudo black

chaff (PBC). This gene is expressed as partial resistance and has been characterized by slow rusting. It is a single gene with modifiers and therefore is additive in nature.

The successful control of stem rust using genetic resistance has resulted in a decline in research activity over the last three decades (Singh *et al.*, 2006). This was not until the emergence of a stem rust pathotype *Ug99* in Uganda in the year 1999 (Pretorius *et al.*, 2000) with virulence to *Sr31*. The *Sr31* gene was introgressed into wheat from rye, *Secale cereale* (Todorovska *et al.*, 2009) and is known to be present in many wheat cultivars throughout the world. The pathogen was found to be virulent to materials planted in Uganda on CIMMYT nurseries in 1998, which was initially thought to be resistant to the stem rust. It was later designated as TTKS (Wanyera *et al.*, 2006) under the North American pathotype nomenclature system (Roelfs and Martens, 1988); and was later confirmed to be virulent to most of the stem rust resistance genes commonly present in wheat germplasm, through simultaneous screening in Kenya and Ethiopia in 2005. The screening linked all isolates from Kenyan fields to the TTKS (Jin *et al.*, 2007; 2008a).

In 2005 to 2007, a number of resistant genes effective against *Ug99* were identified at the wheat research station in Kenya. This was based on seedling infection type and field disease severity (Jin *et al.*, 2008a; Njau *et al.*, 2010). Stem rust resistant gene *Sr24* originally transferred from the tall wheat grass (*Elitrigia elongata*) to bread wheat (Smith *et al.*, 1968) initially exhibited high level of resistance. It was later noticed in 2007 that the mutant race of *Ug99*, TTKST overcame the *Sr24* (Jin *et al.*, 2008a; Njau *et al.*, 2010). Another *Ug99* mutant race TTTSK has overcome *Sr36* (Jin *et al.*, 2008b). The *Sr36* is derived from Sanduri wheat (*T. timopheevii*) (Allard and Shands, 1954). *Sr24* virulence had also been detected in India (Bhardwaj, 1990) and South Africa (Le Roux, 1987). In Australia and South Africa, virulence has been detected to *Sr27* (http://www.fera.defra.gov.uk, accessed on 11 may 2012). Screening in Kenya and Ethiopia has identified a low frequency of resistant wheat varieties and breeding materials, mostly from CIMMYT (Singh *et al.*, 2011). Among 107 advanced lines screened in CIMMYT-Turkey and the National plant breeding station in Kenya, 21 CIMMYT lines were resistant to the five aggressive Kazakhstan stem rust races (Kokhmetova *et al.*, 2011).

2.4.1 The International Centre for Maize and Wheat Improvement (CIMMYT) Approach

CIMMYT's primary goal is to develop broadly adapted wheat germplasm with high yield stability, durable disease resistance, and acceptable end-use quality; most of its expense is spent

on developing varieties with partial and durable resistance to stem rust (Bartos *et al.*, 2002). Since its inception in 1970's, CIMMYT'S wheat breeding program has made significant impact which has seen the developing countries release varieties directly from CIMMYT advanced lines or had at least one CIMMYT parent (Rajaram, 1996). This has been achieved through utilization of various breeding methodologies such as shuttle-breeding programs and multi-locational testing through the distribution of the international screening nurseries and yield trials (ISNYT) (CIMMYT, 2003; Singh *et al.*, 2008)). There are 12 breeding domains (Mega Environments, ME) targeted by these programs. Breeding methodologies that were utilized in early 1970s are the top (or three-way) crosses and double crosses. However, double crosses were dropped by late 1970s due to poor results. From then onwards, all crosses utilized were either backcrosses and/or top crosses (CIMMYT, 2003). The methodology of selection has since been modified from pedigree selection to modified pedigree-bulk selection approach that allows one experienced breeder to evaluate all segregating population in timely fashion (Singh *et al.*, 2011).

The main aim of CIMMYT's bread wheat improvement program for almost 40 years now has been development of germplasm with durable resistance to all rust diseases of wheat (Marasas *et al.*, 2003; Singh *et al.*, 2008). The APR gene *Sr2* confers slow rusting to stem rust. When in combination with other unknown slow rusting resistance genes possibly originating from Thatcher and Thatcher-derived cultivar Chris, commonly known as "*Sr2* complex" can confer durable resistance to stem rust (McIntosh, 1988). Breeding for complex APR resistance requires accumulation of four to five slow rusting resistance genes. This has involved use of molecular markers and/or phenotypic marker PBC during selection in an area with a higher disease pressure.

2.4.2 The Kenya Agricultural and Livestock Research Organization (KALRO)-CIMMYT Shuttle Breeding Program

The collaborative shuttle-breeding program initiated under the leadership of Cornell University, BGRI and DRRW aims at identifying new stem rust resistant genes; improving surveillance, multiplying and distributing rust-resistant wheat seed to farmers (www.globalrust.org/db/attachments, accesed on 21st may 2013). The five-year breeding cycle was initiated in 2006 and has proved a great success, as new high-yielding varieties with desirable levels of disease resistance are produced by the end of each breeding cycle (Singh *et al.*, 2011). The process that begins in Mexico by crossing and selection of promising materials in

different sites with different soil types, temperatures, environmental, and disease pressures (Singh *et al.*, 2008; Singh *et al.*, 2011) ends with selection of lines in advanced breeding populations. The lines are then grown and selected at screening nurseries in Njoro, Kenya for two generations (F3 and F4) under high *Ug99* pressure and back to Mexico for screening and selection for resistance to other biotic and abiotic stresses (Singh *et al.*, 2008).

2.5 Genotype × Environment Interaction and Stability Analyses

The term "Genotype" represents the whole plant genome and hence relates to the numerous genes constituting the complex biological framework of the cultivar (Bull *et al.*, 1992). Genotype by Environment (GE) interaction is the differential genotypic expression across environments and is common with most quantitative traits (Breese, 1969; Yan and Hunt, 2002). It shows a high influence of the environment and influences the association between phenotypic and genotypic values therefore complicating the plant breeders' aim of developing high yielding varieties with high adaptability (Yan and Hunt, 2002). However, measurement of GE interaction enables the plant breeder to determine the attribute of stability in genotypes over a range of environments and an optimum breeding strategy for releasing genotypes with adequate adaptation to a target environment (Ahmad *et al.*, 1996).

Rust diseases of wheat are such an unpredictable variable that affect the yield, and expression of resistance varies across locations according to the prevalent pathotypes and the climatic conditions. Negative relationship of *Ug99* race of stem rust to growth and yield of barley has been documented (Mwando *et al.*, 2012). The same relationship with yellow rust disease severity in wheat has also been reported (Ali *et al.*, 2007). According to Johnson (1981), a cultivar is believed to have durable resistance if it survives for a longer period and on a large scale without losing its resistance in an environment that supports the disease, or various locations with many races of the pathogen. With regard to the interaction, multi-location trials therefore provide useful information on genotypic adaptation and stability (Crossa, 1990).

Several biometrical methods have been developed to assess genotypic stability. Combined analysis of variance (ANOVA), is often used to identify the existence of GE interactions in multi-location experiments, and it is valid if the error terms from different environments are homogeneous (Gauch *et al.*, 1992). Finlay and Wilkinson (1963) regression analysis, Wricke's ecovalence (*Wi*) (Wricke, 1962) have also been used to assess the phenotypic stability. Site regression analysis (SREG) also called the GGE (genotype main effect plus

genotype-environment interaction) is a linear-bilinear model that removes the effect of location and expresses the answer as a function of genotype and GE interaction effect (Cornelius *et al.*, 1996; Crossa *et al.*, 2002). This can be represented graphically in an easily interpretable and informative biplot that shows both main effects and GEI. The model has been widely used effectively over the past few years to analyze crop stability (Crossa, 1990; Yau, 1995; Yan and Hunt, 1998).

2.6 Gene Action Controlling Quantitative Traits

For a successful breeding program, there should be a vast knowledge on inheritance of the targeted traits, with emphasis on the type of gene action *i.e.* whether additive or non-additive. In order to obtain information on genetic variation of plant characters, the diallel cross technique as advocated by Hayman (1954) offers a method especially in the self-fertilized crops like wheat to assess the crosses in F_1 generation. In this technique, the total sum of squares is partitioned into various components: additive *a*, the dominance effect *b* (which is further sub-divided into effects due to directional dominance b_1, parental contribution of varying degree of dominant alleles b_2 and specific gene interaction b_3), maternal *c*, and non-maternal *d*. This approach has been applied in various studies that have depicted the role of partial dominance and over-dominance gene action in controlling various economic traits in wheat (Khan *et al.*, 2000; Kashif *et al.*, 2003). Akram *et al.* (2008) used diallel approach to study gene action in; days to maturity, plant height, flag leaf area and grain filling period, number of tillers, and days to heading. Additive gene action with partial dominance was observed for plant height, number of tillers per plant, spike length, number of spikelets and grain yield per plant, and over-dominance for peduncle length (Ullah *et al.*, 2010). The technique has also been used in genetic analysis for disease resistance in wheat and a range of crops. Wagoire *et al.* (1998) disclosed the major role of additive gene action in inheritance of adult field resistance to yellow rust (*Puccinia striiformis*) of wheat. The importance of additive genetic effects as compared to non-additive effects has been reported in inheritance of ascochyta blight caused by *Ascochyta rabiei* in chickpea (*Cicer arietinum* L.) (Danehloueipour *et al.*, 2007). Preponderance of additive gene action over dominant gene action was also reported for inheritance of resistance to scab (*Sphaceloma sp.*) of Cowpea (*Vigna unguiculata* L. Walp) (Tumwegamire *et al.*, 1998). Major role of non-additive gene action has also been revealed in inheritance of resistance to Wheat streak mosaic virus in wheat (Hakizimana *et al.*, 2004).

2.7 References

Ahmad, J., Choundhrey, M. H., Salah-ud-Din, S., & Ali, M. A. (1996). Stability for grain yield in wheat. *Pakistan Journal of Botany, 28*, 61-65.

Akram, Z., Ajmal, S. U., Munir, M., & Shabir, G. (2008). Genetic determination of yield related attributes in bread wheat. *Sarhad Journal of Agriculture, 24*, 431-438.

Ali, S., Shah, S. J. A., & Ibrahim, M. (2007). Assessment of wheat breeding lines for slow yellow rusting (*Puccinia striiformis* west. *tritici*). *Pakistan Journal of Biological Sciences, 10*, 3440-3444.

Allard, R. W., & Shands, R. G. (1954). Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevi*. *Phytopathology, 44*, 266-274.

Australian Centre for Plant Functional Genomics (ACPFG) (2008). The Biology of *Triticum aestivum* L. em Thell. (Bread wheat). Retrieved from http://www.acpfg.com (verified 23 June 2012).

Ayliffe, M., Singh, R., & Lagudah E. (2008). Durable resistance to wheat stem rust needed. In de M. Juliette, K. Maarten (Eds.) *Current Opinion in Plant Biology* (pp. 187-192). Elsevier

Bartos, P., Sip, V., Chrpova, J., Vacke, J., Stuchlikova, E., Blazkova, V., & Hanzalova, A. (2002). Achievements and prospects of wheat breeding for disease resistance. *Czech Journal of Genetics and Plant Breeding*, *38*, 16-28.

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

Breese, E. L. (1969). The measurement and significance of genotype-environment interaction in grasse. *Heredity, 24*, 27-44.

Brown, J. K. M., & Hovmøller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science, 297*, 537-542.

Bull, J. K., Cooper, M., Delacy, I. H., Basford., & Woodruff, D. R. (1992). Utility of repeated checks for hierachical classification of data from plant breeding trials. *Field Crops Research, 30*, 79-95.

Cornelius, P. L., Crossa, J., & Seyedsadr, M. S. (1996). Statistical test and estimators of multiplicative models for genotype-by-environment interaction. In M. S. Kang & H. G. Gauch (Eds.). *Genotype-by-environment interaction* (pp. 199-234). Boca Raton, FL, CRC Press.

Crossa, J. (1990). Statistical analyses of multilocation trials. *Advances in Agronomy, 44*, 55-85.

Crossa, J., Cornelius, P. L., & Yan, W. (2002). Biplots of linear-bilinear models for studying crossover genotype - environment interaction. *Crop Science, 42*, 619-633.

Danehloueipour, N., Yan, G., Clarke, H. J., & Siddique, K. H. M. (2007). Diallel analyses revealed the genetic control of resistance to ascochyta blight in diverse chickpea and wild *Cicer* species.

Eberhart, S. A., & Russell, W. A. (1966). Stability parameters for comparing varieties. *Crop Science, 6*, 36-40.

Feldman, M. & Kislev, M. E. (2007). Domestication of emmer wheat and evolution of free threshing tetraploid wheat. *Israel Journal Plant Science, 55,* 207-221.

Finlay, K. W., & Wilkinson, G. N. (1963). The analysis of adaptation in a plant breeding program. *Australian Journal of Agricultural Research, 14*, 742-754.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology, 9*, 275-296.

Food and Agriculture Organization (FAO) (2012). Crop prospects and food situation. Retrieved from www.fao.org/giews/ (verified 26 July 2012).

Gauch, H. G. Jr. (1992). AMMI and related models. In M. S Kang & H. G. Gauch (Ed.), *Genotype by environment interaction* (pp. 1-40). CRC Press, Boca Raton.

Hakizimana, F., Ibrahim, M. H. A., Langham, A. C. M., Haley, D. S., & Rudd, C. J. (2004). Diallel analysis of Wheat streak mosaic virus resistance in Winter wheat. *Crop Science 44*, 89-92.

Hayman, B. I. (1954). The theory and analysis of diallel crosses. *Genetics, 39*, 789-809.

Hillman, G. (1978). On the origins of domestic rye Secale cereale: the finds from Aceramic Can Hasan III in Turkey. *Anatolian Studies, 28,157*-174.

International Centre for Maize and Wheat Improvement (CIMMYT) (2003). Documentation of the CIMMYT Wheat Breeding Programs. Wheat Program, CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

International Food Policy Research Institute, IFPRI Discussion paper 00910. (2009). Combating stem and leaf rust of wheat. Retrieved from www.ifpri.org/millionsfed (verified 20 February 2012).

Jin, Y., Pretorius, Z. A., Singh, R. P., Fetch, T. (2008a). Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Diseases, 92*, 923-926.

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

Jin, Y., Szabo, L. J., & Pretorius, Z. A. (2008b). Virulence variation within the *Ug99* lineage. In R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntyre & P. Sharp (Eds), *Proceedings of 11th International Wheat Genetics Symposium* (pp. 342). Brisbane, Australia: Sydney University.

Johnson, R. (1981). Durable resistance: Definition of genetic control and attainment in plant breeding. *Phytopathology, 71*, 567-568.

Kashif, M., Ahmed, J., Chowdhry, M. A., & Perveen, K. (2003). Study of genetic architecture of some important agronomic traits in durum wheat (*Triticum durum* Desf.). *Asian Journal of Plant Science, 2*, 708-712.

Khan A. S., Khan M. K. R., & Khan, T. M. (2000). Genetic analysis of plant height, grain yield and other traits in wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Biology, 2*, 129-132.

Kihara, H. (1944). Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. *Agricultural Horticulture, 19*, 889-890.

Kokhmetova, A., Morgounov, A., Rsaliev, S., Rsaliev, A., Yessenbekova, G., & Typina, L. (2011). Wheat germplasm screening for stem rust resistance using conventional and molecular techniques. *Czech Journal of Genetics and Plant Breeding, 47*, 146-154.

Le Roux, J., & Rijkenberg, F. H. J. (1987). Pathotypes of *Puccinia graminis* f. sp. *Tritici* with increased virulence for *Sr24*. *Plant Diseases, 71*, 1115-1119.

Leonard, K. J., & Szabo, L. J. (2005). Pathogen profile: Stem rust of small grains and grasses caused by *Puccinia graminis*. *Molecular Plant Pathology, 6*, 99-111.

Lev-Yadun, S., Gopher, A., & Abbo, S. (2000). The cradle of agriculture. *Science, 288*, 1602–1603.

Lilienfeld, F., & Kihara, H. (1934). *Triticum timopheevi* Zhuk. *Cytologia, 6*, 87-122.

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

Marasas, C. N., Smale, M., & Singh, R. P. (2003). The economic impact of productivity maintenance research: Breeding for leaf rust resistance in modern wheat. *Agricultural Economics, 29*, 253-263.

McFadden, E. S., & Sears, E. R. (1944). The artificial synthesis of *Triticum spelta*. *Genetic Society in America, 13,* 26-27.

McIntosh, R. A. (1988). The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In N. W. Simmonds, S. Rajaram (Eds.), *Breeding strategies for resistance to the rusts of wheat* (pp. 1-9). CIMMYT: Mexico, D.F.

McIntosh, R. A., & Brown, G. N. (1997). Anticipatory breeding for resistance to rust diseases in wheat. *Annual Review of Phytopathology, 35,* 311-26.

McIntosh, R. A., Hart, G. E., Devos K. M., & Gale M. D., Rogers, W. J. (1998). Catalogue of gene symbols for wheat. In A. E. Slinkard (Ed.), *Proceedings of the 9th International Wheat Genetics Symposium 2–7 August 1998* (pp.1-235). Saskatoon, Canada

Morris, R., & Sears, E. R., (1967). The cytogenetics of wheat and its relatives. In K. S. Quisenberry, & L. P. Reitz (Eds.), *Wheat and Wheat Improvement* (pp. 19-87). Madison Wisconsin: American Society of Agronomy.

Mwando, E. K., Tabu, I. M., Otaye, D. O., & Njau, P. N. (2012). Effect of *Ug99* race of Stem rust (*Puccinia garaminis* f. sp. *tritici*) on growth and yield of Barley (*Hordeum vulgare* L.) in Kenya. *Journal of Agricultural Science, 4*, 161-168.

Nesbitt, M. & Samuel, D. (1996). From stable crop to extinction? The archaeology and history of the hulled wheats. In S. Padulosi, K. Hammer & J. Heller (Eds). *Hulled Wheats. Proceedings of the First International Workshop on Hulled Wheats* (pp. 41-48). International Plant Genetic Resources Institute (IPGRI), Italy.

Nesbitt, M. (2001). Wheat evolution: integrating archaeological and biological evidence. In P. D. S. Caligari & P. E Brandham. *Wheat Taxonomy: The legacy of John Percival* (pp. 37-59). Academic Press, London.

Njau, P. N., Bhavani, S. Huerta-Espino, J., Keller, B., & Singh R. P. (2013). Identification of QTL associated with durable adult plant resistance to stem rust race *Ug99* in wheat cultivar 'Pavon 76'. *Euphytica, 190*, 33-44.

Njau, P. N., Jin, Y., Huerta-Espino, J., Keller, B., & Singh, R. P. (2010). Identification and evaluation of sources of resistance to stem rust race *Ug99* in wheat. *Plant Disease, 94*, 413-419.

Peterson, R. F., Campbell, A. B., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust severity on leaves and stems of cereals. *Canadian Journal of Genetics and Cytology C, 26*, 496-500.

Pretorius, Z. A., Pakendorf, K. W., Marais, G. F., Prins, R., & Komen, J. S. (2007). Challenges for sustainable control of cereal rust diseases in South Africa. *Australian Journal of Agricultural Research, 58, 593*-601.

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., & Payne, T. S. (2000). Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in wheat. *Plant Diseases, 84*, 203-207.

Priyamvada, A., Saharan, M. S., & Ratan, T. (2011). Durable resistance in wheat. *International Journal of Genetics and Molecular Biology, 3*, 104-114.

Rajaram, S., Braun, H. J., & Van Ginkel, M. (1996). CIMMYT's approach to breed for drought tolerance. *Euphytica, 92*, 147-153.

Roelfs, A. P., & Martel, L. B. (1984). Uredospore dispersal from a point source within a wheat canopy. *Phytopathology, 74*, 1262-67.

Roelfs, A. P., & Martens, J. W. (1988). An international system of nomenclature for *Puccinia graminis* f. sp. *tritici. Phytopathology, 78*, 526-533.

Roelfs, A. P., Singh, R. P., & Saari, E. E. (1992). *Rust diseases of wheat: Concepts and methods of disease management.* Mexico, D.F: CIMMYT.

Schumann, G. L., & Leonard, K. J. (2000). Stem rust of wheat (black rust). In *The plant health instructor.* DOI:10.1094/PHI-I-2000-0721-01 (Updated 2011).

Sears, E. R. (1965). Nullisomic-tetrasomic combinations in hexaploid wheat. In *Chromosome Manipulations and Plant Genetics*. *A Supplement of Heredity, 20,* 29-45.

Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany, 60,* 1537-1553.

Singh, D., Girma, D., Njau, P., Badebo, A., Sign, R. P, Bhavani, S., Singh, R. P., Huerta-Espino, J., Woldeab, G., and Ward, R. (2009). Stem rust screening in East Africa. In *Borlaug Global Rust Initiative: 2009 Technical workshop March 17-20*. Cd Obregon, Sonora, Mexico

Singh, R. P, Hodson, D. P, Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel S., & Ward, R.W. (2008). Will stem rust destroy the world's wheat crop? *Advances Agronomy, 98*, 271-309.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau P, Herrera-Foessel S., Singh P. K., Singh, S., & Govindan, V. (2011). The emergence of *Ug99* races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology, 49,* 465-481.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M. G, Wanyera, R., Njau, P., & Ward, R.W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 1,* 054-058.

Singh, R. P., William, H. M., Huerta-Espino, J., & Rosewarne, G. (2004). Wheat rust in Asia: Meeting the challenges with old and new technologies. In *Proceedings of the 4th International Crop Science Congress* (pp. 1-13). Brisbane, Australia.

Smith, E. L., Schlehuber, A. M., Young, H. C, & Jr, Edwards, L. H. (1968). Registration of agent wheat. *Crop Science, 8*, 511-512.

Stakman, E. C, Steward, D. M., & Loegering, W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici*. United States Department of Agriculture, Agricultural Research Service, 1962.

Sunderwirth, S. D., & Roelfs, A. P. (1980). Greenhouse characterization of the adult plant resistance of *Sr2* to wheat stem rust. *Phytopathology, 70,* 634-41.

Symko, S. (1999). From a single seed: Tracing the Marquis wheat success story in Canada to its roots in the Ukraine. Research Branch Agriculture and Agri-Food Canada. Retrieved from http://www4.agr.gc.ca/AAFC-AAC (verified 10 November 2012).

Todorovska, E., Christov, N., Slavov, S., Christova, P., & Vassilev, D. (2009). Biotic stress resistance in wheat breeding and genomic selection implications. *Biotechnology & Biotechnological Equipment, 23,* 1417-1426.

Tumwegamire, S., Rubaihayo, P.R., & Adipala, E. (1998). Genetics of resistance to *Sphaceloma* scab of Cowpea. *African Crop Science, 6,* 227-240.

Ullah, S., Khan, A. S., Raza, A., & Sadique, S. (2010). Gene action analysis of yield and yield related traits in spring wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Biology, 12,* 125-128.

United States Department of Agriculture - Food and Agriculture Service (USDA-FAS). (2011). Global agricultural information network: Kenya wheat report. Retrieved from http://gain.fas.usda.gov/ (verified 31 March 2012).

Wagoire, W. W., Stolen, O., & Ortiz R. (1998). Inheritance of adult field resistance to yellow rust disease among broad-based hexaploid spring wheat germplasm. *Theoretical and Applied Genetics, 97,* 502-506.

Wanyera, R., Kinyua, M. G., Jin, Y., & Singh, R. (2006). The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici,* with virulence on *Sr31* in wheat in Eastern Africa. *Plant disease, 90,* 113-118.

Wricke, G. (1962). A method of understanding the biological diversity in field research. Z. *Pflanzenzüchtg, 47,* 92-96.

Yan, W., & Hunt, L. A. (1998). Genotype by environment interaction and crop yield. *Plant Breeding Review, 16,* 35-178.

Yan, W., & Hunt, L. A. (2002). Biplot analysis of diallel data. *Crop Science, 42*, 21-30.

Yau, S. K. (1995). Regression and AMMI analyses of genotype x environment interactions: An empirical comparison. *Agronomy Journal, 87*, 121-126.

CHAPTER THREE

GENOTYPIC PERFORMANCE OF ADVANCED WHEAT (*Triticum aestivum* L.) LINES FOR RESISTANCE TO STEM RUST (*Puccinia graminis* f. sp. *tritici*) AND YIELD IN KENYA

3.1 Abstract

Stem rust (*Puccinia graminis* f. sp *tritici*) is a destructive disease of bread wheat (*Triticum sp.*) and a major challenge to wheat production in Kenya as well as other wheat growing countries of Africa and Asia. The objectives of this study were to; i) determine resistance of wheat genotypes to stem rust at both seedling and adult plant development stages in Kenya ii) identify high yielding and stable wheat genotypes under natural stem rust disease infection across locations in Kenya. Sixty-four advanced Kenyan wheat genotypes including two check varieties were evaluated for disease resistance and yield. The seedling resistance of the lines was evaluated in a greenhouse at Kenya Agricultural and Livestock Research Organization, Njoro (0^o20'S, 35^o56'E and 2185 m a.s.l), under artificial inoculation while the adult plant resistance and yield were assessed across three sites in Kenya; Njoro (0^o20'S, 35^o56'E and 2185 m a.s.l), Timau (0^o5'S, 37^o20'E and 2640 m a.s.l) and Mau Narok (0°38' S, 35°47′ E and 2185 m a.s.l). The field experiments followed an alpha lattice design with three replications. Seedling disease infection type (IT) ranged from "0" (immune) to "4" (susceptible), while adult plant infection ranged from Coefficient of Infection (CI) means of 0.2 to 1.7, and Area Under Disease Progress Stairs (AUDPS) means of 30.2 to 1174.2. The mean grain yield ranged from 2.0 to 7.8 t ha^{-1}. Genotype, location and genotype x location interaction for the AUDPS, CI and yield were significant ($P < 0.05$). Considering the disease response yield performance, genotypes KSL 42 and KSL 3 consistently ranked among the top performers. These genotypes are high yielding and appeared to possess desired level of resistance and are therefore suitable candidates for utilization in yield and stem rust resistance improvement programs in Kenya and potentially across the east African region.

Key words: Adult plant resistance, Seedling resistance, Stability

3.2 Introduction

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is one of the major diseases of Wheat (*Triticum aestivum* L.) and has historically constrained wheat production in Kenya as well as other wheat growing countries of Africa and Asia (Wanyera *et al.*, 2006; Singh *et al*., 2006, 2008; Admassu *et al*., 2008; Njau *et al*., 2009). Currently, yield losses associated with stem rust under on farm conditions can go up to 100% (Wanyera *et al*., 2008). Although wheat breeding programs in Kenya have attempted to develop resistant varieties, virulence has been reported on most of the varieties at both seedling stage and adult plant resistance stages (Njau *et al*., 2009). Seedling resistance to stem rust is regulated by major genes which can be completely effective against some races of the pathogen, but can also be vulnerable to at least one other race of the same pathogen hence not reliable (Lowe *et al*., 2011). On the other hand, adult plant resistance (APR) is based on minor genes (Ginkel and Rajaram, 1993) and can be used over a large area, for a long time especially when the host is exposed to a wide range of the pathogen but still remains resistant (Johnson, 1983).

For several decades in recent years, the stem rust had been contained to some extent especially through utilization of more than fifty resistance genes in wheat (McIntosh *et al*., 1998). This resulted in a decline in research activity over the last three decades (Singh *et al*., 2006) until the emergence of a stem rust pathotype *Ug99* in Uganda in the year 1999 (Pretorius *et al*., 2000). This pathotype is virulent to *Sr31* which was introgressed into wheat from rye, *Secale cereale* (Todorovska *et al*., 2009) and is known to be present in many wheat cultivars throughout the world (Singh *et al*., 2006). In 2007, a mutant race of *Ug99*, TTKST overcame the *Sr24* (Jin *et al*., 2008a; Njau *et al*., 2010) which was originally transferred from the tall wheat grass (*Elitrigia elongata*) to bread wheat (Smith *et al.*, 1968). Another *Ug99* mutant race (TTTSK) has overcome the *Sr36* (Jin *et al.*, 2008b). The *Sr36* is derived from Sanduri wheat (*Triticum timopheevii*) (Allard and Shands, 1954). However, the APR genes when combined with such major genes offer a primary means of durable resistance and can confer desired levels of resistance near immunity (Singh *et al*., 2005). A Mexico-Kenya shuttle breeding program was initiated in 2006 in a bid to transfer the APR identified in CIMMYT wheat to a range of important wheat germplasm (Singh *et al*., 2008). Through this program, CIMMYT germplasm are used in wheat breeding activities for durable resistance in Kenya have been developed (Njau *et al*., 2010).

Quantitative traits such as APR and yield are usually influenced by genotype, environment and genotype × environment (GE) interaction (Breese, 1969; Yan and Hunt, 2002). The cross over GE interaction type results in inconsistent performance of genotypes across environments (Yan and Hunt, 2002). It complicates the plant breeders' aim of developing varieties that are best performing and most stable, hence reducing the progress from selection in any one environment. This can be managed by selecting genotypes that are broadly adapted to a range of environments (Yau, 1995). For stem rust disease, the pathogen as well as seasonal variation is part of the environment, which strongly affects resistance and its durability (Parlevliet, 1993). Therefore, to increase the level of durable resistance, the breeder should select the lines with lower levels of disease severity continuously over seasons or locations where they will be exposed to a wide spectrum of the pathogen races (Johnson, 1983; Parlevliet and Van Ommeren, 1988). On the other hand, a durably resistant wheat variety to stem rust will be of less importance to the farmer unless it has other important traits such as yield. Measurement of GE interactions for disease resistance and yield enables the plant breeder to identify broadly adapted genotypes that offer stable performance across all sites, as well as under specific conditions such as high disease pressure (Yan and Tinker, 2005). This aids in development of an optimum breeding strategy for releasing genotypes adapted to a target environment (Ahmad *et al*., 1996). The objectives of this study were to; i) asses advanced Kenyan wheat lines for sources of resistance to stem rust, and ii) identify high yielding and stable wheat genotypes under stem rust disease across three Kenyan locations.

3.3 Materials and Methods

3.3.1 Race Determination

Ten seeds of a stem rust susceptible cultivar *Cacuke* were planted in each of the ten square pots (5 cm span) filled with a potting mix in a greenhouse at KARI, Njoro. The pots were then placed in plastic trays in fixed positions. The 9 day old seedlings were then inoculated with stem rust urediniospores collected from trap nursery of stem rust resistance screening nursery (SRRSN), Njoro. The spores were suspended in light mineral oil (Soltrol 170, Chevron Philips chemical Co. Philtex Plant Spur 119 Borger, TX) at a concentration of 6×10^6 spores/ml of oil. Inoculation was done by spraying indirectly on the leaves using a hand held atomizer. Inoculated plants were then air-dried for one hour and incubated for 24 hrs in dark dew chamber kept moist

by frequently spraying with distilled water to maintain humidity of 80% to 100%. Temperatures were maintained between 18^{o}C and 20^{o}C. The seedlings were then transferred to the greenhouse at 23^{o}C after incubation. For the first two hours after transfer, misty condition was created by spraying indirectly on the leaves with distilled water at an interval of 30 minutes in order to maintain high relative humidity. For single pustule isolation, a vigorous plant with a single well isolated spore near the leaf base was selected. The leaf blade above the pustule and other parts of the seedling were removed. The spores were then scooped out by a glass slide cover and air dried for one hour. They were then put in a tube and stored in a freezer at 4^{o}C. This was used as inoculum for the next transfer generation until more inoculum was obtained.

For race identification a set of 20 differentials, each with a different single stem rust (*Sr*) resistance gene (Table 1) was used. Four seeds of each differential line were planted in each corner of the square pot (5 cm span) filled with a potting mix. The single pustule inoculum derived as explained in section 3.3.1 was suspended in light mineral oil (Soltrol 170) at a concentration of 6×10^{6} spores/ml of oil. Spore quantification was done using a hemacytometer. Inoculation and incubation was done as explained in section 3.3.1. The disease infection type (IT) was observed 15 days after inoculation following the procedure of Stakman *et al.* (1962) (Appendix 1a), where ITs “0”, “;”, “1”, “2”, or combinations indicated low (L). Infection type “3” to “4” was considered high (H).

3.3.2 Seedling Resistance Test

Sixty four advanced wheat breeding lines obtained from CIMMYT and selected from the Njoro, stem rust resistance screening nursery (SRRSN) and two checks; *Cacuke* and *Robin* (Table 2) were evaluated in a greenhouse at KARI, Njoro. Ten seeds of each entry were planted in 5 cm wide square pots filled with a vermiculite potting mix and placed in a plastic tray with each pot in a fixed position. The 9 day old seedlings were inoculated with the single pustule derived inoculum obtained as described in section 3.3.1. The spores were suspended in light mineral oil (Soltrol 170) at a concentration of 6×10^{6} spores/ml of oil. The procedures for inoculation and incubation were followed as previously described in section 3.3.1. The disease infection type (IT) was observed 15 days after inoculation following the procedure of Stakman *et al.* (1962), where “0” is no disease and the genotype is resistant while “4” shows the highly susceptible genotype/s. ITs “0”, “;”, “1”, “2”, or combinations indicated resistance, while, ITs “3” to “4” indicated susceptibility.

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

Set	Gene	LIT	Diff line for world distribution, April 2009	Origin/Pedigree	Source
1	Sr5	0	ISr5-Ra CI 14159	Thatcher/Chinese Spring	Jin, USDA
	Sr21	1-	T monococcum/8*LMPG-6 DK13	Einkorn CI 2433	Fetch, AAFC
	Sr9e	1- to2-	Vernstein PI 442914	Little Club//3* Gabo /2* Charter /3/3* Steinwedel / CI 7778	Jin, USDA
	Sr7b	2	ISr7b-Ra CI 14165	Hope/Chinese Spring	Jin, USDA
2	Sr11	; to 2-	Yalta PI 155433	Kenya C6402/Pusa4//Dundee	Park, Australia
	Sr6	0;	ISr6-Ra CI 14163	Red Egyptian/Chinese Spring	Jin, USDA
	Sr8a	2- to 2	Mentana W1124 PI 221154	Rieti/Wilhelmina // Akagomughi	Park, Australia
	Sr9g	2-	Acme CI 5284	Selection from Kubanka (CI 1516)	Pretorius, SA
3	Sr36	0;	W2691SrTt-1 CI 17385	CI 12632 T. timopheevii	Jin, USDA
	Sr9b	2	Prelude*4/2/Marquis*6/Kenya 117A	Kenya 117A	Fetch, AAFC
	Sr30	1+ to 2	Festiguay W2706 PI 330957	Festival / Uruguay C10837	Park, Australia
	Sr17	;1	Prelude/8*Marquis*2/2/Esp 518/9	Esp 518/9	Fetch, AAFC
4	Sr9a	1- to 2-	ISr9a-Ra CI 14169	Red Egyptian/Chinese Spring	Jin, USDA
	Sr9d	1- to 1	ISr9d-Ra CI 14177	Hope/Chinese Spring	Jin, USDA
	Sr10	;1N to 3C	W2691Sr10 CI 17388	Marquis*4/Egypt NA95/2/2*W2691	Jin, USDA
	SrTmp	2-	CnsSrTmp	Triumph 64 (CI 13679)/Chinese Spring	Jin, USDA
5	Sr24	1- to2-	LcSr24Ag	Little Club/Agent (CI 13523)	Jin, USDA
	Sr31	1- to 2	Kavkaz/Federation4	Kavkaz	Pretorius, SA
	Sr38	X=	Trident	Spear*4/VPM (PI 519303)	Park,Australia
	SrMcN	2-	McNair 701 (CI 15288)		Jin, USDA

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

Table 2**:** The origin and pedigrees of wheat (*Triticum aestivum* L.) evaluated in greenhouse and field across the three sites in Kenya during 2012-2013 cropping season.

No.[a]	Origin	Pedigree	Selection history
KSL1	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-15ZTM-01Y-0B-01Y-0B
KSL2	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-15ZTM-01Y-0B-03Y-0B
KSL3	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-23ZTM-03Y-0B-06Y-0B
KSL4	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-40ZTM-04Y-0B-08Y-0B
KSL5	PYTRF/11	PREMIO/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/PIFED	CMSA07WM00086S-050Y-2ZTM-06Y-0B-02Y-0B
KSL6	PYTRF/11	PREMIO/5/TUI//2*SUNCO/SA1166/3/TUI/4/FINSI	CMSA07WM00088S-050Y-4ZTM-01Y-0B-02Y-0B
KSL7	PYTRF/11	SW89-3218/VORONA//SUNCO/2*PASTOR	CMSA07WM00011S-050Y-040ZTM-040ZTY-48ZTM-010Y-0B
KSL8	PYTRF/11	SW89-3218/VORONA//SUNCO/2*PASTOR	CMSA07WM00011S-050Y-040ZTM-040ZTY-55ZTM-010Y-0B
KSL9	PYTRF/11	MERCATO/VORB	CMSA07WM00076S-050Y-040ZTM-040ZTY-77ZTM-010Y-0B
KSL10	PYTRF/11	MERCATO/5/CHEN/AE.SQ//2*OPATA/3/BAV92/4/JARU	CMSA07WM00078S-050Y-040ZTM-040ZTY-36ZTM-010Y-0B
KSL11	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-040ZTM-040ZTY-8ZTM-010Y-0B
KSL12	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-040ZTM-040ZTY-28ZTM-010Y-0B
KSL13	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-040ZTM-040ZTY-29ZTM-010Y-0B
KSL14	PYTRF/11	MERCATO//JNRB.5/PIFED	CMSA07WM00080S-050Y-040ZTM-040ZTY-102ZTM-010Y-0B
KSL15	PYTRF/11	PREMIO/BERKUT	CMSA07WM00082S-050Y-040ZTM-040ZTY-88ZTM-010Y-0B
KSL16	PYTRF/11	PREMIO/VORB	CMSA07WM00083S-050Y-040ZTM-040ZTY-63ZTM-010Y-0B
KSL17	PYTRF/11	PREMIO/5/TUI//2*SUNCO/SA1166/3/TUI/4/FINSI	CMSA07WM00088S-050Y-040ZTM-040ZTY-149ZTM-010Y-0B
KSL18	C30 SAWSN/11	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA (TAUS)/4/WEAVER/5/...	CMSA06Y00262S-040ZTP0Y-040ZTM-040SY-37ZTM-0Y-0B
KSL19	C30 SAWSN/11	KRICHAUFF/2*PASTOR/3/CETA/AE.SQUARROSA (327)//2*JANZ/4/PARUS/PASTOR	CMSA06M00011T-037(CSLV34HET)Y-040ZTM-030(CSLV34POS)ZTY-14ZTM-0Y-0B
KSL20	C30 SAWSN/11	EGA BONNIE ROCK/6/CP18/GEDI/3/GOO//ALB/CRA/4/AE.QQUARROSA (208)/5/2*WESTONIA	CMSA05M00023S-0130ZTM-039(LR34HOM+HET)ZTY-040ZTM-040SY-6ZTM-0Y-0B
KSL21	C30 SAWSN/11	EGA BONNIE ROCK/6/CP18/GEDI/3/GOO//ALB/CRA/4/AE.QQUARROSA (208)/5/2*WESTONIA	CMSA05M00023S-0130ZTM-039(LR34HOM+HET)ZTY-040ZTM-040SY-8ZTM-0Y-0B
KSL22	C30 SAWSN/11	VORB*2/3/PFAU/WEAVER//KIRITATI	CMSA06M00111T-040Y-040ZTM-0NJ-0NJ-22Y-3B-0Y-0B
KSL23	29TH SAWSN/11	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA (224)//…	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-2ZTM-01Y-0B
KSL24	29TH SAWSN/11	SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE	CMSA05Y01220T-040M-040ZTP0Y-040ZTM-040SY-9ZTM-02Y-0B
KSL25	29TH SAWSN/11	SOKOLL*2/ROLFO7	CMSA05Y01226T-040M-040ZTP0Y-040ZTM-040SY-17ZTM-03Y-0B
KSL26	RF ELITE/11	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA (224)//OPATA/7/PASTOR//MILAN/KAUZ/3/BAV92	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-6ZTM-01Y-0B
KSL27	M6SRRSN/11	BAV92//IRENA/KAUZ/3/HUITES*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	CMSS06Y00969T-099TOPM-099Y-099ZTM-099Y-099M-7WGY-0B
KSL28	M6SRRSN/11	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	CMSS06B00959T-099TOPY-099ZTM-099NJ-099NJ-2WGY-0B
KSL29	M6SRRSN/11	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	CMSS06B01005T-099TOPY-099ZTM-099NJ-099NJ-28WGY-0B
KSL30	M6SRRSN/11	WAXWING/KIRITATI*2//YANAC	CMSS06Y00689T-099TOPM-099Y-099ZTM-099NJ-099NJ-37WGY-0B
KSL31	M6SRRSN/11	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/HAR311	CMSS06Y00723T-099TOPM-099Y-099ZTM-099Y-099M-17WGY-0B
KSL32	M6SRRSN/11	PBW343*2/KUKUNA/3/PGO/SERI//BAV92	CMSS06B00495S-0Y-099ZTM-099NJ-099NJ-27WGY-0B

Table 2: *continue*

No.[a]	Origin	Pedigree	Selection history
KSL33	PC BWIR/11	KIRITATI//ATTILA*2/PASTOR/3/AKURI	CMSS07Y00143S-0B-099Y-099M-099Y-9M-0WGY
KSL34	PC BWIR/11	KIRITATI//ATTILA*2/PASTOR/3/AKURI	CMSS07Y00143S-0B-099Y-099M-099Y-17M-0WGY
KSL35	PC BWIR/11	KIRITATI//PRL/2*PASTOR/3/FRANCOLIN #1	CMSS07Y00156S-0B-099Y-099M-099Y-6M-0WGY
KSL36	PC BWIR/11	KIRITATI/WBLL1//FRANCOLIN #1	CMSS07Y00174S-0B-099Y-099M-099Y-5M-0WGY
KSL37	PC BWIR/11	MILAN/S87230//BAV92/3/KINGBIRD #1	CMSS07Y00177S-0B-099Y-099M-099Y-36M-0WGY
KSL38	PC BWIR/11	MILAN/S87230//BAV92/3/KINGBIRD #1	CMSS07Y00177S-0B-099Y-099M-099Y-36M-0WGY
KSL39	PC BWIR/11	MILAN/S87230//BAV92/3/KINGBIRD #1	CMSS07Y00174S-0B-099Y-099M-099Y-36M-0WGY
KSL40	PC BWIR/11	MILAN/S87230//BAV92/3/KINGBIRD #1	CMSS07Y00177S-0B-099Y-099M-099Y-36M-0WGY
KSL41	PC BWIR/11	PFAU/SERI.1B//AMAD/3/WAXWING/4/AKURI #1	CMSS07Y00201S-0B-099Y-099M-099Y-15M-0WGY
KSL42	PC BWIR/11	KIRITATI//PRL/2*PASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZ/6/KIRITATI//PRL/2*PASTOR	CMSS07Y00728T-099TOPM-099Y-099M-099Y-2M-0WGY
KSL43	PC BWIR/11	ALTAR84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65	CMSS07Y00855T-099TOPM-099Y-099M-099Y-48M-0WGY
KSL44	PC BWIR/11	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/WHEAR	CMSS07Y01201T-099TOPM-099Y-099M-099Y-15M-0WGY
KSL45	PC BWIR/11	PFAU/SERI.1B//AMAD/3/WAXWING/4/JUCHI	CMSS07Y00199S-0B-099Y-099M-099Y-33M-0RGY
KSL46	PC BWIR/11	KINGBIRD #1/INQALAB 91//INQALAB 91*2/KUKUNA	CMSS07Y01113T-099TOPM-099Y-099M-099Y-6M-0RGY
KSL47	PC BWIR/11	QUAIU #3*2/3/TAM200/PASTOR//TOBA97	CMSS07Y01188T-099TOPM-099Y-099M-099Y-1M-0RGY
KSL48	PC BWIR/11	MUU/KBIRD	CMSS07B00151S-099M-099Y-099M-7WGY-0B
KSL49	PC BWIR/11	KISKADEE #1//KIRITATI/2*TRCH	CMSS07B00258S-099M-099Y-099M-9WGY-0B
KSL50	PC BWIR/11	WBLL1*2/KURUKU*2/4/PFAU/SERI.1B//AMAD/3/WAXWING	CMSS07B00686T-099TOPY-099M-099Y-099M-5WGY-0B
KSL51	PC BWIR/11	SHORTENEDSR26TRANSLOCATION//WBLL1*2/BRAMBLING/3/VILLA JUAREZ 2009	CMSS07Y00151S-0B-099Y-099M-099Y-3WGY-0B
KSL52	PC BWIR/11	SHORTENEDSR26TRANSLOCATION//WBLL1*2/BRAMBLING/3/VILLA JUAREZ 2009	CMSS07Y00172S-0B-099Y-099M-099Y-6WGY-0B
KSL53	PC BWIR/11	SITE/MO//PASTOR/3/TILHI/4/MUNAL #1	CMSS07Y00236S-0B-099Y-099M-099NJ-099NJ-3WGY-0B
KSL54	PC BWIR/11	TUKURU//BAV92/RAYON/3/MUNAL #1	CMSS07Y00236S-0B-099Y-099M-099NJ-099NJ-5WGY-0B
KSL55	PC BWIR/11	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WBLL1*2/BRAMBLING	CMSS07Y00765T-099TOPM-099Y-099M-099NJ-099NJ-7RGY-0B
KSL56	PC BWIR/11	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WBLL1*2/BRAMBLING	CMSS07Y00770T-099TOPM-099Y-099M-099NJ-099NJ-2RGY-0B
KSL57	URUGUAY 11	R09 RC1F5-5292	
KSL58	URUGUAY 11	PARULA	
KSL59	TURKEY 11	PAMUKOVA-97*3/3/88 ZHONG 257//CNO79/PRL	STAE-09-10
KSL60	TURKEY 11	PAMUKOVA-97/AROSTOR	STAE-09-10
KSL61	TURKEY 11	CARISMA	STAE-09-10
KSL62	TURKEY 11	SVEVO	Orijinal
KSL63	BRAZIL	BR 35/CEP 9291/4/BR 32/3/CNO 79/PF 70354/MUS"S"	FCEP. CRISTALINO
KSL64	BRAZIL	PF 70100/J15157-69	MN07330-8
65	ROBIN	BABAX/LR42//BABAX*2/3/TUKURU (Resistant check)	
66	CACUKE	CANADIAN/CUNNINGHAM//KENNEDY (Susceptible check)	

KSL: Kenyan Selection, PYTRF/11: Preliminary Yield Trial-Rain Fed 2011, SAWSN/11: Semi-Arid Wheat Screening Nursery 2011, RF ELITE/11: Elite Rain-fed lines 2011, SRRSN/11: Stem Rust Resistant Screening Nursery 2011PC BWIR/11: *Parcela chica* "small plots" Bread Wheat Irrigated Lines 2011.

[a]: Entry number

3.3.3 Field Tests

The plant materials evaluated in this study are described under section 3.3.2 on seedling resistance test (Table 2). The trials were carried out during 2012 cropping season across three locations in Kenya; Njoro (0^o20'S, 35^o56'E and 2185 m a.s.l), Timau (0^o5'S, 37^o20'E and 2640 m a.s.l) and Mau Narok (0°38' S, 35°47′ E and 2185 m a.s.l) with annual rainfall of 939 mm (15 years), 896 mm (15 years) and 1200 mm (15 years), respectively and average annual minimum and maximum temperatures of 9°C and 24°C, 5°C and 23°C, and 11°C and 24.5°C, respectively. The experimental design used at all the three locations was alpha-lattice (22 rows × 3 columns) with three replications. Each entry was planted in plots of 2 rows × 1.2 m long × 0.2 m apart at a seed rate of 125 kg ha^{-1}. The entries were separated by 0.3 m and 0.5 m wide alleyways within and between the blocks, respectively. Susceptible wheat cultivar, *Cacuke* was planted around the trial plot and in the middle of the 0.5 m alleyway on both sides of plots to facilitate uniform inoculum build up and serve as spreader. Nitrogen and Phosphorus were applied at the rate of 22.5 kg N ha^{-1} and 25.3 kg P ha^{-1}. Buctril MC (225 g L^{-1} Bromoxynil octanoate and 225 g L^{-1} MCPA Ethylhexylester), a post emergence herbicide was sprayed at tillering stage at the rate of 7 ml L^{-1} of water to control broad-leaved weeds. The trial was top dressed with 30 kg N ha^{-1} at jointing. Manual weeding by hand was done two times between stem elongation and booting stages to eradicate grasses. The field trials across sites were under natural infection.

Assessment of plants for APR was done from milk to early dough stage (Zadok's growth stage 75 to 85) (Zadoks *et al*., 1974) of grain development. The adult plant response to infection was classified according to Roelfs *et al.* (1992) (Appendix 1b) into four categories; R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible and overlapping responses between two categories was denoted using a dash (−) between the categories for example MR/MS. The stem rust severity was determined by use of the modified Cobb scale where the severities ranged from 5%-100% (Peterson *et al.*, 1948) (Appendix 1c). Disease observations were made three times on an eight day interval.

Data on 1000 kernel weight was obtained by weighing 1000 grains from each entry, using a beam balance. Total plants in each entry were harvested for grain yield assessment. The initial moisture content of the grains was determined using a grain moisture meter. Grain yield was then adjusted for 12.5% seed moisture before conversion to tons ha^{-1} for statistical analysis. The formula for adjustment was;

$$Y_2 = \frac{12.5Y_1}{I}$$

Where Y_1 is the initial grain weight at initial grain moisture I, and Y_2 is the adjusted grain weight at 12.5% moisture content.

3.4 Data Analysis

3.4.1 Adult Plant Resistance to Stem Rust

Mean disease severity was used to calculate the area under disease progress stairs (AUDPS) (Ali *et al.*, 2012). The coefficient of infection (CI) was calculated by taking into account the disease severity and the host response to infection of the final disease observation where; 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 represented immune, resistant (R), moderately resistant (MR), moderately resistant to moderately susceptible (M), moderately susceptible (MS) and susceptible (S), respectively (Roelfs *et al.*, 1992). Since disease observations were made on a regular interval, the following formula as described by Simko and Piepho (2012) was applied;

$$\text{AUDPS} = \bar{y} \times \frac{Dn}{n-1}$$

Where, $\bar{y}$ is the arithmetic mean of all assessments, D is time duration (in days) between the first and the last observation and, n is the number of observations.

3.4.2 Analyses of Variance and Genotype × Environment (GE) Interaction

A combined analysis of variance for AUDPS, CI, TKW, and yield across sites was performed using the Statistical Analysis Software (SAS) version 9.1 procedural PROC GLM (SAS Institute Inc., Cary, 2002) with genotypes as fixed effects while, location, replicates and blocks within replicates were random. The following statistical model was used;

$$Y_{ijk} = \mu + G_i + L_l + R_j + B_{(kj)} + GL_{il} + \varepsilon_{ijk}$$

Where; Y_{ijk} = observations; μ = mean of the experiment; G_i = effect of the i^{th} genotype; L_l = effect of l^{th} location; R_j = effect of the j^{th} replicate (superblock); $B_{(jk)}$ = effect of the k^{th} incomplete block within the j^{th} replicate; GL_{il} = interaction between i^{th} genotype and l^{th} location and ε_{ijk} = experimental error. The least significant difference was determined at $P<0.05$. For analysis of static stability in disease resistance, genotypic variance (S_i^2) (Lin *et al.*, 1986) and used by Francis and Kannenburg (1978) was computed using IBWorkFlowSystem. The analysis follows the equation;

$$S_i^2 = \sum_{j=1}^{q} X_{ij}(X_{ij} - \ddot{X}_i)^2 / \, q-1$$

Where, X_{ij}= disease value (AUDPS/CI) of genotype i in location j; $\ddot{X}_i$ = mean of genotype i across all locations; $X_{ij}-\ddot{X}_i$ = deviation from the average disease value, and q = number of locations.

3.4.3 Regression Analysis

General linear regression analysis was also conducted to find the best equation and equation and line that predicted wheat yield from disease level. These analyses were performed using GenStat 14th edition statistical software (VSN international, Ltd 2010). For testing the adequacy of the model, a scaling test known as regression coefficient analysis was used. Departure of the regression coefficient (*b*) from zero was tested using (*b*-0)/s.e*b* while departure of *b* from unity was tested using (1-*b*)/s.e*b*, where s.e is the standard error. Significant deviation of regression coefficients from Zero but not from unity indicated the adequacy of model in explaining the relationships of yield and the two disease parameters.

3.4.4 Yield Stability and Genotypic Adaptability across Locations

Finlay and Wilkinson's analysis (Finlay and Wilkinson, 1963), Wricke's ecovalence (Wi) (Wricke, 1962), and GGE biplot models in IBWorkFlowSystem were used to analyze genotype sensitivity, stability and for graphic exploration of relationships between genotypes and/or sites, respectively. The joint regression analysis according to Finlay and Wilkinson (1963) follows the ANOVA statistical model described in section 3.4.2. However, *GL* is partitioned into a component due to regression b_i of the i^{th} genotype on the location mean and a deviation d_{ij}.

$$GL_{il} = b_i L_j + d_{ij}$$

The ecovalence W_i or stability of the i^{th} genotype is its interaction with the environments, squared and summed across environments, and expressed as;

$$W_i = [\bar{y}_{ij} - \bar{y}_{i.} - \bar{y}_{.j} - \bar{y}_{..}]^2$$

Where $\bar{y}_{ij}$ is the mean performance of genotype i in the j^{th} environment; $\bar{y}_{i.}$ and $\bar{y}_{.j}$ are the genotype and environment mean deviations, respectively; $\bar{y}_{..}$ is the overall mean.

3.5 Results

3.5.1 Seedling Reaction to Stem Rust

Based on a set of differentials, the inoculum used in seedling experiment was identified as TTKST (*Ug99* with virulence on *Sr24*) (Table 3). The results for seedling reactions of the

genotypes are presented in table 4. Twenty-six genotypes exhibited resistance (IT's of ";", "1", "2" or combinations), one line, KSL 31 showed a heterogeneous reaction "x" while the rest 39 lines showed susceptible reactions. The two checks *Robin* (resistant check) and *Cacuke* (Susceptible check) showed resistance (1+) and susceptibility (4), respectively.

3.5.2 Analyses of Variance and Genotype × Location (GL) Interaction for Adult Plant Resistance to Stem rust

The rate of stem rust disease progress and final disease observation (severity and host response) are represented in the AUDPS and CI values, respectively. Significant ($P<0.05$) main effects of genotypes (G), locations (L), and interaction between genotype (G) and location (L) (GL) were obtained for CI, AUDPS and yield (Table 5). The main effects and interactions were not significant except for location main effect ($P < 0.05$) for TKW. The presence of GL for the traits indicated that the mean yield and host reaction to stem rust varied across locations. Therefore, further analyses were conducted for the disease parameters and yield.

3.5.3 Adult Plant Resistance to Stem Rust and Stability Analyses

The CI, AUDPS, and stability values for the genotypes that proved better than the resistant check based on AUDPS values are presented in Table 6. Based on the LSD mean separation at $P < 0.05$, the three sites were significantly different from each other. Timau had the lowest disease pressure as evidenced by its mean values of CI (0.5) and AUDPS (69.3), followed by Njoro with CI and AUDPS values of 0.8 and 273.3, respectively. Mau Narok had the highest disease pressure with AUDPS and CI values of 1.2 and 441.6, respectively. Genotypes that ranked among the best performers with regard to rate of disease development as indicated by AUDPS means include KSL 42, KSL 51, and KSL 3 with AUDPS values of 30.2, 42.7 and 74.7, respectively. These genotypes also had low CI values of 0.2, 0.4, and 0.5, respectively. The susceptible check *Cacuke* displayed the highest mean values of CI (1.7) and AUDPS (671.4). This implies that AUDPS and CI were positively related. The best genotypes with regard to AUDPS stability values included; KSL 42 (1400), KSL 59 (2110), and KSL 51 (2440). The

Table 3: Stem rust race identification

Seedling infection type data for differential sets

Diffrential set	Sr gene	Infection Type	Reaction	Code
I	5	3	H	T
	21	3+	H	
	9e	3+	H	
	7b	3	H	
II	11	3+	H	T
	6	4	H	
	8a	3+	H	
	9g	4	H	
III	36	0	L	K
	9b	3+	H	
	30	3	H	
	13+17	3	H	
IV	9a	3	H	S
	9d	4	H	
	10	3	H	
	Tmp	1	L	
V	24	4	H	T
	31	3	H	
	38	3	H	
	Mcn	3	H	
				TTKST

H=High infection type
L=Low infection type

First 16 consonants of the English alphabet used as code for each set of differentials (stem rust)

North American Stem Rust Nomenclature Code Sheet

	Four gene differential sets				
	Sr5	*Sr21*	*Sr9e*	*Sr7b*	Set 1
	Sr11	*Sr6*	*Sr8a*	*Sr9g*	Set 2
	Sr36	*Sr9b*	*Sr30*	*Sr17*	Set 3
	Sr9a	*Sr9d*	*Sr10*	*SrTmp*	Set 4
Pgt letter	*Sr24*	*Sr31*	*Sr38*	*SrMcN*	Set 5
B	L	L	L	L	
C	L	L	L	H	
D	L	L	H	L	
F	L	L	H	H	
G	L	H	L	L	
H	L	H	L	H	
J	L	H	H	L	
K	L	H	H	H	
L	H	L	L	L	
M	H	L	L	H	
N	H	L	H	L	
P	H	L	H	H	
Q	H	H	L	L	
R	H	H	L	H	
S	H	H	H	L	
T	H	H	H	H	

H = High Infection Type (3-4 on standard evaluation scale)
L = Low Infection Type (0-2 on standard evaluation scale)

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

Table 4: Seeding infection type to stem rust (*Puccinia graminis*) of the wheat (*Triticum aestivum* L.) genotypes evaluated in a greenhouse.

Genotype	IT[1]	Genotype	IT[1]
KSL 1	2	KSL 34	4
KSL 2	3+	KSL 35	3+
KSL 3	4	KSL 36	3-
KSL 4	3	KSL 37	3+
KSL 5	; 1+	KSL 38	4
KSL 6	3+	KSL 39	3+
KSL 7	3-	KSL 40	2+
KSL 8	3+	KSL 41	4
KSL 9	; 1	KSL 42	;1
KSL 10	0	KSL 43	3
KSL 11	2+	KSL 44	;1
KSL 12	0	KSL 45	3-
KSL 13	1+	KSL 46	;1
KSL 14	2	KSL 47	3+
KSL 15	3+	KSL 48	3
KSL 16	;1+	KSL 49	3
KSL 17	3+	KSL 50	3-
KSL 18	4	KSL 51	3-
KSL 19	4	KSL 52	;1
KSL 20	3+	KSL 53	2
KSL 21	1	KSL 54	3-
KSL 22	3+	KSL 55	;1
KSL 23	4	KSL 56	;1
KSL 24	;1	KSL 57	3
KSL 25	1+	KSL 58	3+
KSL 26	3-	KSL 59	3
KSL 27	3+	KSL 60	3
KSL 28	3+	KSL 61	4
KSL 29	3-	KSL 62	1+
KSL 30	;1	KSL 63	2+
KSL 31	x	KSL 64	2+
KSL 32	3+	Robin	1+
KSL 33	2+	Cacuke	4

[1]IT: Infection type, KSL: Kenyan Selection

Table 5: Mean squares for stem rust disease parameters and grain yield of 66 wheat genotypes evaluated across three locations in Kenya during 2012-2013 cropping season.

Source of variance	d.f	CI	AUDPS	TKW	YIELD t/ha
Location	2	26.57*	6884551.22*	0.0029*	27.11*
Rep(Loc)	2	0.03	5726.49	0.0003	0.07
Block(Rep)	2	0.10	70133.76*	0.0001	0.30
Genotype	65	0.71*	252686.29*	0.0003	7.59*
Genotype×Location	130	0.17*	80119.25*	0.0002	4.62*
Error	367	0.03	9602.40	0.0002	0.45
CV%		20.81	37.49	43.19	12.87
R_A^2		0.90	0.91	0.36	0.86

*Significant at $P < 0.05$, R_A^2 Adjusted coefficient of determination

Table 6: The stem rust Coefficient of Infection (CI), Area Under Disease Progress Stairs (AUDPS), and stability values for the wheat genotypes that proved better than the resistant check as evaluated across three locations in Kenya. Genotypes were ranked according to their AUDPS means across locations.

	CI						AUDPS					
	Mau Narok	Njoro	Timau	Mean	Stability (1000)	Rank	Mau Narok	Njoro	Timau	Mean	Stability (1000)	Rank
KSL 42	0.6	0.1	0.0	0.2	0.01	2	72.0	18.7	0.0	30.2	1.40	2
KSL 51	0.8	0.2	0.1	0.4	0.15	32	98.7	24.0	5.3	42.7	2.44	5
KSL 3	0.9	0.3	0.2	0.5	0.16	35	178.7	32.0	13.3	74.7	8.20	10
KSL 57	1.1	0.1	0.7	0.6	0.26	47	173.3	2.7	82.7	86.2	7.29	9
KSL 13	0.7	0.4	0.2	0.4	0.04	6	141.3	120.0	0.0	87.1	5.81	8
KSL 7	1.0	0.4	0.2	0.5	0.16	34	216.0	34.7	18.7	89.8	12.01	14
KSL 34	1.0	0.3	0.1	0.5	0.26	50	245.3	34.7	8.0	96.0	16.90	18
KSL 59	0.9	0.3	0.6	0.6	0.09	12	152.0	64.0	85.3	100.4	2.11	4
KSL 5	0.9	0.4	0.1	0.5	0.18	37	202.7	101.3	8.0	104.0	9.48	12
KSL 62	0.9	0.4	0.1	0.5	0.18	36	216.0	98.7	5.3	106.7	11.14	13
KSL 29	1.0	0.9	0.5	0.8	0.07	9	152.0	162.7	45.3	120.0	4.21	7
KSL 8	1.0	0.3	0.6	0.6	0.13	22	256.0	34.7	80.0	123.6	13.67	15
KSL 9	1.0	0.5	0.3	0.6	0.11	17	269.3	98.7	10.7	126.2	17.30	19
KSL 61	1.1	0.8	0.3	0.7	0.14	27	232.0	160.0	45.3	145.8	8.86	11
KSL 39	1.1	0.7	0.3	0.7	0.19	38	306.7	125.3	32.0	154.7	19.51	21
KSL 58	0.9	0.6	0.7	0.7	0.03	4	229.3	125.3	114.7	156.4	4.01	6
KSL 63	1.2	0.3	0.6	0.7	0.23	41	349.3	45.3	85.3	160.0	27.29	27
KSL 43	1.1	0.7	0.4	0.7	0.13	23	298.7	138.7	48.0	161.8	16.11	17
KSL 30	1.1	0.3	0.3	0.6	0.20	39	413.3	29.3	48.0	163.5	46.88	40
Robin[a]	1.0	0.6	0.3	0.6	0.12	19	333.3	162.7	26.7	174.2	23.61	24
Cacuke[b]	2.0	2.0	1.1	1.7	0.24	45	1693.3	229.3	1174.2	671.4	671.79	66
Means	**1.2**	**0.8**	**0.5**				**441.6**	**273.3**	**69.3**			
LSD (0.05)[c]				**0.16**						**90.81**		
LSD (0.05)[d]				**0.03**						**19.36**		

KSL; Kenya selection, [a]; resistant check, [b]; susceptible check, [c]; LSD for comparing means within locations and [d]; LSD for comparing means between locations.

AUDPS means for these genotypes were also not significantly different from each other ($P < 0.05$). It is also notable that KSL 42 was the most stable of the presented genotypes in regard to CI stability values. A simple regression analysis of disease on yield of the wheat genotypes revealed a significant linear and inverse relationship ($P < 0.01$) (Figure 1 and 2). The regression coefficient deviated significantly from Zero but not from unity for CI (P=0.001). This indicated the adequacy of model in explaining the relationship of yield and the disease CI disease parameters. However, regression coefficient for AUDPS significantly deviated from zero and also from unity (P=0.001) indicating partial adequacy of the regression in explaining the relationship between AUDPS and yield.

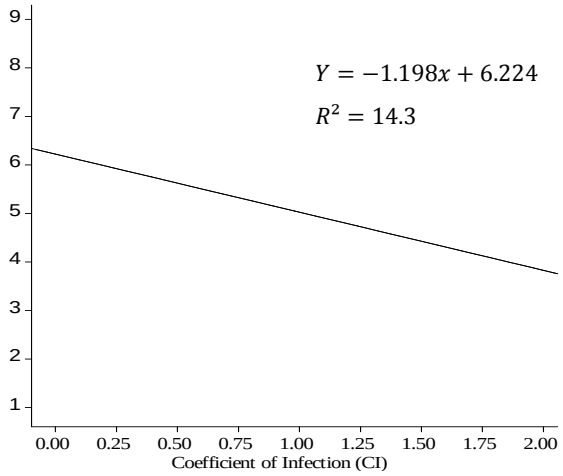


Figure 1: Association of grain yield with CI for the all the tested wheat genotypes.

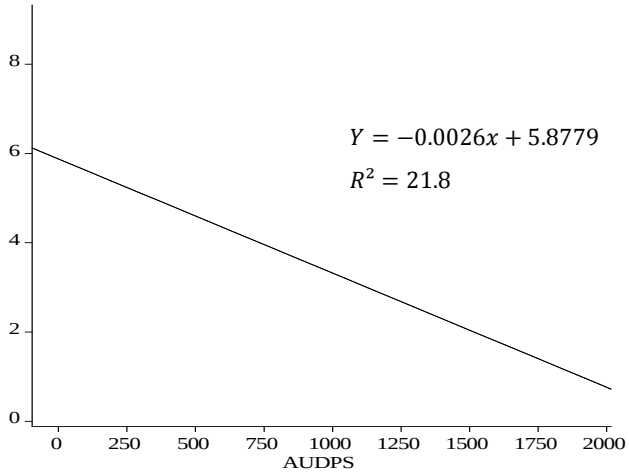


Figure 2: Association of grain yield with stem rust AUDPS for all the tested wheat genotypes.

3.5.4 Genotypic Stability and Yield Performance across Sites

Significant ($P < 0.05$) main effects of genotypes (G), locations (L), and interaction between genotype (G) and location (L) (GL) were obtained for grain yield (Table 5). This showed the importance of location in differentiating the genotypes with regard to yield. The mean yields for the best 20, and the two checks of wheat genotypes evaluated across sites are presented in Table 7. From the results of genotypic means across locations, Njoro (5.4 t ha^{-1}) and Timau (5.4 t ha^{-1}) recorded equal high yields, while Mau Narok (4.8 t ha^{-1}) recorded the lowest yield. Genotypes KSL 42, KSL 3, and KSL 9 ranked the highest with; 7.8 t ha^{-1}, 6.6 t ha^{-1} and 6.6 t ha^{-1} mean yields across sites, respectively. Results of Wricke's stability are also presented in Table 7. Genotypes are ranked based on their stability values. From the table, KSL 42 was the most stable genotype followed by KSL 3 with stability values of 0.17 and 0.21, respectively. The two genotypes also ranked best in regard to yield (Table 7) although they were significantly different from each other at $P < 0.05$.

3.5.5 The Finlay and Wilkinson Adaptability for Yield

Further analysis of genotype (G) × location (L) interaction in Finlay and Wilkinson (1963) modified joint regression analysis for yield of the evaluated genotypes showed significant genotypes (G), location (L) and sensitivities ($P < 0.05$) (Table 8). The sensitivities and average Table 8: Analysis of variance in Finlay and Wilkinson modified joint regression analysis of yield for the wheat genotypes evaluated across three sites in Kenya during 2012-2013 cropping season yield performance for 30 genotypes representing specific adaptability to favorable environments, general adaptability, and specific adaptability for unfavorable environments across the locations are presented in a two dimension scatter plot (Figure 3). From the scatter plot, the most adapted genotypes to unfavorable locations are located towards the bottom of the plot. Such genotypes are; KSL 13 (26), KSL 26 (30), KSL 34 (29), and KSL 8 (28). The most adapted genotypes to favorable location are located towards the top of the plot, such genotypes are KSL 20 (3), KSL 63 (2), and KSL 2 (4). The generally adapted genotypes to all locations are located on or close to b=1.0 regression coefficient line. The further a genotype is to the right, the higher the yield. With regard to general adaptability and yield, KSL 3 (19) and KSL 42 (18) were the best since they were close to the b=1.0 regression line and farthest to the right.

Table 7: The mean yields and Wricke's stability values for the best 20 and the two checks of wheat genotypes evaluated across the three sites in Kenya during 2012-2013 cropping season. Selection and ranking of the genotypes was based on their mean yields.

			Yield t ha^{-1}				Rank*
	Genotype	Means	Mau Narok	Njoro	Timau	Stability	(Stability)
1	KSL 42	7.8	7.6	8.2	7.6	0.17	4
2	KSL 3	6.6	6.5	6.7	6.6	0.21	5
3	KSL 9	6.6	5.1	8.0	6.8	2.54	39
4	KSL 4	6.5	7.4	6.7	5.5	3.21	42
5	KSL 61	6.5	4.9	7.3	7.4	2.07	37
6	KSL 7	6.3	7.1	7.3	4.6	5.73	55
7	KSL 29	6.3	7.2	6.4	5.3	3.22	26
8	KSL 11	6.2	7.2	4.6	6.7	5.44	54
9	KSL 13	6.2	7.7	5.7	5.2	5.93	56
10	KSL 14	6.2	6.5	6.0	6.3	0.74	18
11	KSL 28	6.2	5.1	7.1	6.3	0.91	25
12	KSL 58	6.1	5.3	6.0	6.9	0.60	14
13	KSL 34	6.0	7.7	5.9	4.5	7.53	60
14	KSL 10	5.9	5.1	5.9	6.5	0.43	9
15	KSL 60	5.9	3.5	7.8	6.6	6.15	6
16	KSL 63	5.9	2.9	7.0	7.8	10.27	64
17	KSL 26	5.8	7.8	5.1	4.7	8.71	62
18	KSL 32	5.8	5.2	6.8	5.3	1.13	29
19	KSL 55	5.8	4.8	5.9	6.6	0.70	16
20	KSL 12	5.7	7.2	5.6	4.3	6.39	58
21	ROBIN	4.9	3.8	5.3	5.6	0.72	17
22	CACUKE	3.6	1.4	1.7	7.5	22.06	66
Means			**4.8**	**5.4**	**5.4**		
CV				**12.87**			
LSD (0.05)[a]				**0.62**			
LSD (0.05)[b]				**0.13**			

KSL: Kenyan Selection, [a]; LSD for comparing means within locations, [b]; LSD for comparing means between locations.
*Rank was based on stability values

Table 8: Analysis of variance in Finlay and Wilkinson modified joint regression analysis

Source	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Genotypes	65	493.92	7.60	8.44	<0.001
Locations	2	54.24	27.12	30.12	<0.001
Sensitivities	65	363.75	5.60	6.22	<0.001
Residual	461	415.04	0.90		
Total	593	1326.95	2.24		

3.5.6 The Genotype and Genotype × Environment (GGE) Biplot for Yield

The GGE biplot presentation of the average grain yield under natural stem rust disease pressure across sites is presented in Figure 4. The first and the second principal components (PC1 and PC2) explained more than 79% of the total variation across sites. There was an acute angle between the environmental axes of Njoro and Mau Narok, indicating similarity between the two sites with regard to genotype yield performance. The same relationship was detected for Timau and Njoro. With regard to disease, the two sites had slightly different means (Table 6). However, an obtuse angle between Timau and Mau Narok indicated a negative correlation in genotypic yield performance between the two sites. Mau Narok made the larger contribution of the GE interaction as it had a longer projection from the biplot origin. Also, the site was more discriminative being the farthest from the biplot origin (Figure 4) and had a higher disease pressure (Table 6); making it a more suitable site for evaluating grain yield under the disease pressure. High yielding genotypes are concentrated on the right side of the biplot while low yielding ones are located on the left side of the biplot. These include KSL 42 (42) on the extreme

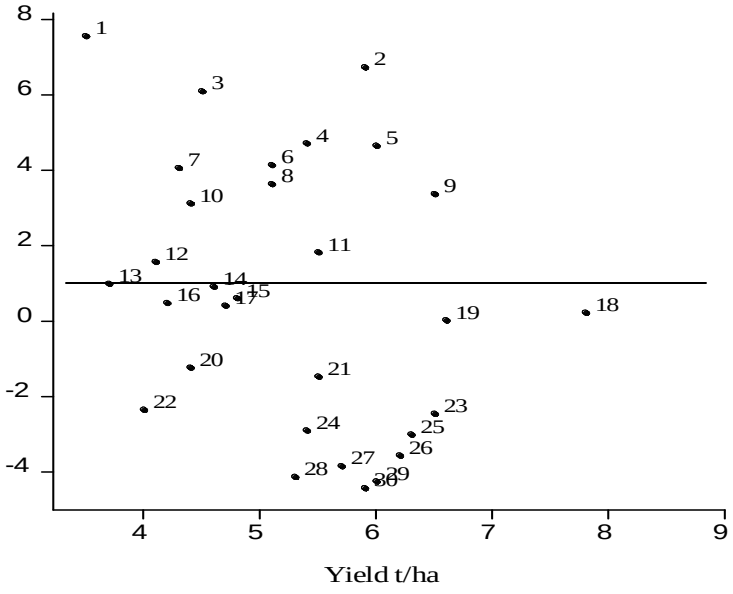


Figure 3: Relationship of genotype adaptation (Finlay and Wilkinson Sensitivity) and genotypic mean yield for the genotypes representing specific adaptability to favorable environments, general adaptability, and specific adaptability for unfavorable environments across the locations.

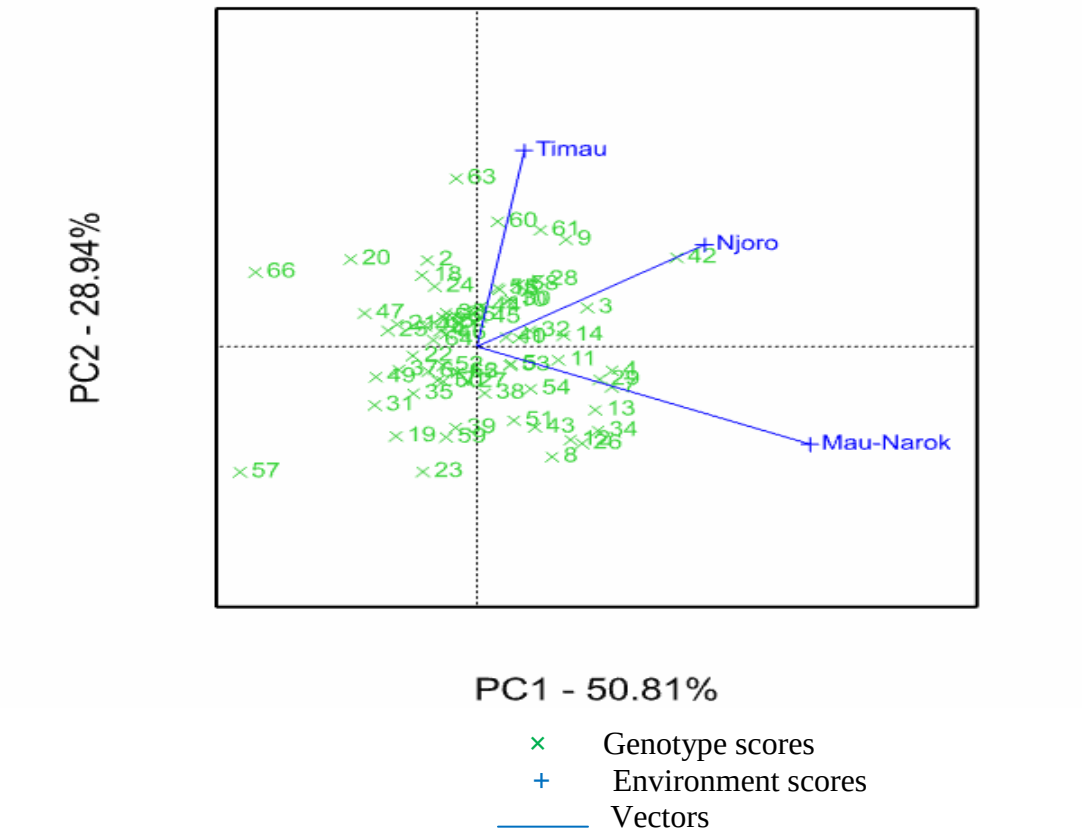


Figure 4: The GGE biplot presentation of the average grain yield across the sites.

right and KSL 57 (57) on the extreme left (and below the origin). Genotype KSL 63 (63) has a good yield as it is situated on the right side of the biplot and projected on Timau axis above origin indicating a positive interaction with that site. Genotype KSL 42 interacts positively with Njoro as it projected on that site's vector. On the other hand, KSL 13 (13) interacts positively with Mau Narok as its vector is close to that site's vector. These three genotypes were best performers in their respective sites in spite of the stem rust disease pressure.

3.6 Discussion

Stem rust is among the most destructive of wheat diseases and can cause heavy yield loss if uncontrolled. It is however possible to mitigate this yield loss through the use of fungicides, but this has a serious cost implication to a resource poor farmer. Consequently, plant breeders have emphasized the use of genetic sources of resistance. Major gene resistance/seedling resistance can offer complete protection and significant economic benefits to farmers. Nevertheless, this kind of resistance is known to lack durability (Johnson, 1983). Adult plant resistance (APR) is not complete and not limited to specific physiological races of the pathogen

but unlike major gene resistance, it can be durable, hence a major concentration for wheat breeders and valuable to farmers. However, the APR genes can render the plant completely susceptible to the pathogen at seedling stage.

From the field experiments, the high stem rust infection of *Cacuke* which was used as a susceptible check and disease spreader implies that the disease response recorded in all the three sites was predominantly due to TTKST (*Ug99* + *Sr24* virulent). This postulation is also supported by previous reports of Singh *et al.* (2011) which revealed TTKST as the predominant race of *Ug99* in Kenyan fields by 2011. Nonetheless, there was evidence of significant genetic variability in disease response and severity among the genotypes across locations. However, most lines exhibited some resistance. Similar variations among wheat genotypes have previously been reported (Tabassum, 2011; Macharia and Wanyera, 2012). Some lines exhibited high susceptibility at seedling stage but low severity in the field e.g. KSL 3. Similar trends of genotypes showing susceptible reactions at the seedling stage and maintaining low severity in the field across a range of environments have been reported; this phenomenon is common with genotypes based on the *Sr2* gene (Njau *et al.*, 2010; Singh *et al.*, 2011). The stem rust resistant gene *Sr2* is so far the only characterized minor gene and it can offer moderate levels of resistance when alone (Mago *et al*., 2005; Singh *et al.*, 2005) but in case of a high disease pressure, the resistance offered may not be effective unless it is in combination with other unknown APR genes (Singh *et al.*, 2005). This might explain the high values of AUDPS and CI in Mau Narok. Effective major gene/s in combination with APR gene/s may explain the kind of resistance in KSL 42 which showed resistant reactions both at seedling stage and in the adult plants across sites. The pedigree information also shows that KSL 42 has *Kiritati* in the parentage, this CIMMYT genotype is known to possess the *Sr2*; this hypothesizes the source of APR gene/s in KSL 42. According to Johnson (1983), there is a need to employ adult plant resistance since it is durable and can be used for a long time especially when the host is exposed to a wide range of the pathogen. In our stability analysis, KSL 42 and KSL 3 were the most stable genotypes across the three locations. The two genotypes also displayed high mean yields. Besides disease resistance, farmers' preference is also for high yielding and stable varieties, hence yield stability and adaptability is an important concept in wheat breeding.

Yield variability existed across locations; this was due to diverse genetic backgrounds for the genotypes (as depicted by pedigree information), location, and GL interaction. There was a

negative relationship between disease and yield. This was evidenced by low performance of genotypes in Mau Narok with mean yield of 4.8 t ha^{-1}. Apparently, the site had the highest means for the two disease parameters. Similar negative relationship between yield and the disease has been reported by Bathal *et al.* (2003), Ali *et al.* (2007; 2009), Macharia and Wanyera (2012). Most genotypes that showed high values of CI and AUDPS had lower yields, however some contradiction were observed, for example, KSL 4 which did not rank better than *Robin*, ranked among top five high yielders. This may be attributed to tolerance of the line and therefore detailed studies should be performed to explore this phenomenon. The weak relationship between the disease and yield is because variation in yield is not only due to disease response but also other sources of variability mentioned above. The existence of such variation enables the breeder to select both high yielding and disease resistant genotypes across sites and in presence of disease pressure. Genotypes KSL 42 and KSL 3 combined both field disease resistance and high yield. Such genotypes are potential candidate lines for variety release.

According to Wricke's ecovalence values (*Wi*) (Wricke, 1962), the lower the values, the more stable the genotype is; and hence KSL 3 and KSL 42 proved to be the most stable genotypes compared to *Robin* (a check variety) with regard to grain yield. These two genotypes emerged among the best in yield as well as phenotypic stability *i.e.* they performed consistently across locations. Such genotypes are useful to farmers because they would give consistent yields that can withstand unpredictable and transient environmental fluctuations. Selection for specific adaptability is also useful because farmers are able to utilize high yielders for their respective environment. From the scatter plot, most genotypes had regression coefficients $b<1$. Such genotypes, for example, KSL 13 (26), KSL 26 (30), KSL 34 (29), and KSL 8 (28) were highly adapted to poor environment (such as Mau Narok which had high disease pressure). This was further evidenced by these genotypes' close association with Mau Narok in a GGE biplot. They were able to endure environmental change (above average stability), and therefore had specific adaptability to low yielding environments. However, according to Finlay and Wilkinson (1963), plant breeders ignore the results obtained in low yielding environments on the sense that they have very low yields and are therefore not able to differentiate between the selections characterized. The genotypes with $b>1$, for example, KSL 20 (3), KSL 63 (2), and KSL 2 (4) were sensitive to environmental changes and are therefore suitable for cultivation under favorable conditions such as Timau which had low disease pressure as shown in Table 6. The

close associated of these genotypes with Timau is also depicted in biplot. Therefore, such specifically adapted genotypes have high yields and are able to be differentiated from the selections. Similar findings have been reported by Mevlüt *et al.* (2009). According to Finlay and Wilkinson (1963), genotypes characterized by *b*=1.0 are considered to have average phenotypic stability and hence adapted to all environments. Genotypes KSL 3 (19), and KSL 42 (18) were distributed close to *b*=1.0 regression line, thus phenotypically stable. On the other hand, KSL 42 combined high yield and phenotypic stability.

The GGE biplots afford a platform for breeders to graphically explore the relationship/s between genotypes and/or environments; the closer the genotypes and/or environments the higher the similarity (Malosetti *et al.*, 2013). This is determined by the angle between the vectors for each factor projected to the biplot origin. It shows those sites which are ideal and representative environment for experimentation and the effect of specific traits of interest e.g. stem rust resistance for each wheat genotype on yield performance, adaptability, and stability across environments. In this study, Njoro and Mau Narok were similar in genotypic yield performance under the disease pressure due to an acute angle between the environmental axes of the two sites. The same relationship was detected for Timau and Njoro. However, there was a negative correlation in genotypic yield performance between Timau and Mau Narok. Mau Narok proved to be a good site for wheat selection for yield under stem rust pressure since it had a larger projection from the biplot origin, indicating that it made the largest contribution of the GL interaction. According to Malosetti *et al.* (2013), the projection of a genotype onto a site vector reflects the performance of that genotype in that environment. Therefore, KSL 63 has a good yield and is positively associated with Timau; KSL 42 interacted positively with Njoro while KSL 13, KSL 4, KSL 26 and KSL 34 interacted positively with Mau Narok. This positive association of genotypes with sites was in spite of the stem rust disease in these sites. The GGE biplots have been previously used to identify superior wheat genotypes with regard to yield and other agronomic traits (Mohammadi *et al.*, 2011).

3.7 Conclusion

The screening of stem rust disease in a greenhouse and the three field locations allowed evaluation of stem rust at both levels. The field experiments also allowed assessments of yield potential of the genotypes in presence of the disease. The results of this study revealed existence of variation in stem rust resistance and grain yield among the evaluated germplasm. This study

therefore allowed for identification of wheat lines which combined both good yield and disease resistance. With regard to disease resistance yield performance, the genotypes KSL 42 and KSL 3 consistently ranked among the top performers and thus are recommended for utilization in wheat breeding programs in Kenya for improvement of stem rust resistance and yield. These outstanding lines can be included in regional and national trials and used as parental lines for obtaining a segregating population for stem rust disease resistance and yield related traits. Therefore, it is necessary to carry out genetic analysis to identify the kind of gene action to guide in effective introgression of these important traits into the Kenyan adapted but stem rust susceptible commercial cultivars.

3.8 References

Admassu, B., Lind, V., Friedt, W., & Ordon, F. (2008). Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of *Ug99*. *Plant Pathology 58,* 362-369.

Ahmad, J., Choundhrey, M. H., Salah-ud-Din, S., & Ali, M. A. (1996). Stability for grain yield in wheat. *Pakistan Journal of Botany, 28,* 61-65.

Ali, S., Jawad, S., Shah, A., Rahman, H., Saqib, M. S., Ibrahim, M., & Sajjad, M. (2009). Variability in wheat yield under yellow rust pressure in Pakistan. *Turkish Journal of Agricultural Forage, 33,* 537–546. doi:10.3906/tar-0812-27.

Ali, S., Shah, S. J. A., & Ibrahim, M. (2007). Assessment of wheat breeding lines for slow yellow rusting (*Puccinia striiformis* west. *tritici*). *Pakistan Journal Biological Sciences, 10,* 3440-3444.

Allard, R. W., & Shands, R. G. (1954). Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevi*. *Phytopathology, 44,* 266-274.

Bathal, J. S., Loughman, R., & Speijers, J. (2003). Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and *Septoria nodorum* blotch. *European Journal of Plant Pathology, 109,* 435-443.

Breese, E. L. (1969). The measurement and significance of genotype-environment interaction in grasses. *Heredity, 24*, 27-44.

Finlay, K. W., & Wilkinson, G. N. (1963). The analysis of adaptation in a plant breeding program. *Australian Journal of Agricultural Research, 14*, 742-754.

Francis, T. R., & Kannenburg, L. W. (1978). Yield stability studies in short-season maize. I. A descriptive method for grouping genotypes. *Canadian Journal of Plant Science, 58,* 1029-1034.

Ginkel, M., & Rajaram, S. (1993). Breeding for durable resistance to diseases in wheat: In T. Jacobs, & J. E. Parlevliet (Eds.), *An international perspective. Durability of disease resistance*. Kluwer Academic Publishers: Norwell, MA, pp. 259-272.

Jin, Y., Pretorius, Z. A., Singh, R. P., & Fetch, T. (2008a). Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease, 92,* 923-926.

Jin, Y., Szabo, L. J., & Pretorius, Z. A. (2008b). Virulence variation within the *Ug99* lineage In R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntyre & P. Sharp (Eds), *Proceedings of 11th International Wheat Genetics Symposium. Brisbane, Australia*: Sydney University, pp. 342.

Johnson, R. (1983). Genetic background of durable resistance. In F. Lamberti, J.M Waller, & N.A Van der Graaff (Eds), *Durable Resistance in Crops*. New York, Plenum, pp. 5-24.

Lin, C. S., Binns, M. R., & Letkovitch, L. P. (1986). Stability analysis: Where do we stand? *Crop Science, 26,* 894-899.

Lowe, I., Cantu, D., & Dubcovsky, J. (2011). Durable resistance to the wheat rusts: integrating systems biology and traditional phenotype-based research methods to guide in deployment of resistant genes. *Euphytica, 179,* 69-79.

Macharia, J. K., & Wanyera, R. (2012). Effects of stem rust race *Ug99* on grain yield and yield components of wheat cultivars in Kenya. *Journal of Agricultural Science and Technology, 2*, 423-431.

Mago, R., Bariana, H. S., Dundas, E. I. S, Spielmeyer, W., Lawrence, G. I., Pryor A. J. & Ellis, J. G. (2005). Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. *Theoretical and Applied Genetics, 111*, 496-504.

Malosetti, M., Ribaut, J., & Van-Eeuwijk, F.A. (2013). The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. *Frontiers in Physiology, 4*, 44-46.

McIntosh, R. A., Hart, G. E., Devos, K. M., Gale, M. D., & Rogers, W. J. (1998). Catalogue of gene symbols for wheat, In A. E. Slinkard (Ed), *Proceedings of the 9th International Wheat Genetics Symposium*, Saskatoon: Canada, pp.1-235.

Mevlüt, A., Yuksel, K., & Seyfi, T. (2009). Evaluation of durum wheat genotypes using parametric and non-parametric stability statistics. *Turkish Journal of Field Crops, 14*, 111-122.

Mohammadi, M., Karimizadeh, R., Hosseinpour, T., Kalateharabi, M., Khanzadeh, H., Sabaghnia, N., Mohammadi, P., & Hasanpour Hosni, M. (2011). Analysis of genotype, environment, and genotype × environment interaction in bread wheat in warm rainfed areas of Iran. *Crop Breeding Journal, 2*, 63-70.

Njau, P. N., Jin, Y., Huerta-Espino, J., Keller, B., & Singh, R. P. (2010). Identification and evaluation of sources of resistance to stem rust race *Ug99* in wheat. *Plant Disease, 94*, 413-419.

Njau, P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R., & Keller, B. (2009). Resistance in Kenyan bread wheat to recent eastern African isolate of stem rust, *Puccinia graminis* f. sp. *tritici*, *Ug99*. *Journal of Plant Breeding and Crop Science*, *1*, 22-27.

Parlevliet, J. E, & Van Ommeren, A. (1988). Accumulation of partial resistance in barley to barley leaf rust and powdery mildew through recurrent selection against susceptibility. *Euphytica, 37,* 261-274.

Parlevliet, J. E. (1993). What is durable resistance, a general outline, In T. Jacobs, & J.E Parlevliet (Eds.), *Durability of disease resistance.* Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 23-39.

Peterson, R. F., Campbell, A. B., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust severity on leaves and stems of cereals. *Canadian Journal of Genetics and Cytology, 26*, 496-500.

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., & Payne, T.S. (2000). Detection of virulence to wheat stem rust (*Puccinia graminis* f. sp. *Tritici*) resistance gene *Sr31* in wheat. *Plant Disease, 84,* 203-207.

Roelfs, A. P., Singh, R. P., & Saari, E. E. (1992). *Rust diseases of wheat: Concepts and methods of disease management.* Mexico, D.F: CIMMYT.

SAS Institute Inc. (2002). The SAS system for Windows, release 9.1.3 SAS Institute, Cary, NC.

Simko, I., & Piepho, H. P. (2012). The area under the disease progress stairs: calculation, advantage, and application. *Phytopathology, 102,* 381-389.

Singh, R. P, Hodson, D. P, Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel S., & Ward, R.W. (2008). Will stem rust destroy the world's wheat crop? *Advances Agronomy, 98,* 271-309.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau P, Herrera-Foessel S., Singh P. K., Singh, S., & Govindan, V. (2011). The emergence of *Ug99* races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology, 49,* 465-481.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M. G, Wanyera, R., Njau, P., & Ward, R.W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 1,* 054-058.

Singh, R. P., Huerta-Espino, J., & Manilal, H. M. (2005). Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turkish Journal of Agriculture and Forestry, 29,* 121-127.

Smith, E. L., Schlehuber, A. M., Young, H. C., & Jr, Edwards, L. H. (1968). Registration of agent wheat. *Crop Science, 8,* 511-512.

Stakman, E. C., Steward, D. M., & Loegering, W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici*. United States Department of Agriculture, Agricultural Research Service, 1962.

Tabassum, S. (2011). Evaluation of Advance Wheat Lines for Slow Yellow Rusting (*Puccinia striiformis* f. sp. *tritici*), *Journal of Agricultural Science, 3*(1), 239–249.

Todorovska, E., Christov, N., Slavov, S., Christova, P., & Vassilev, D. (2009). Biotic stress resistance in wheat breeding and genomic selection implications. *Biotechnology and Biotechnological Equipment, 23(4),* 1417-1426.

Wanyera, R. (2008). Status and impact of TTKS (*Ug99*) in Kenya. In G. P. Singh, K. V. Prabhu, A. M. Singh (Eds), *Proceeding of International Conference on Wheat Stem Rust Ug99- A Threat to Food Security*, Indian Agricultural Research Institute, New Delhi, India, pp 12-14.

Wanyera, R., Kinyua, M. G., Jin, Y., & Singh, R. (2006). The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. *Plant disease, 90,* 113-118.

Wricke, G. (1962). A method of understanding the biological diversity in field research. Z. *Pflanzenzüchtg, 47,* 92-96.

Yan, W., & Hunt, L. A. (2002). Biplot analysis of diallel data. *Crop Science, 42,* 21-30.

Yan, W., & Tinker, N. A. (2005). An integrated system of biplot analysis for displaying, interpreting, and exploring genotype by environment interactions. *Crop Science, 45,* 1004-1016.

Yau, S. K. (1995). Regression and AMMI analyses of genotype x environment interactions: An empirical comparison. *Agronomy Journal, 87,* 121-126.

Zadoks, J. C., Chang, T. T. & Konzak, C.F. (1974). A decimal code for growth stages of cereals. *Weed Research, 14,* 415-421.

CHAPTER FOUR

GENETIC ANALYSIS OF ADULT PLANT RESISTANCE TO STEM RUST *(Puccinia graminis* f. sp. *tritici)* AND YIELD IN WHEAT (*Triticum aestivum* L.)

4.1 Abstract

Stem rust (*Puccinia graminis* f. sp. *tritici*) disease is a major challenge to wheat (*Triticum sp.*) production in Africa and other wheat growing countries of the world. Genetic resistance is a viable option to minimize yield losses due to the disease. The objectives of the study were to (i) estimate the kind of gene action involved in inheritance of adult plant resistance to stem rust and yield related traits in wheat and (ii) determine heritability of these traits. Six genotypes, four with known reaction to stem rust and two genotypes adapted to Kenyan growing environments were crossed in complete 6×6 diallel fashion. The F_1s and parents resulting from crosses were grown in alpha-lattice design replicated three times under natural infection of stem rust. Results revealed that both general combining ability (GCA) and specific combining ability (SCA) effects were significant ($P<0.01$) for all traits studied. Nonetheless, GCA effects were predominant for all the traits. This establishes predominance of additive genetic effects (fixable variation) over non-additive effects. Narrow sense heritability estimates were moderate (0.33 for grain yield) to high (0.78 for days to heading). Additionally, the *Wr/Vr* graph revealed partial dominance for stem rust infection, the number of days to heading and the number of productive tillers while over-dominance was observed for grain yield and plant height. Since all the traits were highly fixable, gamete selection will be effective in development of high yielding and disease resistant wheat varieties. Inclusion of parents KSL 13 and KSL 42 as well as crosses KSL 34/KSL 52, *NjBwII*/KSL 42, *Kwale*/KSL 13, KSL 34/KSL 42 in a breeding program would produce desired segregants. These could therefore be exploited successfully in enhancement of stem rust disease resistance as well as yield in areas prone to stem rust infection.

Key words: Combining ability, General predictability ratio, Griffing's, Hayman's

4.2 Introduction

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a major fungal diseases of bread wheat (*Triticum aestivum* L.) that can cause significant yield and economic yield losses in Kenya and other wheat growing countries of Africa (Wanyera *et al.*, 2006; Njau *et al.*, 2009) and Asia

(Singh *et al.*, 2008; Singh *et al.*, 2011). Common bread wheat production in Kenya was estimated to be 0.25 million tons in 2012 against the demand of 0.9 million tons in the same year (FAO, 2012). This deficit in wheat production is due to the stem rust disease among other biotic and abiotic challenges. And this is met by importing wheat from wheat sufficient countries around the world. Stem rust can cause up to 100% yield loss in case of an epidemic, or when a susceptible cultivar is grown (Park, 2007).

While several options including chemical, cultural etc. are advocated for managing rust diseases, genetic resistance still remains the most viable and sustainable option. Attempts have been made to develop resistant cultivars. This had been achieved for over 30 years up to 90's through characterization and utilization of more than 60 stem rust (*Sr*) resistant genes, of which all except *Sr2* were major genes which are dominant in action (McIntosh *et al.*, 1995; Singh *et al.*, 2011). But the rust pathogen is always mutating and is known to cause susceptibility in previously resistant wheat varieties (Luig, 1983; Park, 2007). Considering the constant mutation of the pathogen and occurrence of new variants especially in stem rust 'hot spots', there is a need for durable and broad-spectrum disease resistance conditioned by minor genes having additive effects. *Sr2* is an adult plant resistant (APR) gene known to confer slow rust resistance or durable resistance (Sunderwirth and Roelfs, 1980). When in combination with other unknown minor or some of the already characterized major genes, *Sr2* can confer adequate and durable levels of resistance (Knott, 1988). Existence of unidentified stem rust APR minor genes has been reported by Knott (2001). In other studies, a number of quantitative trait loci (QTL) conferring APR to stem rust have been identified (Bhavani *et al.*, 2011; Njau *et al.*, 2013). This shows some evidence of quantitative and complex inheritance of APR to stem rust. The usefulness of carrying out genetic studies to explore the mechanism of inheritance of APR to stem rust is indicated by the fact that breeders must keep ahead of the pathogen by fast tracking breeding.

Researchers at the International Center for Maize and Wheat Improvement (CIMMYT) continue to develop and select for wheat germplasm that have durable resistance to stem rust as well as desirable yield potential which are released as cultivars in countries they are adaptable (Singh *et al.*, 2011). The yield potential as well as APR to stem rust in the CIMMYT materials and Kenyan old varieties can be utilized in wheat improvement programs aimed at increasing wheat yields (Njau *et al.*, 2010, Nzuve *et al.*, 2012). However, to fast track success, information

on genetics of inheritance of the targeted traits, with emphasis on the type of gene action (whether additive or non-additive) and heritability is critical.

The diallel cross technique reported by Griffing's (1956), and Hayman (1954) have been extensively used to assess the F_1 crosses in self-pollinated crops. These methods have previously been used by many workers; for example Sangwan and Chaudhary (1999) reported equal contribution of additive and non-additive gene effects in inheritance of tillers per plant and grains per ear in wheat. Additive gene action and non-additive have been reported for days to maturity and plant height in wheat, respectively by Akram *et al.* (2008). For grain yield, the study by Muhammad *et al.* (2000) revealed non-additive genetic effects. In addition to the yield and yield related traits, the diallel cross technique has been used to study the genetics of resistance to rust diseases of wheat. For instance, additive with partial dominance was displayed in inheritance of leaf rust incidence (Hussain, 2005) and the major importance of additive effects with non-additive (dominance and epistasis) gene effects to lesser extent for yellow rust resistance (Wagoire *et al.*, 1998). But these reports have proved inconsistent and the results obtained are restricted to the samples used and may not necessarily apply to other samples. Notably, limited information exists on the studies on inheritance of stem rust. Therefore, independent genetic studies are essential for any targeted population. This study aimed to determine (i) the kind of gene action associated with adult plant resistance and yield related traits and (ii) heritability of the traits, in a diallel analysis of wheat genotypes adapted to key wheat growing environments in Kenya.

4.3 Materials and Methods

4.3.1 Experimental Site

The experimental was conducted at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro situated at $0^{o}20'S$, $35^{o}56'E$ at an elevation of 2185 m a.s.l., has an average annual rainfall of 939 mm (average of 15 years), and average annual minimum and maximum temperatures of 9°C and 24°C (average of 15 years). This site is one of the widely considered 'hot-spots' for stem rust disease and heavy natural epidemics of the pathogen are observed in most seasons.

4.3.2 Diallel Experiment: Parental genotypes and field procedure

Four advanced wheat breeding lines namely; KSL 13, KSL 34, KSL 42 and KSL 51 and two locally adapted but stem rust susceptible cultivars *NjBw II* and *Kwale* were crossed using diallel mating design (Table 9). The four advanced lines were selected from an earlier field screening trial. Selection of lines was based on high levels of resistance to stem rust and desirable agronomic traits. The two locally adapted cultivars were previously reported to be susceptible (Njau *et al.*, 2009). Crosses were made following a 6x6 complete diallel model. Subsequently, 30 F_1s and six parents were planted in an alpha lattice design (12 rows and 3 columns) with three replications. Each entry was planted in two rows of 1.5 m length; the plant to plant and row to row spacing was 0.1 m and 0.2 m, respectively. Incomplete blocks (columns) and replicates were separated by a space of 0.5 m. The highly susceptible wheat cultivar, *Cacuke* was planted around the experimental plot and in the middle of the 0.5 m alleyway on both sides of the entries to enhance inoculum build up, and serve as spreader. At planting, Nitrogen and Phosphorus were applied at the rate of 22.5 kg N ha^{-1} and 25.3 kg P ha^{-1}, respectively. Buctril MC (225 g L^{-1} Bromoxynil octanoate and 225 g L^{-1} MCPA Ethylhexylester), a post emergence herbicide was sprayed at tillering stage at the rate of 7 ml L^{-1} of water to control broad-leafed weeds. To control of insect pest, Buldock Duo (225 g L^{-1} Beta-Cyfluthrin) was sprayed at the rate of 10 ml L^{-1} of water. Manual weeding by hand was done two times between stem elongation and booting stages to eradicate grasses.

Table 9: Description of bread wheat (*Triticum aestivum* L.) genotypes used in a 6×6 diallel cross

Genotype	Source	Pedigree/ Selection History	Attributes
NjBw II	Kenya	TNMU,CM 81812-12Y-06PZ-4Y-5M-0Y-2AL-0Y-2AL-0AL-0M	Stem rust susceptible, locally adapted
KWALE	Kenya	Kinglet,CM33089-W	Stem rust susceptible, locally adapted
KSL 13	CIMMYT	MERCATO//JNRB.5/PIFED	Moderately resistant to stem rust
KSL 34	CIMMYT	KIRITATI//ATTILA*2/PASTOR/3/AKURI	Moderately resistant to stem rust
KSL 42	CIMMYT	KIRITATI//PRL/2*PASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZ/6/KIRITATI//PRL/2*PASTOR	Moderately resistant to stem rust
KSL 51	CIMMYT	SHORTENEDSR26TRANSLOCATION//WBLL1*2/BRAMBLING/3/VILLA JUAREZ 2009	Moderately resistant to stem rust

KSL: Kenyan Selection, CIMMYT: Center for Maize and Wheat Improvement, NjBw II: Njoro bread wheat II.

Days to heading for each entry was recorded as the number of days from planting to 50% of plants booting *i.e.* Zadok's stage 57 (three fourths of ear emerged) (Zadok *et al.*, 1974).

Assessment of plants for APR to stem rust was done on dough stage (Zadoks 83 of grain development) and when the spreader reached 50% severity. The adult plant response to infection was classified according to Roelfs *et al.* (1992) into four categories; R = resistant, MR= moderately resistant, MS = moderately susceptible, S = susceptible. Overlapping responses between two categories was denoted using a dash between the two categories. The stem rust severities ranging from 5 to 100% were determined by use of the modified Cobb scale (Peterson *et al.*, 1948). Coefficient of infection (CI) for all genotypes were then calculated by taking into account the disease severity and their infection response where; 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 represented immune, resistant (R), moderately resistant (MR), moderately resistant to moderately susceptible (M), moderately susceptible (MS) and susceptible (S), respectively as proposed by Roelfs *et al.* (1992). The average plant height (cm) was measured from the base of the plant to the base of the spike in ten randomly selected plants. Moreover, average number of productive tillers of 10 randomly selected plants was determined. Lastly, average grain weights of all the plants per entry were extrapolated to obtain the grain yield per plot and standardized at 12% grain moisture content.

4.4 Statistical and Genetic Analyses

Data collected from the 36 wheat genotypes were subjected to an analysis of variance (ANOVA) to determine significance of the genotypic differences in traits under consideration. This was performed using the Statistical Analysis Software (SAS) version 9.1 procedure PROC GLM (SAS Institute Inc., Cary 2002) by implementing the statistical model;

$$Y_{ijk}=\mu + G_i + R_j + B_{(jk)} + \varepsilon_{ijk}$$

Where; Y_{ijk} = observed phenotype of the i^{th} genotype, in the k^{th} incomplete blok of j^{th} replicate; μ = mean of the experiment; G_i = effect of the i^{th} genotype; R_j = effect of the j^{th} replicate; $B_{(jk)}$ = effect of the k^{th} incomplete block within the j^{th} replicate and ε_{ijk} = experimental error.

4.4.1 Combining Ability Analyses

The "Diallel-SAS05" routine of the SAS programme was used to perform Griffing's method 1 model 1 analysis (Zhang *et al.*, 2005) based on the relationship:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + r_{ij} + \varepsilon_{ij}$$

Where; $\mu =$ general mean, g_i and $g_j =$ general combining ability effects of the i^{th} and j^{th} parent, respectively, $s_{ij} =$ specific combining ability effects of the cross (ixj), $r_{ij} =$ reciprocal effect, $\varepsilon_{ij} =$ experimental error. General predictability ratio, GPR was calculated as $2MS_{GCA}/2MS_{GCA}+ MS_{SCA}$ as suggested by (Baker, 1987) and narrow sense heritability h^2 as $V_A/V_A+ V_D + V_E$, where $V_A=$ additive genetic component of variance, V_D = non-additive genetic component of variance and V_E = error variance (Kearsey and Pooni, 1996).

4.4.2 Additive-Dominance Model

Hayman's (1954) approach was used to partition the components of variation for the traits that showed significant variation into: *a* (additive); *b* (the dominance effect); [dominance effects *b* was further sub-divided into b_1 (directional dominance), b_2 (asymmetrical distribution of dominance) and b_3 (peculiarity of dominance to some crosses)]; *c* (maternal); and *d* (non-maternal). This was performed by the GenStat 14th edition statistical software (VSN international Ltd, 2010) using the following additive dominance model;

$$Y=\mu + block + a + b + c + d + (a \times block) + (b \times block) + (c \times block) + (d \times block)$$

Where; *Y* - observed effect; μ - grand mean; *block* - block effects; *a* - additive effects; *b* - dominance effects; *c* - additive maternal effects; *d* - maternal interaction effects. Then, $a \times block + b \times block + c \times block + d \times block$ is the interaction of the blocks with the model components. The *F*-test was then performed to test for the significance as the ratio of the means square of an item in the model and the block*effect interaction mean square. Test for homogeneity of the interactions were done according to Bartlett test (Steel and Torrie, 1980). However, for testing of b_1, b_2 and b_3, the homogeneity of their interaction with blocks was first tested and since they were homogeneous, all the three effects were tested against block $\times$ *b*. For *a*, *b*, *c*, and *d*, their interactions with the blocks were also tested for homogeneity. These were then pooled together since they were homogeneous and the pooled error was used to test each of the items.

The graphical approach (Hayman, 1954) was used to test for: i) the adequacy of the dominance-additive model, ii) the degree of dominance, *i.e.* whether partial, complete or over-dominance and, iii) distribution of dominance and recessive genes. For testing the adequacy of the model, a scaling test known as regression coefficient analysis was used according to Hayman

(1954). Regression coefficient was generated from a plot of the covariance (*Wr*) of family means with non-recurrent parents against variance (*Vr*) of the family means within an array. Departure of the regression coefficient (*b*) from zero was tested using (*b*-0)/s.e$_b$ while departure of *b* from unity was tested using (1-*b*)/s.e$_b$, where s.e is the standard error. Significant deviation of regression coefficients from Zero but not from unity, *i.e.* uniformity of *Wr*, *Vr* indicated the validity of assumptions for diallel analysis made by Hayman (1954).

4.5 Results

Studies on evaluation of genotypic variation among the wheat genotypes under natural infection of stem rust disease revealed significant genotypic differences at $P < 0.05$ for all the studied traits indicating genetic variability (Table 10).

4.5.1 Combining Ability Effects

Results of Griffing's method 1 model 1 analyses showed significant general combining ability (GCA) ($P < 0.001$) and specific combining ability (SCA) ($P < 0.05$) effects for all the

Table 10: Mean squares derived from analysis of variance for stem rust disease resistance and yield components of wheat genotypes.

Source	df	Days to heading	No. of tillers	Plant height	Grain yield/plot (kg)	CI
Rep	2	3.86	14.51	17.12	0.06	6.57
Block (in rep)	2	12.47*	5.43	2.68	0.02	10.58*
Genotype	35	93.38*	79.29*	72.31*	0.04*	28.61*
Error	68	9.06	10.22	12.82	0.01	4.40
CV%		4.11	14.01	4.91	22.21	35.87
R^2A		83.15	78.57	72.73	66.26	76.51

* represent significance at $P < 0.05$; df, degrees of freedom; CI, coefficient of infection; R^2A, adjusted R-squared

Table 11: Variance analysis results

Source of variation	d.f.	Heading	No. of tillers	Plant height (cm)	Grain yield/plot (Kg)	CI
GCA	5	564.689***	378.941***	205.214***	0.136***	137.519***
SCA	15	25.687**	42.230***	56.657***	0.042***	11.679*
MAT	5	8.340	14.856	43.727	0.016	4.528
NMAT	10	6.310	17.989	45.707	0.006	8.072
Reciprocal	15	6.989	16.940	45.057	0.009	6.890
Error		9.156	10.090	12.532	0.010	5.088
GPR		0.977	0.947	0.878	0.866	0.959

h^2	0.782	0.592	0.361	0.333	0.65

* represent significance at $P < 0.05$; ** represent significance at $P < 0.01$; *** represent significance at $P < 0.001$; d.f, degrees of freedom; h^2, narrow-sense heritability; CI, coefficient of stem rust infection.

studied traits (Table 11). However, GCA effects were greater than SCA effects across all traits. General predictability ratio (GPR) ranged from 0.866 (for grain yield) to 0.977 (for the number of days to heading). This shows the predominance of GCA effects in control of traits measured. Based on predominance of GCA effects of parents, performance of a single-cross derived progeny will be highly predictable. There was no significance in maternal, non-maternal and reciprocal effects. Narrow sense heritability estimates were low (0.33 for grain yield) to high (0.78 for days to heading). General combining ability effects of the parents for the studied traits are presented in Table 12.

Early maturing wheat varieties are desirable because the risks of grain loss due to factors such as disease and water stress can be reduced. Therefore, negative GCA effects for days to heading are desirable. KSL 13 and KSL 34 had negative and significant ($P < 0.001$) GCA effects for this trait. This indicates the usefulness of the two parents in breeding for early maturing wheat. For number of tillers, positive GCA effects are desirable; *NjBw II* and *Kwale* had positive and significant ($P < 0.001$) GCA effects which indicate that the two parents increased the number of tillers. The desired GCA effects for plant height should be negative; GCA effects for parents KSL 13 and KSL 51 were negative and significant ($P < 0.001$ and $P < 0.5$, respectively). For grain yield, positive GCA effects are important; parents KSL 13 and KSL 42 enhanced grain yield with positive and significant ($P < 0.001$) GCA effects. Lastly, the desired GCA effects for disease resistance should be negative; apart from having significant positive GCA effects for yield, parents KSL 42 and KSL 13 were also important in contributing negative significant ($P < 0.05$) GCA effects for disease resistance.

Table 12: General combining ability effects of parents for the studied traits of wheat at Njoro, Kenya

Parent	Days to heading	No. of tillers	Plant height (cm)	Grain yield/plot (Kg)	CI
NjBw II	2.444***	3.12***	1.375*	-0.012	2.420***
Kwale	6.305***	4.037***	2.441***	0.001	0.143
KSL 13	-5.222***	-4.435***	-4.116***	0.075***	-1.451***
KSL 34	-2.083***	0.814	-0.116	-0.043*	1.409***
KSL 42	-0.583	-1.824***	1.566**	0.06***	-2.985***

KSL 51	-8.61	-1.712***	-1.105*	-0.084***	0.464

* represent significance at $P < 0.05$; ** represent significance at $P < 0.01$; *** represent significance at $P < 0.001$; CI, stem rust coefficient of infection.

Results of SCA of the wheat crosses for evaluated traits under stem rust disease are presented in Table 13. Crosses *Kwale*/KSL 13 and *Kwale*/KSL 51 displayed negative and significant ($P < 0.001$) SCA for number of days to heading. Notably, these crosses are derived from parents with negative GCA effects for the trait. The cross KSL 34/KSL 42 displayed positive and significant ($P < 0.05$) SCA effects for number of tillers per plant. For plant height cross KSL 34/KSL 51 had significant ($P < 0.01$) SCA effects; both parents in this cross showed negative GCA effects. There was positive and significant SCA ($P < 0.05$) effects in cross *NjBw II*/ KSL 42 for grain yield. The same cross also displayed negative and significant SCA effects for disease CI. This implies that apart from yield, this cross also shows high levels of resistance to stem rust disease.

Table 13: Specific combining abilities of F_1 wheat crosses for traits evaluated under stem rust infection.

Cross	Days to heading	No. of tillers	Height	Grain yield/plot (Kg)	CI
NjBw II/ Kwale	-0.167	-1.648	0.011	-0.075	1.124
NjBw II/ KSL 13	0.528	0.657	4.386***	0.092	-1.281
NjBw II/ KSL 34	-0.278	-0.259	0.497	0.026	2.357**
NjBw II/ KSL 42	-4.444	-0.120	0.303	0.098*	-2.348**
NjBw II/ KSL 51	1.972	-0.500	1.347	-0.413***	2.788
Kwale/ KSL 13	-3.666***	-0.093	-1.481	0.059	-0.837
Kwale/ KSL 34	1.028	-4.676***	0.297	0.010	-1.364
Kwale/ KSL 42	2.36*	-2.870*	4.09**	-0.039	-0.404
Kwale/ KSL 51	-7.167***	-10.916***	4.247	-0.065	1.011
KSL 13/ KSL 34	-1.278	0.963	3.056*	-0.055	0.896
KSL 13/ KSL 42	-1.944	-2.231*	0.478	-0.027	0.957
KSL 13/ KSL 51	-2.694	-2.056	5.872*	0.023	0,833
KSL 34/ KSL 42	1.416	2.019*	-1.544	0.051	-0.070
KSL 34/ KSL 51	1.278	-3.139	-7.772**	-0.078	-1.888
KSL 42/ KSL 51	2.111	0.889	6.67**	-0.067	-2.450

CI; coefficient of infection, *, **, and *** represent significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

4.5.2 Additive-Dominance Model

Mean squares for the disease and yield components according to Hayman's analysis (Hayman, 1954) of evaluated genotypes are presented in Table 14. The additive component of variation *a* was highly significant for days to heading, number of tillers, grain yield per plot and stem rust CI ($P < 0.001$) and plant height ($P < 0.05$). These results agree with combining ability analysis. For plant height, dominance was more significant ($P < 0.01$), but based on the magnitude of the mean squares, additive component was more important.

Table 14: Mean squares for the stem rust disease infection and yield components of wheat genotypes in Hayman analysis

Source	df	Days to heading	No. of Tillers	Height	Grain yield/plot	CI
Block	2	4.23	17.95	26.19	0.07	25.47
a	5	561.77***	379.45***	187.03*	0.14***	134.99***
b	15	25.52**	39.99***	61.57**	0.04***	12.44*
b_1	1	11.85ns	171.14***	85.44ns	0.21**	0.23ns
b_2	5	32.69*	64.58**	82.44ns	0.05**	5.58ns
b_3	9	23.06*	11.75ns	47.32**	0.02ns	17.60*
c	5	8.19ns	15.87ns	37.71ns	0.02ns	3.95ns
d	10	6.61ns	15.57ns	31.99ns	0.01ns	5.67ns
Total	35	94.25	78.35	67.63	0.04	27.82
Block x a	10	10.74	15.46	13.71	0.01	5.41
Block x b	30	8.44	10.37	12.33	0.01	5.10
Block x b_1	2	2.11	5.28	15.4	0.02	2.79
Block x b_2	10	8.48	9.53	16.44	0.01	5.15
Block x b_3	18	9.12	11.4	9.71	0.01	5.33
Block x c	10	11.81	8.85	15.47	0.02	5.49
Block x d	20	8.15	7.82	17.79	0.01	4.22
Block x Total	70	9.16	10.15	14.54	0.01	4.95

df, degrees of freedom; b_1, direction of dominance; b_2, asymmetry of alleles; b_3, residual dominance effects; CI, coefficient of infection; *, **, and *** represent significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$; ns, non-significance.

The dominance component of variation was significant for all the traits. There was no significance in maternal *c* and non-maternal *d*, suggesting there was no need to retest *a* and *b*. Further partitioning of dominance *b*, direction of dominance b_1 was significant for number of tillers at $P < 0.001$ and grain yield at $P < 0.01$. Asymmetry in gene distribution b_2 was significant ($P < 0.05$) for days to heading. Lastly, residual dominance effects (b_3) were also significant ($P < 0.01$) for plant height suggesting that some dominance for this trait were peculiar

to some F_1 crosses. Regressions of covariance (*Wr*) on variance (*Vr*) for the traits trait are presented in figures 5 to 9. For the number of days to heading (Figure 5), the *Wr*/*Vr* regression was highly significant ($P < 0.001$) from zero, with a regression coefficient not significantly different from unity. This satisfies the assumption of the absence of non-allelic (epistatic) interaction.

According to Hayman (1954), in the absence of non-allelic interaction and with independent distribution of genes among parents, *Wr* is related to *Vr* by a straight regression unit slope. The first part towards the origin includes 75% or more dominant genes, the second part with 50 to 75% dominant genes, the third part between 25 and 50% dominant genes and in the last fourth part below 25% dominant genes. The point where the regression line cuts the *Wr*-axis provides a measure of average degree of dominance. For days to heading, regression line intercepted the *Wr* axis above the *Wr* origin (Figure 5), this revealed partial dominance of genes. Parent KSL 13 contributed most of the dominant genes towards days to heading, since it was close to the origin of the slope. *Kwale* contributed the most recessive genes since it was furthest on the regression slope. Parent KSL 34 contributed both dominant and recessive genes since it was equidistant to the line.

For inheritance of number of tillers per plant (Figure 6), regression coefficient was significant from zero, but not from unity. Regression line intersected *Wr*-axis above the origin also depicting partial dominance. All the parents except *Kwale* were clustered near the origin of the slope suggesting important contribution of dominant alleles. *Kwale* which was at the furthest end of regression line contributed the recessive genes.

The *Wr*/*Vr* regression was significant from zero but not from unity for plant height (Figure 7). The line touched *Wr*-axis below the point of origin indicating over-dominance of genes. Distribution of array points along regression line showed that *NjBw II* contributed most of the dominant alleles since it was the closest to the origin, while KSL 13 which was furthest from the origin contributed most of the recessive alleles. Parents KSL 42, *Kwale* and KSL 51 were situated at the middle of the regression line; this indicated contribution of both dominant and recessive alleles by the three parents.

For grain yield, *Wr*/*Vr* regression was significant ($P < 0.05$) from zero but not from unity (Figure 8). Over-dominant type of gene action was revealed as intercept point on Wr-axis was negative. *NjBw II* contributed most of the recessive genes as it was the furthest from the

origin while KSL 42 seemed to be the closest to the origin implying that it contributed most of the dominant genes.

Lastly, *Wr*/*Vr* regression line for CI was highly significant ($P < 0.001$) from zero but not from unity. The line touched *Wr*-axis above the origin indicating the partial dominance kind of gene effects. Parent *NjBw II* was the farthest from the origin suggesting that this parent contributed most of the recessive alleles for disease susceptibility, while KSL 42 contributed most of the dominant alleles for disease resistance since it was the closest to the origin (Figure 9).

The means of array variance and covariance for studied yield components are shown in Table 15. The very low *Wr-Vr* value but high *Wr+Vr* value for *Kwale* (days to heading and number of tillers), KSL 13 (plant height), *NjBw II* (grain yield and disease CI) and was further confirmation that recessive genes were controlling the inheritance of these traits in these parents. It can be deduced that for all traits studied, there was superiority of parental means over array means. However, a few instances of higher array means than parental means were also observed; this confirmed that dominance also played a role in inheritance of the traits.

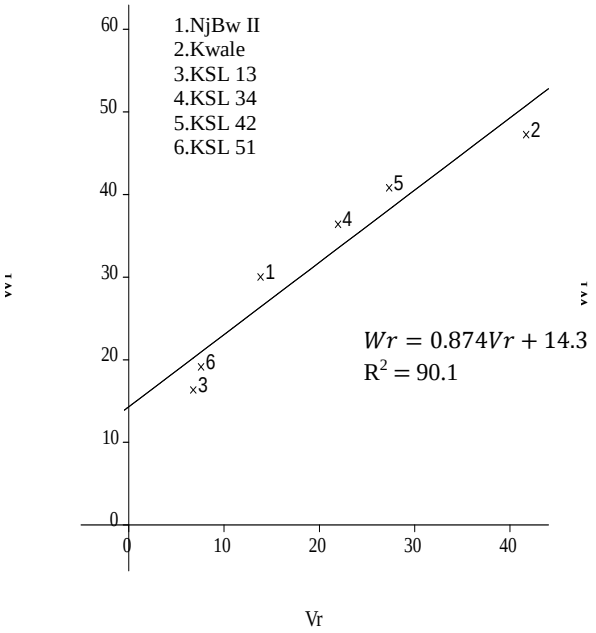


Figure 5: Linear regression of *Wr/Vr* for days to heading

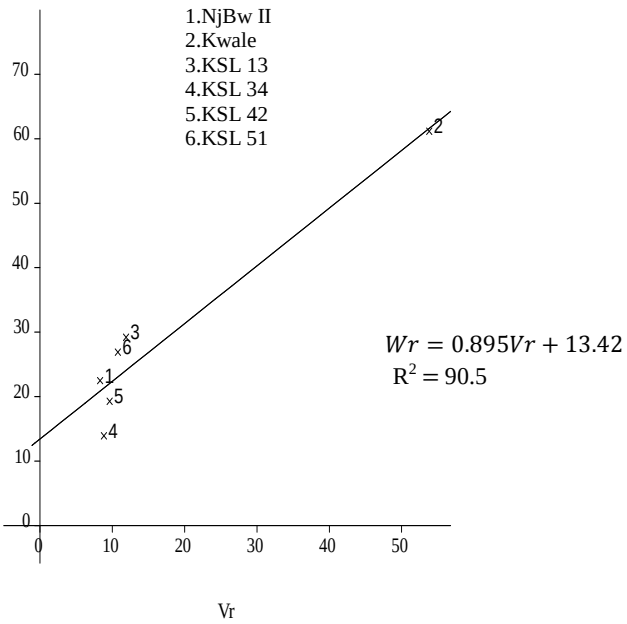


Figure 6: Linear regression of *Wr/Vr* for number of tillers

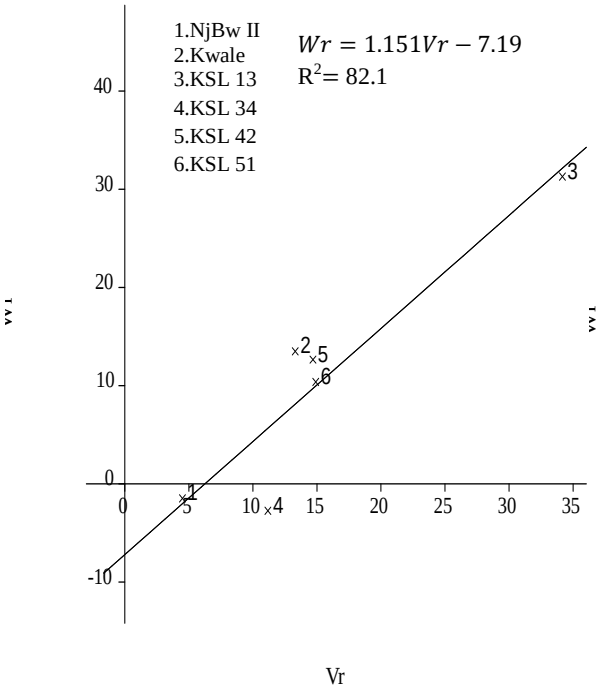


Figure 7: Linear regression of *Wr/Vr* for plant height

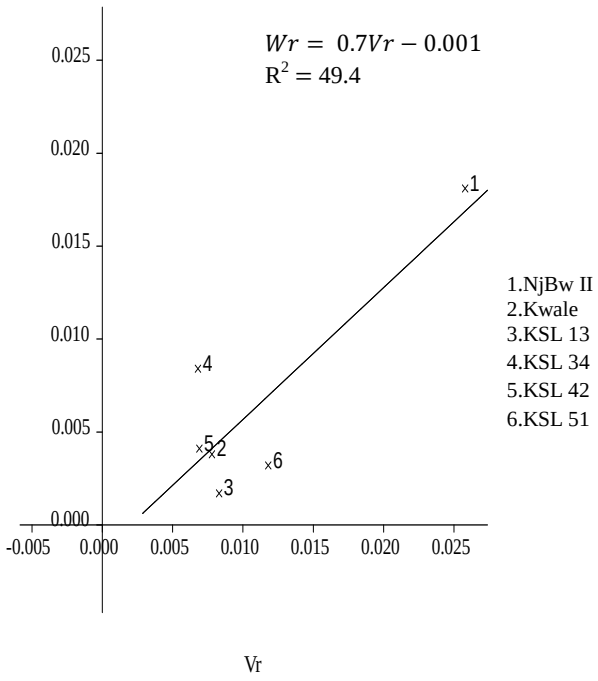


Figure 8: Linear regression of *Wr/Vr* grain yield per plot

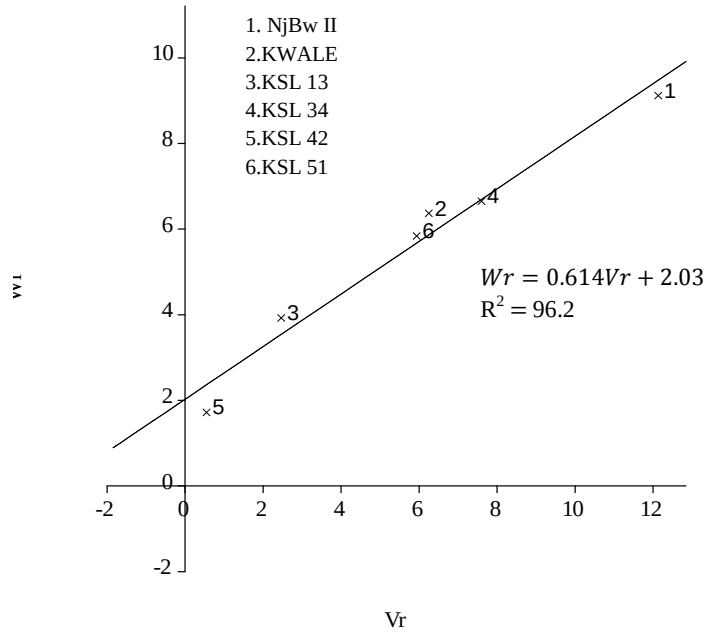


Figure 9: Linear regression of *Wr*/*Vr* for disease coefficient of infection

Table 15: Means of array variance and covariance for the traits associated with resistance to stem rust

Array	Array mean	Parent Mean	*Vr*	*Wr*	*Wr-Vr*	*Wr+Vr*
Days to heading						
NjBw II	75.7	77.3	13.827	30.011	16.184	43.838
Kwale	79.6	89.7	41.707	47.267	5.559	88.974
KSL 13	68.1	67.3	6.763	16.344	9.582	23.107
KSL 34	71.2	68.0	21.949	36.411	14.462	58.360
KSL 42	72.7	70.3	27.322	40.822	13.500	68.144
KSL 51	72.4	71.3	7.585	19.144	11.559	26.730
No. of tillers						
NjBw II	26.0	30.0	8.305	22.474	14.169	30.779
Kwale	26.8	41.0	53.827	61.174	7.347	115.001
KSL 13	18.5	15.3	11.889	29.211	17.322	41.100
KSL 34	23.5	27.7	8.838	13.930	5.092	22.768
KSL 42	20.7	20.3	9.616	19.282	9.666	28.897
KSL 51	21.0	19.0	10.756	26.889	16.133	37.644
Plant height (cm)						
NjBw II	74.2	72.3	4.496	-1.483	-5.979	3.013
Kwale	75.3	74.2	13.300	13.496	0.196	26.796
KSL 13	69.1	58.5	34.178	31.277	-2.901	65.455
KSL 34	73.1	75.3	11.174	-2.751	-13.925	8.423
KSL 42	74.4	71.0	14.681	12.646	-2.036	27.327
KSL 51	71.6	74.5	14.912	10.378	-4.533	25.290
Grain yield (kg)						
NjBw II	0.46	0.75	0.0258	0.0181	-0.0076	0.0439
Kwale	0.47	0.52	0.0078	0.0038	-0.0041	0.0116
KSL 13	0.55	0.67	0.0083	0.0017	-0.0066	0.01
KSL 34	0.42	0.40	0.0068	0.0084	0.0016	0.0153
KSL 42	0.53	0.67	0.0069	0.0041	-0.0028	0.0109
KSL 51	0.39	0.40	0.0118	0.0032	-0.0086	0.015
Stem rust coefficient of infection (CI)						
NjBw II	8.1	9.3	12.1341	9.1185	-3.0156	21.2526
Kwale	6.0	6.3	6.2506	6.3667	0.1161	12.6172
KSL 13	4.3	3.0	2.4639	3.9222	1.4583	6.3861
KSL 34	7.2	8.6	7.5963	6.6519	-0.9444	14.2481
KSL 42	2.9	2.0	0.5523	1.7185	1.1662	2.2708
KSL 51	6.2	6.0	5.9407	5.837	-0.1037	11.7778

Vr; Variance, *Wr*; covariance, *Wr-Vr*; differences over the arrays, *Wr+Vr*; parental order of dominance

4.6 Discussion

Disease coefficient of infection (CI) is one of the parameters that have been used to evaluate the level of host reaction to the stem rust disease of wheat. This has enabled measurement and quantification of genetic variation for resistance to the disease. Results of the study showed significance in GCA and SCA effects for stem rust CI and yield related traits evaluated under the disease infection. The traits included days to heading, plant height, number of productive tillers and grain yield per plot. The GCA effects had relatively higher magnitudes as compared to the SCA effects. According to Griffing (1956), high GCA effects are related to additive or additive x additive interaction effects, the components that respond to selection. Baker ratio (Baker, 1978) further confirms the importance of additive gene effects. Therefore, with regard to the GCA effects and the Baker ratios, additive gene effects controlled inheritance of all the traits under stem rust disease pressure. Similar findings of predominance of GCA effects in wheat, although in absence of disease pressure have previously been reported for; days to heading (Zare-kohan and Heidari 2012) number of tillers per plant (Chowdhry *et al.*, 1992) and grain yield (Hassan *et al.*, 2007). However, this did not conform to other previous reports for; days to heading (Iqbal, 2007) and both the number of productive tillers and grain yield (Shabbir *et al.*, 2011). The dominance of GCA effects for plant height conformed to previous reports of Zare-kohan and Heidari (2012) but not to that of Shahzad and Chowdhry (1998) which reported domination of SCA effects. The results of Hayman's analyses highlighted the primary importance of additive properties of gene effects in inheritance of all the traits studied. This confirms the results of combining abilities in the current study for these traits.

Both Griffing's and Hayman's analyses indicated that even though inheritance of stem rust disease resistance and yield related traits under stem rust infection was mainly due to additive type of gene action, dominant genetic effects also played a role. From the *Wr*/*Vr* analyses, the genetic model proved to be adequate for the data set since the regression coefficients deviated significantly from zero but not from unity for all the traits. This indicated the absence of non-allelic (epistatic) interaction.

An early maturing wheat crop has advantages in a sense that the risks of crop losses due to vagaries such as the stem rust disease, water stress etc. are minimized. It is therefore an important trait that can be invested on by the breeders. In addition to the additive gene action in inheritance of days to heading in stem rust conditions, the *Wr*/*Vr* graph also revealed partial

dominance. Similar results with days to heading in wheat genotypes have previously been obtained (Siddique *et al.*, 2004) but in absence of the disease. In order to improve on maturity, the parents used in crosses should have negative and significant GCA effects. From the current study, KSL 13 and KSL 34 proved to be the best parents for reduction of the number of days to heading due to their negative and significant ($P < 0.001$) GCA effects.

According to Otteson *et al.* (2008), grain yield depends substantially on the number of productive tillers. Therefore, selection based on productive tillering is essential in enhancing wheat productivity. Inheritance of number of tillers per wheat plant was governed by partial dominant kind of gene action in addition to the major action of additive genetic effects; this conformed to the findings of Ullah *et al.* (2010). Positive GCA effects are desired for improvement of the number of productive tillers. *NjBw II* and *Kwale* had positive and significant ($P < 0.001$) GCA effects. These are presented as the best parents for improvement of the trait.

Plant height is also a critical contributor to grain yield. In many wheat improvement programs, short wheat plants are desired as they are resistant to lodging and responsive to fertilizer application. This precedence was set in green revolution following which incorporation of dwarfing genes in breeding population became a routine. Inheritance of this trait was mainly governed by additive genetic effects with over-dominance. Negative GCA effects are desired for plant height, which was displayed by parents KSL 13. Therefore, this parent is important in reducing the plant height. For grain yield (Figure 8), in addition to the major role of additive gene action, over-dominant type of gene action was also revealed. This conformed to previous findings of Muhammad *et al.* (2000). Positive GCA effects are important for improvement of grain yield; therefore in the current study, parents KSL 42 and KSL 13 are recommend because of their positive and significant ($P < 0.001$) GCA effects. The crosses that would provide superior segregants for shorter time to maturity are *Kwale*/KSL 13 and *Kwale*/KSL 51, while KSL 34/KSL 42 can be utilized for number of tillers per plant. For shorter plants, KSL 34/KSL 51 proved valuable. The cross *NjBw II*/KSL 42 would produce desired segregants for grain yield and resistance to stem rust disease.

In conclusion, the present study provided useful information by way of indicating the nature of inheritance of stem rust disease resistance and yield related components under stem rust disease infection. The results showed that all the studied traits were mainly governed by additive type of gene action and displayed low to high values of heritability. This implies that the genes

are fixable in wheat lines. The low heritability values for grain yield (0.33) and plant height (0.36) also showed that non-additive gene action was also important in inheritance of these traits. Therefore, gamete selection is advocated for since it would result in accumulation of genes from different sources. It was further possible to classify the parents on the basis of the type of alleles present in them and this provides useful clues for the selection of parents which are likely to give better segregates. KSL 13 was the best general combiner for days to heading, plant height and grain yield, in addition to being a good combiner for resistance to stem rust. KSL 42 was also the best combiner for resistance to stem rust as well as a good combiner for grain yield. Therefore, inclusion of parents KSL 13 and KSL 42 as well as crosses KSL 34/KSL 51, *NjBw II*/KSL 42, *Kwale*/KSL 13, KSL 34/KSL 42 in a breeding program would provide favorable alleles for enhancement of stem rust disease resistance as well as yield in areas prone to stem rust infection in Kenya.

4.7 References

Akram, Z., Ajmal, S. U., Munir, M., & Shabir, G. (2008). Genetic determination of yield related attributes in bread wheat. *Sarhad Journal of Agriculture, 24*, 431-438.

Baker, R. J. (1978). Issues in diallel analysis. *Crop Science, 18,* 533-536.

Bhavani, S., Singh, R. P., Argillier, O., Huerta-Espino, J., Singh, S., Njau, P., Brun, S., Lacam, S., & Desmouceaux, N. (2011). Mapping durable adult plant resistance to the race *Ug99* group in six CIMMYT wheats. In. R. McIntosh (Ed) *Proceedings of Borlaug global rust initiative technical workshop*. Saint Paul USA: Minnesota. pp. 43-53.

Chowdhry, M. A., Rafiq, M., & Alam, M. (1992). Genetic architecture of grain yield and certain other traits in bread wheat. *Pakistan Journal of Agricultural Research, 13,* 216-220.

Food and Agriculture Organization (FAO) (2012). Crop prospects and food situation. Retrieved from www.fao.org/giews/ (verified 26 July 2012).

Griffing, B. (1956). A generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity, 10,* 31-35.

Hassan, G., Mohammad, F., Afridi, S. S., & Khalil, I. (2007). Combining ability in the F_1 generations of diallel cross for yield and yield components in wheat. *Sarhad Journal of Agriculture, 23*, 937-942.

Hayman, B. I. (1954). The theory and analysis of diallel crosses. *Genetics, 39,* 789-809.

Hussain F. (2005). Genetic analysis of adult plant leaf rust resistance in spring wheat (*Triticum aestivum* L.). Ph.D. Thesis, Department of Biological Sciences/Quaid-i-Azam University Islamabad.

Iqbal, M., Navabi, A., Salmon, D. F., Yang, R. C., Murdoch, B. M., Moore, S. S., & Spaner, D. (2007). Genetic analysis of flowering and maturity time in high latitude spring wheat. *Euphytica, 154,* 207-218.

Kearsey, M. J., & Pooni, H. S. (1996). *The genetical analysis of quantitative traits*. Chapman and Hall, London.

Knott, D. R. (1988). Using polygenic resistance to breed for stem rust resistance in wheat: In N. W. Simmonds, & S. Rajaram (eds), *Breeding strategies for resistance to the rusts of wheat*. CIMMYT, Mexico, D.F., pp39-47.

Knott, D. R. (2001). The relationship between seedling and field resistance to two races of stem rust in Thatcher wheat. *Canadian Journal of Plant Science, 81,* 415-418.

Kolmer, J. A., Garvin, D. F., & Jin, Y. (2011). Expression of a Thatcher wheat adult plant stem rust resistance QTL on chromosome arm 2BL is enhanced by *Lr34*. *Crop Science, 51,* 526-533.

Luig, N. H. (1983). A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Verlag, Paul Parey, Berlin.

McIntosh, R. A., Wellings, C. R., & Park, R. F. (1995). Wheat rusts: an atlas of resistance genes. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Muhammad, A., Ihsan, K., & Aslam, C. (2000). Genetic analysis for some metric traits in hexaploid wheat. *Pakistan Journal of Biological Sciences, 3*, 525-527.

Njau, P. N., Bhavani, S. Huerta-Espino, J., Keller, B. & Singh R. P. (2013). Identification of QTL associated with durable adult plant resistance to stem rust race *Ug99* in wheat cultivar 'Pavon 76'. *Euphytica, 190*, 33-44.

Njau, P. N., Jin, Y., Huerta-Espino, J., Keller, B., & Singh, R. P. (2010). Identification and evaluation of sources of resistance to stem rust race *Ug99* in wheat. *Plant Disease, 94*, 413-419.

Njau, P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R., & Keller, B. (2009). Resistance in Kenyan bread wheat to recent eastern African isolate of stem rust, *Puccinia graminis* f. sp. *tritici*, *Ug99*. *Journal of Plant Breeding and Crop Science, 1*, 22-27.

Nzuve, F. M., Bhavani, S., Tusiime, G., Njau, P., & Wanyera, R. (2012). Evaluation of bread wheat for both seedling and adult plant resistance to stem rust. *African Journal of Plant Science, 6,* 426-432.

Otteson, B. N., Mergoum, M., Ransom, J. K., & Shatz, B. (2008). Tiller contribution to spring wheat yield under varying seed and nitrogen management. *Agronomy Journal, 2*, 406-413.

Park, R.F. (2007). Stem rust of wheat in Australia. *Australian Journal of Agricultural Research, 58,* 558-566.

Peterson, R. F., Campbell, A. B., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust severity on leaves and stems of cereals. *Canadian Journal of Genetics and Cytology C, 26,* 496-500.

Roelfs, A. P., Singh, R. P., & Saari, E. E. (1992). Rust diseases of wheat: concepts and methods of disease management. Mexico, D.F: CIMMYT.

Sangwan, V. P. & Chaudhary, B. D. (1999). Diallel analysis in wheat (*Triticum aestivum* L.). *Annals of Biology Ludhiana, 15,* 181-183.

SAS Institute Inc. (2002). The SAS system for Windows, release 9.1.3 SAS Institute, Cary, NC.

Shabbir, G., Ahmad, N. H., Akram, Z., & Tabassum, M. I. (2011). Genetic behaviour and analysis of some yield traits in wheat (*T. aestivum* L.) genotypes. *Journal of Agricultural Research, 49*, 1-9.

Shahzad, K., & Chowdhry, M. A. (1998). Study of combining ability for plant height, yield and some leaf characteristics in bread wheat. *Journal of Animal and Plant Science, 8*, 23-25.

Siddique, M., Ali, S., Malik, M. F. A., & Awan, S. I. (2004). Combining ability estimates for yield and yield components in spring wheat. *Sarhad Journal of Agriculture, 20(4),* 285-287.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau P, Herrera-Foessel S., Singh P. K., Singh, S., & Govindan, V. (2011). The emergence of *Ug99* races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology, 49*, 465-481.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel S., & Ward, R.W. (2008). Will stem rust destroy the world's wheat crop? *Advances Agronomy, 98*, 271-309.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M. G., Wanyera, R., Njau, P., & Ward, R. W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. *Perspectives of Agriculture, Veterinary Science, Nutrition and Natural Resources, 1***,** 054-058.

Steel, R. G. D, & Torrie, J. H. (1980). *Principles and procedures of statistics.* McGraw-Hill Book company, New York.

Sunderwirth, S. D., & Roelfs, A. P. (1980). Greenhouse characterization of the adult plant resistance of *Sr2* to wheat stem rust. *Phytopathology 70,* 634-637.

Ullah, S., Khan, A. S., Raza, A., & Sadique, S. (2010). Gene action analysis of yield and yield related traits in spring wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Biology, 12,* 125-128.

Wanyera, R., Kinyua, M. G., Jin, Y., & Singh, R. (2006). The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. *Plant disease,* 90, 113-118.

Zadok, J. C., Chang, T. T., & Konzak C. F. (1974). A decimal code for growth stages of cereals. *Weed Research, 14,* 415-421.

Zare-kohan, M., & Heidari, B. (2012). Estimation of genetic parameters for maturity and grain yield in diallel crosses of five wheat cultivars using two different models. *Journal of Agricultural Sciences, 8*, 74-85.

Zhang, Y., Kang, M. S., & Lamkey, K. R. (2005). Diallel SAS05: A comprehensive program for Griffing's and Gardener-Eberhart analyses. *Agronomy Journal, 97,* 1097-1106.

CHAPTER FIVE
GENERAL DISCUSSION

Among various strategies for controlling stem rust and reducing yield loss due to the disease, breeding for host-plant resistance remains the most viable strategy. Similarly, due to cost implications, commercial farmers everywhere prefer growing resistant varieties. More so, various national and international breeding programs are aimed at increasing wheat yield through development of stem rust resistant wheat varieties. However, their breeding efforts have proved difficult due to the rapidly mutating nature of the stem rust pathogen. Because of this, it is necessary to cross-check wheat cultivars and breeding lines at every stage. This ensures that novel sources of resistance to the emerging strains of the pathogen as well as good yield potential are identified, gathered and utilized in various wheat improvement programs.

There are two types of resistance for the stem rust disease i.e. seedling and adult plant resistance. There was significant genetic variability in disease response and severity among the tested genotypes. The study revealed that majority of the advanced breeding lines was susceptible to TTKST race of stem rust disease at both levels. Genotypes KSL 42 and KSL 13 maintained low disease severities across sites as well as resistant reaction at the seedling stage. Such genotypes would be desirable for release as varieties or used as parents for improvement of varieties especially if they record high grain yield. Previous studies have shown that seedling stage susceptible wheat genotypes can also maintain low severity at adult plant stage in the field across a range of environments. The phenomenon is observed in KSL 3. Wheat improvement programs are currently emphasizing on the use of adult plant resistance (APR) since this type of resistance can be used for a long time especially when the host is exposed to a wide range of the pathogen (Johnson, 1983). Unlike the APR, seedling resistance is based on major gene/s and can render the plant completely resistance hence offering significant economic benefits to farmers. However, this kind of resistance is not durable, and in the current scenario of constant mutation of the stem rust pathogen. Nonetheless, complete and durable resistance could be achieved in case of combination of both levels of disease resistance i.e. if resistance is based on both effective seedling resistance gene/s and APR genes.

Besides disease resistance, farmers' preference is also for high yielding and stable varieties, hence high yield potential, stability and adaptability is an important concept in wheat breeding. The yield variability recorded across the three sites indicated that the genotypes,

location and also the genotype by location played important roles in determination of yield performance. The negative correlation of the disease parameters also showed the importance of stem rust disease. Effect of stem rust disease was further evidenced by the sites that had high disease levels recording lower yield. The existence of such variation enables the breeder to select both high yielding and disease resistant genotypes across sites and in presence of disease pressure. Genotypes KSL 42 and KSL 3 combined both field disease resistance and high yield. Such genotypes are potential candidate lines for variety release. The two genotypes also emerged among the best with regard to consistent performance across locations. They are there important to farmers in the sense that they would withstand unpredictable and transient environmental fluctuations. However, some researchers believe that selection for specific adaptability is also useful because farmers are able to utilize high yielders for their respective environment. From the scatter plot KSL 13, KSL 26, KSL 34, and KSL 8 were highly adapted to poor environment such as Mau Narok which had high disease pressure. This was further evidenced by these genotypes' close association with Mau Narok in a GGE biplot. They were able to resist environmental change (above average stability), and therefore had specific adaptability to low yielding environments. The genotypes KSL 20, KSL 63, and KSL 2 were sensitive to environmental changes and are therefore suitable for cultivation under favorable conditions such as Timau which had low disease pressure.

The adult plant resistance to stem rust and yield are complex characters. An understanding of the genetics of the two traits is important in any breeding program. To address this, a genetic study was done to estimate the kind of gene action associated with adult plant resistance to stem rust and yield related traits in selected genotypes. Results of the study showed significance in GCA and SCA effects for stem rust CI and yield related traits evaluated under the disease infection. The traits included days to heading, plant height, number of productive tillers and grain yield per plot. The GCA effects had relatively higher magnitudes as compared to the SCA effects suggesting that inheritance of these traits were predominantly controlled by additive or additive x additive interaction effects. These are the components of variation that respond well to selection. The results of Hayman's analyses highlighted the primary importance of additive properties of gene effects in inheritance of all the traits studied. This confirms the results of combining abilities in the current study for these traits and suggests that the traits under study are highly fixable through selection. Both Griffing's and Hayman's analyses indicated that even

though inheritance stem rust disease resistance and yield related traits under stem rust infection was mainly governed by additive type of gene action, dominant genetic effects also played a smaller role. Early maturing wheat crop has advantages because the risks of crop losses due to vagaries such as the stem rust disease, water stress among others are minimized. It is therefore an important trait that breeders need to pay attention to. In order to improve on shorter days to maturity, the parents used in crosses should have negative and significant GCA effects. From the current study, KSL 13 and KSL 34 proved to be the best parents for reduction of the number of days to heading due to their negative and significant ($P < 0.001$) GCA effects. Grain yield depends substantially on the number of productive tillers. Positive GCA effects are desired for improvement of the number of productive tillers. *NjBw II* and *Kwale* had positive and significant ($P < 0.001$) GCA effects. Plant height is also critical contributor of grain yield. In many wheat improvement programs, short wheat plants are desired as they are resistant to lodging and responsive to fertilizer. Negative GCA effects are desired for plant height, which was displayed by parents KSL 13.

5.1 Conclusion

- Screening of wheat genotypes by artificial inoculation in the greenhouse permitted evaluation of genotypes under single race infection and uniform disease pressure. There was variation in seedling infection type ranging from immune to susceptible. Twenty-six genotypes including KSL 42 and KSL 13 exhibited resistance (infection types of ";", "1", "2" or combinations). Line KSL 31 showed a heterogeneous reaction "x" while the other 39 lines showed susceptible reactions.
- The field experiments showed variation in adult plant resistance to a wide range of stem rust races. KSL 42, KSL 51, KSL 3, KSL 57 and KSL 13 were tolerant with low disease infection and also were well adapted across locations. Among these, KSL 51 and KSL 3 showed combined resistance at both seedling and adult plant stage.
- With regard to yield, KSL 42 and KSL 3 were stable and broadly adapted across the three sites. Notably, they also displayed a high level of adult plant resistance to stem rust across the test locations.
- From the genetic analysis, importance of additive and dominant gene action in inheritance of yield related traits was shown. Nonetheless, the additive genetic effects

were more important than dominant genetic effects for all traits. Inclusion of parents KSL 13 and KSL 42 as well as crosses KSL 34/KSL 52, *NjBw II*/KSL 42, *Kwale*/KSL 13, and KSL 34/KSL 42 in a breeding program would produce desired segregants. These could therefore be exploited successfully in enhancement of stem rust disease resistance as well as yield in areas prone to stem rust infection. The desirable alleles in these different sources can be accumulated by gamete selection.

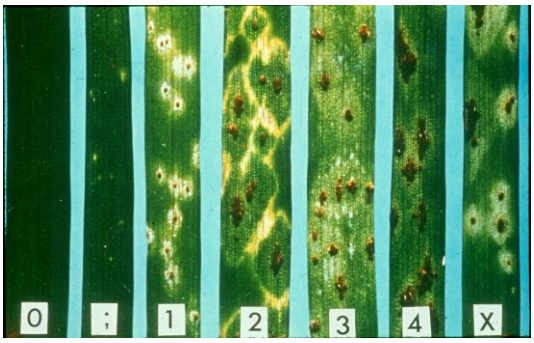
5.2 Recommendation

- Considering the field disease reaction and yield performance across locations, genotypes KSL 42 and KSL 3 consistently ranked among the top performers. These lines can be exploited in wheat breeding programs for development of high yielding and stem rust disease resistant wheat varieties.
- The existence of variation in disease reaction and yield enables the breeder to select both high yielding and disease resistant genotypes across sites and in presence of disease pressure. From the study, Mau-Narok was the most discriminative and therefore a more appropriate site for evaluating wheat grain yield under stem rust pressure.
- Inclusion of parents KSL 13 and KSL 42 as well as crosses KSL 34/KSL 52, *NjBw II*/ KSL 42, *Kwale*/ KSL 13 and KSL 34/KSL 42 in a breeding program would produce desired segregants. These could therefore be exploited successfully in enhancement of stem rust disease resistance as well as yield in areas prone to stem rust infection. The desirable alleles in these different sources can be accumulated by gamete selection.

APPENDICES

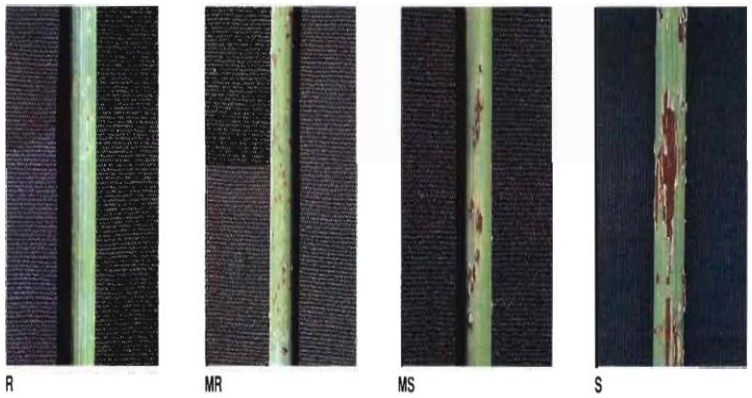
Appendix 1

a) Stakman's Infection Type Scale



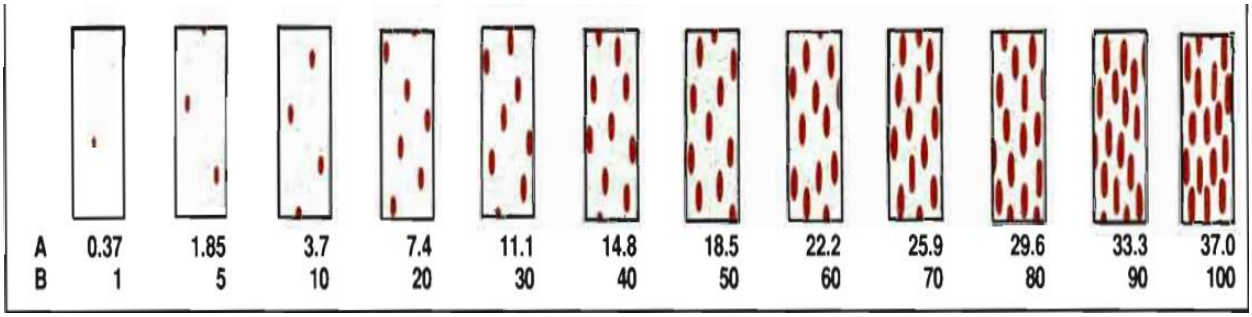
Stakman (1962)

b) Roelfs Field Disease Response to Infection Scale



Roelfs *et al.*, (1992)

c) The Modified Cobb Scale.



A, actual percentage occupied by rust urediniospores; B, rust severities of the modified Cobb scale after Peterson *et al.*, (1948).

Appendix 2

a) SAS ANOVA Procedure for Field Disease and Yield

```
data wheat;
input loc $ rep $ block $ gen $ ci audps tkw yield;
cards;
Njoro  1      1      31     1.57   600    0.033  5.4
,      ,      ,      ,      ,      ,      ,      ,
Timau  3      3      40     0.2    16     0.035  4.9
;
proc glm;
class loc rep block gen;
model ci audps tkw yield=loc rep block gen gen*loc/ss3;
means loc gen/lsd;
test H=gen E=gen*loc;
random loc rep block gen*loc;
run;
```

b) SAS ANOVA Output for Field Disease and Yield

```
                              The SAS System        20:30 Thursday, June 26, 2014  65
                                    The GLM Procedure
                                   Class Level Information

Class       Levels  Values
loc              3  Mau-Naro Njoro Timau
rep              3  1 2 3
block(rep)       3  1 2 3
gen             66  1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
                    29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53
                    54 55 56 57 58 59 60 61 62 63 64 65 66

                           Number of Observations Read         594
                           Number of Observations Used         594


Dependent Variable: ci

      Source                       DF         Sum of Squares     Mean Square    F Value    Pr > F
      Model                       201     121.7547010       0.6057448      19.60    <.0001
      Error                       392      12.1127003       0.0308997
      Corrected Total             593     133.8674013


                    R-Square     Coeff Var      Root MSE       ci Mean
                    0.909517      20.80980      0.175783      0.844714

      Source                       DF     Type III SS     Mean Square    F Value    Pr > F

      loc                           2     53.14475387     26.57237694     859.95    <.0001
      rep                           2      0.06851044      0.03425522       1.11    0.3311
      block(rep)                    2      0.19878923      0.09939461       3.22    0.0411
      gen                          65     46.21226801      0.71095797      23.01    <.0001
      loc*gen                     130     22.13037946      0.17023369       5.51    <.0001
```

Dependent Variable: audps

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	201	40760933.82	202790.72	21.12	<.0001
Error	392	3764140.82	9602.40		
Corrected Total	593	44525074.64			

R-Square	Coeff Var	Root MSE	audps Mean
0.915460	37.48722	97.99184	261.4007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
loc	2	13769102.44	6884551.22	716.96	<.0001
rep	2	11452.98	5726.49	0.60	0.5513
block(rep)	2	140267.53	70133.76	7.30	0.0008
gen	65	16424608.86	252686.29	26.31	<.0001
loc*gen	130	10415502.01	80119.25	8.34	<.0001

Dependent Variable: tkw

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	201	0.05616366	0.00027942	1.30	0.0538
Error	392	0.08400823	0.00021431		
Corrected Total	593	0.14017189			

R-Square	Coeff Var	Root MSE	tkw Mean
0.400677	43.19555	0.014639	0.033891

Source	DF	Type III SS	Mean Square	F Value	Pr > F
loc	2	0.00599186	0.00299593	13.98	<.0001
rep	2	0.00054482	0.00027241	1.27	0.2817
block(rep)	2	0.00022762	0.00011381	0.53	0.5884
gen	65	0.01994589	0.00030686	1.43	0.1517
loc*gen	130	0.02945347	0.00022657	1.06	0.3395

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	201	1150.630404	5.724529	12.73	<.0001
Error	392	176.316801	0.449788		
Corrected Total	593	1326.947205			

R-Square	Coeff Var	Root MSE	yield Mean
0.867126	12.87401	0.670662	5.209428

Source	DF	Type III SS	Mean Square	F Value	Pr > F
loc	2	54.2388215	27.1194108	60.29	<.0001
rep	2	0.1545791	0.0772896	0.17	0.8422
block(rep)	2	0.6086195	0.3043098	0.68	0.5090
gen	65	493.9227609	7.5988117	16.89	<.0001
loc*gen	130	601.7056229	4.6285048	10.29	<.0001

c) The Disease Coefficient of infection (CI), Disease Area Under Disease Progress Stairs (AUDPS), and Stability Values of Genotypic Reaction to Stem Rust for the Evaluated Wheat Genotypes Across Three Sites in Kenya.

	Coefficient of Infection (CI)					Area Under Disease Progress Stairs (AUDPS)				
Genotype	Mau Narok	Njoro	Timau	Means	Stability (1000)	Mau Narok	Njoro	Timau	means	Stability (1000)
KSL 42	0.6	0.1	0.0	0.2	0.01	72.0	18.7	0.0	30.2	1.40
KSL 51	0.8	0.2	0.1	0.4	0.15	98.7	24.0	5.3	42.7	2.44
KSL 3	0.9	0.3	0.2	0.5	0.16	178.7	32.0	13.3	74.7	8.20
KSL 57	1.1	0.1	0.7	0.6	0.26	173.3	2.7	82.7	86.2	7.29
KSL 13	0.7	0.4	0.0	0.4	0.04	141.3	120.0	0.0	87.1	5.81
KSL 7	1.0	0.4	0.2	0.5	0.16	216.0	34.7	18.7	89.8	12.01
KSL 34	1.0	0.3	0.1	0.5	0.26	245.3	34.7	8.0	96.0	16.90
KSL 59	0.9	0.3	0.6	0.6	0.09	152.0	64.0	85.3	100.4	2.11
KSL 5	0.9	0.4	0.1	0.5	0.18	202.7	101.3	8.0	104.0	9.48
KSL 62	0.9	0.4	0.1	0.5	0.18	216.0	98.7	5.3	106.7	11.14
KSL 29	1.0	0.9	0.5	0.8	0.07	152.0	162.7	45.3	120.0	4.21
KSL 8	1.0	0.3	0.6	0.6	0.13	256.0	34.7	80.0	123.6	13.67
KSL 9	1.0	0.5	0.3	0.6	0.11	269.3	98.7	10.7	126.2	17.30
KSL 61	1.1	0.8	0.3	0.7	0.14	232.0	160.0	45.3	145.8	8.86
KSL 39	1.1	0.7	0.3	0.7	0.19	306.7	125.3	32.0	154.7	19.51
KSL 58	0.9	0.6	0.7	0.7	0.03	229.3	125.3	114.7	156.4	4.01
KSL 63	1.2	0.3	0.6	0.7	0.23	349.3	45.3	85.3	160.0	27.29
KSL 43	1.1	0.7	0.4	0.7	0.13	298.7	138.7	48.0	161.8	16.11
KSL 30	1.1	0.3	0.3	0.6	0.20	413.3	29.3	48.0	163.5	46.88
Robin	1.0	0.6	0.3	0.6	0.12	333.3	162.7	26.7	174.2	23.61
KSL 54	1.2	0.5	0.1	0.6	0.29	456.0	56.0	16.0	176.0	59.20
KSL 4	0.9	0.9	0.8	0.9	0.01	202.7	200.0	128	176.9	1.79
KSL 40	1.1	0.2	0.2	0.5	0.28	480.0	34.7	16.0	176.9	68.99
KSL 12	1.0	0.8	0.0	0.6	0.29	293.3	253.3	0.0	182.2	25.30
KSL 32	1.1	0.6	1.0	0.9	0.07	336.0	77.3	152.0	188.4	17.72
KSL 1	1.3	0.6	0.4	0.8	0.20	402.7	120.0	50.7	191.1	34.77
KSL 60	1.3	0.6	0.7	0.9	0.14	418.7	56.0	101.3	192.0	39.05
KSL 36	1.3	0.7	0.6	0.9	0.14	416.0	90.7	72.0	192.9	37.42
KSL 16	1.4	0.6	0.4	0.8	0.31	440.0	101.3	42.7	194.7	46.00
KSL 28	1.1	0.9	0.5	0.8	0.11	373.3	213.3	50.7	212.4	26.03
KSL 10	1.0	0.7	0.3	0.7	0.12	346.7	266.7	26.7	213.4	27.73
KSL 64	1.2	0.6	0.5	0.8	0.14	426.7	189.3	48.0	221.3	36.62
KSL 53	1.2	0.8	0.6	0.9	0.10	453.3	154.7	61.3	223.1	41.93
LSD (0.05)[a]				**0.16**					**90.81**	
LSD (0.05)[b]				**0.03**					**19.36**	

KSL; Kenya selection, [a]; LSD for comparing means within locations and [b]; LSD for comparing means between locations.

Appendix 2c) *continued*

	Coefficient of Infection (CI)					Area Under Disease Progress Stairs (AUDPS)				
Genotype	Mau Narok	Njoro	Timau	Means	Stability (1000)	Mau Narok	Njoro	Timau	Means	Stability (1000)
KSL 14	1.2	0.9	0.6	0.9	0.10	389.3	200.0	88.0	225.8	23.20
KSL 35	1.5	0.8	0.6	1.0	0.24	480.0	136.0	74.7	230.2	47.73
KSL 2	1.2	1.1	0.3	0.9	0.26	376.0	306.7	26.7	236.5	34.21
KSL 55	1.2	0.6	0.5	0.8	0.15	560.0	160.0	50.7	256.9	71.90
KSL 33	1.4	0.9	0.3	0.9	0.31	509.3	280.0	26.7	272.0	58.29
KSL 45	1.3	0.8	0.7	0.9	0.10	533.3	200.0	101.3	278.2	51.25
KSL 15	1.2	1.0	0.8	1.0	0.04	386.7	333.3	149.3	289.8	15.51
KSL 22	1.4	1.3	0.4	1.0	0.31	493.3	333.3	48.0	291.5	50.89
KSL 50	1.4	1.0	0.9	1.1	0.07	506.7	240.0	152.0	299.6	34.11
KSL 17	1.5	1.1	0.5	1.0	0.24	506.7	346.7	48.0	300.5	54.20
KSL 44	1.2	0.9	0.4	0.8	0.13	466.7	373.3	64.0	301.3	44.42
KSL 56	1.4	0.9	0.3	0.9	0.30	600.0	293.3	29.3	307.5	81.57
KSL 11	1.0	1.2	0.2	0.8	0.24	296.0	600.0	26.7	307.6	82.28
KSL 38	1.3	1.2	1.1	1.2	0.01	469.3	280.0	189.3	312.9	20.41
KSL 52	1.6	0.9	0.2	0.9	0.50	786.7	141.3	16.0	314.7	171.02
KSL 41	1.4	1.3	0.4	1.0	0.28	560.0	386.7	40.0	328.9	70.10
KSL 6	1.4	1.4	0.8	1.2	0.14	466.7	440.0	112.0	339.6	39.01
KSL 25	1.4	1.3	0.9	1.2	0.06	506.7	413.3	141.3	353.8	36.03
KSL 48	1.7	0.8	0.4	1.0	0.40	746.7	240.0	74.7	353.8	122.61
KSL 46	1.6	0.9	0.4	1.0	0.38	773.3	240.0	50.7	354.7	140.42
KSL 23	1.2	1.6	0.3	1.0	0.40	373.3	666.7	29.3	356.4	101.76
KSL 27	1.2	1.5	0.9	1.2	0.09	413.3	600.0	122.7	378.7	57.86
KSL 20	1.5	1.3	0.6	1.1	0.26	586.7	480.0	77.3	381.3	72.16
KSL 26	1.1	1.6	0.4	1.0	0.38	272.0	920.0	45.3	412.4	206.05
KSL 24	1.4	1.4	1.0	1.3	0.03	600.0	426.7	221.3	416.0	35.93
KSL 37	1.7	1.2	0.6	1.2	0.32	866.7	306.7	101.3	424.9	156.92
KSL 21	1.4	1.4	0.6	1.1	0.24	800.0	440.0	74.7	438.2	131.53
KSL 31	1.6	1.5	0.9	1.3	0.13	653.3	560.0	165.3	459.5	67.10
KSL 19	1.3	1.6	0.9	1.3	0.12	426.7	773.3	202.7	467.6	82.67
KSL 18	1.6	1.2	0.8	1.2	0.15	866.7	442.7	154.7	488.0	128.28
KSL 47	1.6	1.3	0.3	1.1	0.51	826.7	666.7	26.7	506.7	179.20
KSL 49	1.7	1.5	0.6	1.3	0.37	866.7	693.3	82.7	547.6	169.60
Cacuke	2.0	2.0	1.1	1.7	0.24	1693.3	229.3	1174.2	671.4	671.79
Means	**1.2**	**0.8**	**0.5**			**441.6**	**273.3**	**69.3**		
LSD (0.05)[a]				**0.16**					**90.81**	
LSD (0.05)[b]				**0.03**					**19.36**	

KSL; Kenya selection, [a]; LSD for comparing means within locations and [b]; LSD for comparing means between locations

d) The Mean Yield of the 66 Wheat Genotypes Evaluated across the Three Sites in Kenya During 2012-2013 Cropping Season. Genotypes are Ranked According to the Mean Yields.

		Yield t/ha		
Genotype	**Mean yield**	**Mau-Narok**	**Njoro**	**Timau**
KSL 42	7.8	7.6	8.2	7.6
KSL 3	6.6	6.5	6.7	6.6
KSL 9	6.6	5.1	8.0	6.8
KSL 4	6.5	7.4	6.7	5.5
KSL 61	6.5	4.9	7.3	7.4
KSL 7	6.3	7.1	7.3	4.6
KSL 14	6.3	6.5	6.0	6.3
KSL 29	6.3	7.2	6.4	5.3
KSL 11	6.2	7.2	4.6	6.7
KSL 13	6.2	7.7	5.7	5.2
KSL 28	6.2	5.1	7.1	6.3
KSL 58	6.1	5.3	6.0	6.9
KSL 34	6.0	7.7	5.9	4.5
KSL 60	6.0	3.5	7.8	6.6
KSL 26	5.9	7.8	5.1	4.7
KSL 63	5.9	2.9	7.0	7.8
KSL 10	5.8	5.1	5.9	6.5
KSL 32	5.8	5.2	6.8	5.3
KSL 55	5.8	4.8	5.9	6.6
KSL 12	5.7	7.2	5.6	4.3
KSL 16	5.7	4.6	6.4	6.2
KSL 1	5.6	5.0	6.8	5.0
KSL 44	5.6	5.2	4.7	7.0
KSL 5	5.5	5.5	5.7	5.2
KSL 30	5.5	3.8	8.2	4.6
KSL 54	5.5	5.9	5.9	4.7
KSL 2	5.4	4.3	3.7	8.2
KSL 40	5.4	4.5	7.1	4.6
KSL 43	5.4	6.5	5.4	4.3
KSL 45	5.4	4.7	5.5	6.1
KSL 53	5.4	5.4	5.9	5.0
KSL 8	5.3	6.8	5.6	3.6
KSL 51	5.3	6.4	4.5	4.9
CV (%)		**12.87**		
LSD (0.05)[a]		**0.62**		
LSD (0.05)[b]		**0.13**		

KSL: Kenyan Selection, [a]; LSD for comparing means within locations, [b]; LSD for comparing means between locations.

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Appendix 2d) *Continue*

		Yield t/ha		
Genotype	**Mean yield**	**Mau-Narok**	**Njoro**	**Timau**
KSL 18	5.1	3.5	4.9	6.8
KSL 24	5.1	3.6	5.5	6.3
KSL 27	5.1	5.6	3.8	5.8
KSL 33	5.1	4.0	5.3	5.9
KSL 36	5.1	4.0	5.7	5.6
KSL 38	5.1	5.5	4.8	5.0
KSL 46	5.0	4.4	4.7	5.9
KSL 56	5.0	4.0	5.2	5.9
Robin	4.9	3.8	5.3	5.6
KSL 15	4.8	4.5	5.3	4.7
KSL 41	4.8	3.7	5.7	5.1
KSL 62	4.8	4.5	5.2	4.8
KSL 64	4.8	3.8	5.4	5.1
KSL 50	4.7	4.7	4.4	5.1
KSL 52	4.7	3.9	5.7	4.4
KSL 6	4.6	4.4	4.2	5.2
KSL 17	4.6	4.3	4.9	4.6
KSL 48	4.6	3.1	6.1	4.7
KSL 59	4.6	5.7	3.1	4.9
KSL 20	4.5	2.2	4.3	6.9
KSL 22	4.5	3.9	4.4	5.3
KSL 21	4.4	2.7	5.7	4.8
KSL 25	4.4	3.2	4.5	5.5
KSL 39	4.4	4.4	5.6	3.1
KSL 47	4.3	3.1	3.5	6.4
KSL 35	4.2	3.5	5.4	3.6
KSL 37	4.1	3.0	5.5	3.9
KSL 49	4.1	3.7	3.3	5.2
KSL 23	4.0	5.2	3.2	3.7
KSL 19	3.8	3.6	4.6	3.1
KSL 31	3.7	3.1	4.5	3.6
Cacuke	3.5	1.4	1.7	7.5
KSL 57	2.0	1.4	2.2	2.3
Means		**4.8**	**5.4**	**5.4**
CV (%)		**12.87**		
LSD (0.05)[a]		**0.62**		
LSD (0.05)[b]		**0.13**		

KSL: Kenyan Selection, [a]; LSD for comparing means within locations, [b]; LSD for comparing means between locations.

e) GENSTAT Regression Output for Disease and Yield

Regression analysis

Response variate: Yield_t_ha
Fitted terms: Constant, Coefficient_of_Infection_CI

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	192	191.622	99.92	<.001
Residual	592	1135	1.918		
Total	593	1327	2.238		

Percentage variance accounted for 14.3
Standard error of observations is estimated to be 1.38.

Estimates of parameters

Parameter	estimate	s.e.	t(592)	t pr.	
Constant	6.224	0.116	53.51	<.001	
Coefficient_of_Infection_CI	-1.198	0.120	-10.00	<.001	

Correlations between parameter estimates

Parameter	ref	correlations	
Constant	1	1.000	
Coefficient_of_Infection_CI	2	-0.873	1.000
		1	2
		2	

Regression analysis

Response variate: Yield_t_ha
Fitted terms: Constant, AUDPS

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	291	291.171	166.42	<.001
Residual	592	1036	1.750		
Total	593	1327	2.238		

Percentage variance accounted for 21.8
Standard error of observations is estimated to be 1.32.

Estimates of parameters

Parameter	estimate	s.e.	t(592)	t	pr.
Constant	5.8779	0.0750	78.33	<.001	
AUDPS	-0.002557	0.000198	-12.90	<.001	

Correlations between parameter estimates

Parameter	ref	correlations	
Constant	1	1.000	
AUDPS	2	-0.691	1.000
		1	2

f) Wricke's Ecovalence Stability Values for the 66 Wheat Genotypes Evaluated across Three Sites in Kenya during 2012-2013 Cropping Season. Ranking was Done According to Wricke's Ecovalence Values.

Rank	Genotype	Mean Yield	Ecovalence
1	KSL 17	4.6	0.029
2	KSL 57	2.0	0.037
3	KSL 62	4.9	0.045
4	KSL 42	7.8	0.172
5	KSL 3	6.6	0.210
6	KSL 15	4.8	0.256
7	KSL 45	5.4	0.333
8	KSL 31	3.8	0.392
9	KSL 10	5.9	0.432
10	KSL 5	5.5	0.433
11	KSL 64	4.8	0.478
12	KSL 22	4.5	0.486
13	KSL 50	4.7	0.489
14	KSL 58	6.1	0.595
15	KSL 6	4.6	0.595
16	KSL 55	5.8	0.696
17	ROBIN	4.9	0.732
18	KSL 14	6.2	0.744
19	KSL 36	5.1	0.750
20	KSL 53	5.4	0.755
21	KSL 16	5.7	0.766
22	KSL 46	5.0	0.787
23	KSL 33	5.1	0.806
24	KSL 56	5.0	0.811
25	KSL 28	6.2	0.913
26	KSL 41	4.8	0.919
27	KSL 52	4.7	0.995
28	KSL 38	5.1	1.048
29	KSL 32	5.8	1.127
30	KSL 19	3.8	1.199
31	KSL 25	4.4	1.416
32	KSL 1	5.6	1.633
33	KSL 35	4.2	1.674

KSL: Kenyan Selection

Appendix 2f) *Continue*

Rank	Genotype	Mean Yield	Ecovalence
34	KSL 54	5.5	1.722
35	KSL 37	4.1	2.001
36	KSL 49	4.1	2.038
37	KSL 61	6.5	2.067
38	KSL 24	5.1	2.173
39	KSL 9	6.6	2.537
40	KSL 44	5.6	2.687
41	KSL 48	4.6	2.793
42	KSL 21	4.4	2.822
43	KSL 39	4.4	3.109
44	KSL 4	6.5	3.206
45	KSL 40	5.4	3.222
46	KSL 29	6.3	3.224
47	KSL 27	5.1	3.418
48	KSL 18	5.0	3.491
49	KSL 51	5.3	4.055
50	KSL 23	4.0	4.055
51	KSL 43	5.4	4.079
52	KSL 47	4.3	5.235
53	KSL 59	4.5	5.282
54	KSL 11	6.2	5.439
55	KSL 7	6.3	5.731
56	KSL 13	6.2	5.929
57	KSL 60	5.9	6.149
58	KSL 12	5.7	6.387
59	KSL 8	5.3	7.339
60	KSL 34	6.0	7.525
61	KSL 20	4.5	8.508
62	KSL 26	5.8	8.705
63	KSL 30	5.5	8.977
64	KSL 63	5.9	10.271
65	KSL 2	5.4	11.414
66	*Cacuke*	3.6	22.056

KSL: Kenyan Selection

g) Finlay and Wilkinson's Sensitivity Values and Mean Yields for Evaluated Wheat (*Triticum aestivum* L.) Genotypes.

Genotype	Mean yield	Sensitivity	Genotype	Mean yield	Sensitivity
1	5.6	0.356	34	6	-4.233
2	5.4	4.728	35	4.2	0.497
3	6.6	0.04	36	5.1	2.396
4	6.5	-2.44	37	4.1	1.584
5	5.5	-0.324	38	5.1	-0.744
6	4.6	0.93	39	4.4	-1.217
7	6.3	-2.99	40	5.4	0.756
8	5.3	-4.114	41	4.8	2.092
9	6.6	2.633	42	7.8	0.24
10	5.9	1.976	43	5.4	-2.883
11	6.2	-1.12	44	5.6	2.082
12	5.7	-3.83	45	5.4	1.854
13	6.2	-3.545	46	5	1.877
14	6.2	-0.462	47	4.3	4.077
15	4.8	0.279	48	4.6	2.531
16	5.7	2.294	49	4.1	1.86
17	4.6	0.603	50	4.7	0.425
18	5	4.154	51	5.3	-2.334
19	3.8	-0.408	52	4.7	0.962
20	4.5	6.11	53	5.4	-0.507
21	4.4	3.137	54	5.5	-1.454
22	4.5	1.795	55	5.8	2.398
23	4	-2.334	56	5	2.539
24	5.1	3.647	57	2	1.249
25	4.4	3.044	58	6.1	2.077
26	5.8	-4.411	59	4.5	-1.604
27	5.1	-0.113	60	5.9	4.668
28	6.2	1.97	61	6.5	3.382
29	6.3	-2.46	62	4.9	0.623
30	5.5	1.839	63	5.9	6.747
31	3.8	1.004	64	4.8	1.891
32	5.8	0.437	65	4.9	2.477
33	5.1	2.559	66	3.6	7.571

Appendix 3

a) SAS ANOVA Procedure for Evaluated Traits in Diallel Analysis

```
data dialleldata;
input Replicate  $ Block $ Genotype $ Heading Tillers Height Ci yield;
cards;
1      1      1      67     13     66.1   2      588
.      .      .      .      .      .      .      .
3      3      36     80     28     78     4      403
;
proc glm;
class Replicate Block Genotype;
model Heading Tillers Height Ci  yield = Replicate Block Genotype/ss3;
means Genotype/lsd;
run;
```

b) The Means of the Parents and F_1 Genotypes for All the Evaluated Traits of Kenyan Wheat Genotypes in a Diallel Cross.

Parents	Days to heading	Tillers	Height	CI	Grain /plot (kg)
NjBw II	77.3	30.0	72.3	9.3	0.750
Kwale	89.7	41.0	74.2	6.3	0.526
KSL13	67.3	15.3	58.5	3.0	0.666
KSL34	68.0	27.0	75.3	8.7	0.404
KSL42	70.3	20.3	71.0	2.0	0.663
KSL51	71.3	19.0	75.5	6.0	0.399
Cross					
NjBw II /Kwale	84.0	31.0	77.2	9.0	0.450
NjBw II /KSL13	72.0	22.7	71.0	6.3	0.410
NjBw II /KSL34	72.3	29.0	72.3	10.0	0.477
NjBw II /KSL42	74.3	25.3	75.7	2.1	0.424
NjBw II /KSL51	77.0	22.7	74.9	9.3	0.193
Kwale / NjBw II	79.7	25.7	76.1	10.0	0.316
Kwale /KSL13	70.7	24.3	68.7	3.3	0.570
Kwale /KSL34	78.0	20.0	75.0	6.0	0.407
Kwale /KSL42	82.0	21.3	81.0	2.8	0.441
Kwale /KSL51	75.0	24.7	74.2	5.7	0.337
KSL13/NjBw II	70.0	21.7	78.0	4.7	0.469
KSL13/ Kwale	70.7	20.3	70.7	4.0	0.640
KSL13/KSL34	65.7	18.7	64.2	6.7	0.419
KSL13/KSL42	66.3	14.3	70.3	2.0	0.554
KSL13/KSL51	69.0	16.0	70.4	6.0	0.518
KSL34/NjBw II	74.3	24.0	76.8	14.0	0.401
KSL34/ Kwale	79.0	26.0	75.9	6.0	0.468
KSL34/KSL13	63.7	21.7	79.1	6.7	0.471
KSL34/KSL42	74.7	25.0	70.4	5.3	0.582
KSL34/KSL51	70.7	19.0	67.4	4.0	0.255
KSL42/NjBw II	75.0	22.7	76.5	3.7	0.497
KSL42/ Kwale	80.7	23.0	80.9	2.3	0.546
KSL42/KSL13	64.7	14.3	71.3	2.7	0.605
KSL42/KSL34	69.3	22.7	75.1	3.0	0.496
KSL42/KSL51	72.0	21.7	78.0	4.0	0.425
KSL51/NjBw II	75.0	26.7	67.5	11.0	0.335
KSL51/Kwale	75.7	24.0	75.5	9.7	0.412
KSL51/KSL13	69.0	16.0	64.3	4.0	0.540
KSL51/KSL34	70.3	23.7	65.7	7.7	0.314
KSL51/KSL42	72.3	21.0	72.0	2.0	0.473
LSD (P=0.05)	4.8	5.1	5.9	3.5	0.169
CV (%)	4.1	14.0	4.9	35.8	22.2

c) SAS ANOVA Output for Evaluated Traits in Diallel Analysis

```
                          The SAS System        09:37 Thursday, November 7, 2013   1
                                  The GLM Procedure
                                Class Level Information

Class        Levels  Values
Replicate         3  1 2 3
Block             3  1 2 3
Genotype         36  1 10 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32
                     33 34 35 36 4 5 6 7 8 9
                            Number of Observations Read         108
                            Number of Observations Used         108

Dependent Variable: Heading
                                          Sum of
      Source                     DF         Squares     Mean Square    F Value    Pr > F
      Model                      39     3346.250332       85.801291       9.47    <.0001
      Error                      68      615.999668        9.058819
      Corrected Total           107     3962.250000

                  R-Square     Coeff Var      Root MSE    Heading Mean
                  0.844533      4.108924      3.009787        73.25000

      Source                     DF       Type III SS     Mean Square    F Value    Pr > F
      Replicate                   2        7.722222        3.861111       0.43    0.6547
      Block                       2       24.944776       12.472388       1.38    0.2593
      Genotype                   35     3268.305887       93.380168      10.31    <.0001
```

Dependent Variable: tillers

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	2822.208264	72.364314	7.08	<.0001
Error	68	695.449143	10.227193		
Corrected Total	107	3517.657407			

R-Square	Coeff Var	Root MSE	Tillers Mean
0.802298	14.01151	3.197998	22.82407

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replicate	2	29.018519	14.509259	1.42	0.2491
Block	2	10.865672	5.432836	0.53	0.5903
Genotype	35	2773.949005	79.255686	7.75	<.0001

Dependent Variable: height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	2591.236630	66.441965	5.18	<.0001
Error	68	871.910036	12.822206		
Corrected Total	107	3463.146667			

R-Square	Coeff Var	Root MSE	Height Mean
0.748232	4.914946	3.580811	72.85556

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replicate	2	34.242222	17.121111	1.34	0.2699
Block	2	5.354408	2.677204	0.21	0.8121
Genotype	35	2531.189408	72.319697	5.64	<.0001

Dependent Variable: ci

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	1081.786338	27.738111	6.30	<.0001
Error	68	299.503662	4.404466		
Corrected Total	107	1381.290000			

R-Square	Coeff Var	Root MSE	Ci Mean
0.783171	35.87490	2.098682	5.850000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replicate	2	13.140000	6.570000	1.49	0.2323
Block	2	21.169672	10.584836	2.40	0.0981
Genotype	35	1001.306338	28.608753	6.50	<.0001

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	1624729.077	41659.720	3.86	<.0001
Error	68	734842.803	10806.512		
Corrected Total	107	2359571.880			

R-Square	Coeff Var	Root MSE	wtplot Mean
0.688569	22.20764	103.9544	468.1019

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Replicate	2	0.12728502	0.06364251	5.89	0.0044
Block	2	0.03115151	0.01557576	1.44	0.2437
Genotype	35	1.46440976	0.04184028	3.87	<.0001

d) GENSTAT Commands for Hayman's Diallel Analysis (for Number of Tillers/Plant)

```
Text [Values='1','2', '3', '4', '5', '6']Parents
Matrix [Rows=6;Columns=6] Blockdat[1...3]
Read [serial=yes]Blockdat[]

36      29      22      27      26      21
26      39      23      20      20      22
20      18      16      20      13      18
26      24      17      21      21      18
26      24      17      21      18      18
24      25      21      22      25      19:

27      34      18      30      25      22
25      42      24      16      21      24
23      23      14      18      13      16
22      26      26      26      19      19
22      24      19      16      18      22
26      26      14      26      19      20:

27      30      28      30      25      25
26      42      26      24      23      28
22      20      16      18      17      14
24      28      22      36      28      20
21      19      11      28      25      21
30      21      13      23      19      18:
Diallel [Labels=Parents] Blockdat[]
```

e) The Diallel-SAS05 Programme for Griffing's Method 1 Model 1 Analysis

```
%include 'C:\DIALLEL_SAS_CODES\DialAll_2.1.sas';
DATA DT1;
INPUT I J REP ENTRY HEADING ENV;
if I=J then AVGH=0; else AVGH=1;
cards;
1       1       1       1       67      1
.       .       .       .       .       .
.       .       .       .       .       .
6       6       3       36      70      1
;

proc sql;
create table Dt1 as
select I,J,ENV,REP,ENTRY,avg(HEADING) as avg_Y,AVGH
from Dt1
group by I,J,ENTRY;
run;

title "Griffing model 1 method 1";
%DialAnalFixModel(NUM_P=6,method=1,Yvar=HEADING,ENV=1,rep=3,dsn=dt1);
```