EVALUATION OF KENYAN BREAD WHEAT (*Triticum aestivum* L.) MUTANT LINES FOR RESISTANCE TO STEM RUST (*Puccinia graminis* f.sp *tritici*)

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DECLARATION AND RECOMMENDATION

Declaration

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

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Recommedation

This thesis has been submitted with our approval as university supervisors.

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DEDICATION

This thesis is humbly dedicated to my late father, Mr. Mourice Ogutu who sacrificed everything to ensure my better future, wellbeing and welfare.

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ABSTRACT

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a destructive disease of wheat (*Triticum aestivum* L.) and is known to cause major wheat yield losses in Kenya as well as other wheat growing countries. This study was aimed at; determining resistance of wheat mutant lines to *Puccinia graminis* f. sp. *tritici* races TTKSK and TTKTK in the seedling stage, determining adult plant resistance of wheat mutant lines to stem rust disease across three different locations and determining the variability of disease severity of the wheat mutant lines across different locations. Sixty nine genotypes were evaluated in the greenhouse at the Kenya Agricultural and Livestock Research Organization, Njoro under artificial inoculation and in the field for adult plant resistance and variability of disease severity across three different locations in Kenya, i.e. Njoro, Timau and Mau- Narok. A high frequency (53.6% and 88.4%) of mutant lines were resistance to the TTKTK and TTKSK races respectively at seedling stage with low infection types ranging from flecks (;) to resistant (2+). Additionally, frequency for susceptibility was (46.38%) for TTKTK and (11.59%) for TTKSK on the evaluated genotypes. Adult plant stage infection ranged from 2.6 to 2.8 for coefficient of infection and 13.5 to 25.5 for area under disease progress curve. The mean grain yield for the genotypes ranged from 5.5 to 14.1 t ha^{-1}. Genotype, location and genotype by location interaction for the area under disease progress curve, coefficient of infection and yield were significant at $P<$ 0.05. The relationship between yield and area under disease progress curve displayed negative correlation. R-Square values revealed 0.39 and 0.19 of variation in yield was contributed by area under disease progress curve and coefficient of infection respectively. The area under disease progress curve and coefficient of infection means revealed that Duma 200gry (1026), Duma 200gry (1124), Duma 400gry (1304), NJBWII 100gry (404) and Duma 200gry (1030) had moderate resistance reaction to stem rust. The calculated Variance (*Si*) showed stable genotypes for area under disease progress curve and yield which included Duma 100gry (995) and Kwale 100gry (1483), respectively. High significant level of stem rust severity variability ($P<0.01$) occurred across locations and among the tested genotypes. Durable stem rust resistance genes to be incorporated into locally adapted susceptible wheat varieties for further selection and future release to enhance wheat productivity in Kenya.

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LIST OF ABBREVIATIONS

APR Adult Plant Resistance

AUDPC Area under Disease Progress Curve

CI Coefficient of Infection

DGGW Delivery Genetic Gain of Wheat

IAEA International Atomic Energy Agency

FAO Food and Agriculture Organization

Gry grays (gamma-rays)

IT Infection Type

KALRO Kenya Agriculture and Livestock Research Organization

MASL Meters above Sea Level

NjoroBWII/NJBWII Njoro Bread Wheat II

Pgt *Puccinia graminis* f.sp *tritici*

SRRSN Stem Rust Resistance Screening Nursery

TKW Thousand Kernel Weight

Ug99 Uganda 1999

CHAPTER ONE
INTRODUCTION

1.1 Background

Black stem rust caused by *Puccinia graminis* f.sp *tritici* is a devastating disease in wheat causing a decline in wheat production in many parts of the world (Roelfs, 1978). This is due to the narrow genetic base for stem rust resistance in most of the wheat-producing areas in the world. Saari and Prescott (1985) reported the disease as a major disaster in Europe, Asia, America, Africa and Middle East. The last epidemic of stem rust with the variety Enkoy was in Ethiopia in 1993 and 1994 (Temesgen *et al*., 1995). Rust diseases can cause up to 60% of yield loss for stripe and 100% loss for stem rust (Park, 2007). In Kenya, *Ug99* outbreak caused mean yield loss ranging from 5.6% to 66.3% in 2007, which was about 210,000 tones in commercial production (Macharia and Wanyera, 2012). The economic importance of stem rust could result in loss of grain yield and stability in wheat production (Marasas *et al*., 2004). Stem rust has the ability to spread rapidly and reach epidemic proportions under favorable conditions. This pathogen has the potential to mutate rapidly to overcome the effectiveness of current resistance genes (Singh *et al*., 2011).

The persistence of stem rust as a significant disease in wheat can be attributed to the capacity of the pathogen to produce large number of spores which can be wind-disseminated over long distances. It also has the ability to change to virulent pathotypes through migration, mutation and sexual recombination. Wheat stem rust disease detected in Uganda in 1998 was severe and the race was *Ug99* with virulence on *Sr*3 (Pretorius *et al*., 2000). A new race of *Ug99* with virulence to *Sr*24 was detected in Kenya in 2006 (Jin *et al*., 2007). Due to financial implications and environmental concerns related with the genetic basis of resistance and breeding resistant cultivars to emerging races and pathotypes of stem rust disease has received larger attention.

Seedling and adult plant resistance has been used to monitor the resistance of stem rust in wheat (Kaur *et al*., 2009). Seedling resistance characterized by hyper-sensitive responses is usually expressed throughout the plant growth stages (Navabi *et al*., 2004). Large population and short life cycle of pathogens has led to high disease epidemics (Prakash and Heather, 1988). Avirulent to virulent forms may occur due to the strong selection pressure on the pathogen to avoid its detection either through a single mutation event or deletion of the effector molecule of the host plant. Virulent races of the stem rust may be led by unrecognized effector genes of the selected sexual progeny as it is hypothesized for the *Ug99* (TTKS) race (Ayliffe *et al*., 2008). Adult plant resistance appears susceptible at seedling stage but resistant at the adult

plant stage (Imtiaz *et al*., 2011) thus, a compatible interaction between the plant and pathogen is displayed by having partially resistant adult plants. It is also characterized by genes with additive effects and non-hypersensitive responses (Singh *et al*., 2009).

Most of the available genetic variation used in breeding programs have occurred naturally and exists in the germplasm collections of new and old cultivars, land races and genotypes. This variation is utilized through crosses to produce new and desirable gene recombinations. When existing germplasm fails to provide the desired recombinants, it is necessary to resort to other sources of variation (Maluszynski, 1990). Wheat being self-pollinated (Merezhko, 1998) and has a low natural variation, many breeding techniques are being used to improve on resistance to diseases such as rusts and other traits like yield and quality. However, the major threat of stem rust (*Ug99*) facing wheat production gives the genetic assessment of elite wheat germplasm to be a major priority.

Mutation breeding is one of the breeding programs that has been used to induce variation in wheat and other crops. Additionally, it has been used to improve quantitative and qualitative traits of many crops and also to prevent diseases in most of the wheat growing areas (Ilrijana *et al*., 2007). Induction of many mutants has been important for various plant characters in variety of crops including wheat through treatment with physical and chemical mutagens (Ram Din *et al*., 2003). Induced mutation helps to develop many agronomical important traits such as increased tolerance or resistance to abiotic and biotic factors, improved yield and quality traits, early maturity rate and suitability for rotation use in major crops such as wheat, rice, barley, cotton, peanuts and beans (Kasha, 2002).

1.2 Statement of the problem

Stem rust is a destructive disease that has resulted in decline in wheat production in Kenya and around the world. Due to past experiences, fungicide sprays have become part of the production practice of wheat growers in Kenya. However, this has resulted in increased cost of production. Negative effects have also been associated with the use of chemical fungicides in terms of resistance build up and environmental pollution. Although genetic resistance is currently an effective means of managing the rust diseases of wheat in most of the regions, it has not been fully effective. This is because the populations of the fungi causing stem rust have been mutating resulting to occurrence of new variants. Stem rust fungi have a tremendous reproduction potential and easily moved by wind, resulting in outbreaks of disease throughout a large geographical region. Due to this problem, high host resistance varieties with polygenic genes need to be identified for sustainable resistance against stem rust disease.

1.3 Objectives

1.3.1 Broad objective

To improve wheat production through development of stem rust resistant lines.

1.3.2 Specific objective

1. To determine resistance of wheat mutant lines to *Puccinia graminis* f.sp. *tritici* races TTKSK and TTKTK at seedling stage.
2. To determine adult plant resistance of the wheat mutant lines to stem rust disease across three different locations.
3. To determine the variability of disease severity of the wheat mutant lines across different locations.

1.4 Null Hypotheses

1. There is no resistance of wheat mutant lines to *Puccinia graminis* f.sp. *tritici* races TTTSK and TTKTK at seedling stage.
2. There is no adult plant resistance to stem rust among the wheat mutant lines across the three different locations.
3. There is no difference in the variability of disease severity of the wheat mutant lines across different locations.

1.5 Justification

Stem rust is a destructive disease in most of wheat growing areas; it has contributed to the reduction of the quality and yield of wheat resulting in lower outputs to the farmers. Breeding for resistance has been used as the main method of protection against the stem rust. However, the challenge is host resistance genes and spread of new strains through wind dispersal of the spores. Although fungicides have become an integral part of disease management programs on cereal crops in many countries of the world they are least employed in stem rust management. Major yield losses have been experienced since *Ug99* race was identified in Uganda in 1999 and spread to other parts. This has clearly compromised food security and livelihood in Kenya and other countries of the world. For instance, in 2007, a major wheat yield loss was experienced in Narok, Kenya and fungicides had to be used heavily to protect wheat in late-planted areas.

In Kenya, even though varieties that could offer some resistance have been developed, the rust pathogen keeps mutating into virulent forms. Therefore, there is need to deploy

varieties that offer durable and broad-spectrum resistance. Identification and promotion of new stem rust resistant varieties that will significantly enhance yield potential than current varieties in conjunction with other desirable traits would be the best strategy (Rouse and Jin, 2011). This will ensure their fast adoption and thus succeed in replacing the existing susceptible varieties. This is an achievable objective as most of the current popular varieties were developed during early to mid-1990s and yield potential of new spring wheat germplasm has progressed significantly since then (Njau *et al*., 2009). Testing of new wheat lines with adequate resistance to *Ug99* in various countries has indicated that new wheat varieties with higher yields than current varieties can be a reality. High emphasis is currently being given to seed multiplication of these wheat lines (CIMMYT, 2005). Due to this, it is important to employ more breeding programs such as mutagenesis being one of them to check on the disease resistance as well as yield potential of the breeding lines at every stage. This will ensure that novel sources of resistance to the emerging strains of the pathogen as well as good yield potential are identified, gathered and utilized.

1.6 References

Ayliffe, M., Singh, R. and Lagudah E (2008). Durable resistance to wheat stem rust needed. *Current Opinion of Plant Biology*, 11, 187-192.

CIMMYT. (2005). Sounding the Alarm on Global Stem Rust: *An Assessment of Race Ug99 in Kenya and Ethiopia and the Potential for Impact in Neighboring Countries and Beyond.* Mexico City, Mexico.

Ilirjana, S., Ariana, Y. and Andon, D. (2007). Induced Mutations for Improving Production on Bread and Durum Wheat. *Sixth International Conference of the Balkan Physical Union. AIP. Smithsonian/NASA ADS Physics Abstract Service. Conference Proceedings,* 899, 747-747.

Imtiaz, M., Cromey, M. G., Hampton J. G and Ahmad, M. (2011). Inheritance of durable adult plant resistance to stripe rust (*Puccinia striiformis* f.sp. *tritici*) in Otane wheat (*Triticum aestivum*) *New Zealand Journal of Crop Horticultural Science,* 31(1), 23-31.

Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M., Njau, P., Fetch, T., Pretorius, Z. A. and 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 Disease,* 91, 1096-1099.

Kasha, K. J. (Eds.), (2002). *Mutations, In vitro and Molecular Techniques for Environmentally Sustainable Crop Improvement*. Kluwer Academic Publishers, Dordrecht/Boston/ London.

Kaur, J., Bansal, U. K., Khanna, R., Saini, G. R. and Bariana, H. S (2009). Molecular mapping of stem rust resistance in HD2009/WL711 recombinant inbred line population. *International Journal Plant Breeding,* 3(1), 28-33.

Macharia, J. K. and Wanyera, R. (2012). Effect 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.

Maluszynski, M. (1990). Induced mutations-an integrating tool in genetics and plant breeding. . In: Gene manipulation in plant improvement Gustafson JP (eds), *Proceedings of 19th Stadler Genetics and Symposium* (pp. 127-162) Plenum Press, New York.

Marasas, C. N., Smale, M. and Singh, R. P. (2004). *The Economic Impact in Developing Countries of Leaf Rust Resistance Breeding in CIMMYT- Related Spring Bread wheat. Economic program paper,* 04-01. D.F. CIMMYT, Mexico.

Merezhko, A. F. (1998). Impact of plant genetic resources on wheat breeding. *Euphytica,* 100, 295-303.

Navabi, A., Singh Ravi, P., Tewari J. P. and Briggs, K. G. (2004). Inheritance of High Levels of Adult Plant Resistance to Stripe Rust in Five Spring Wheat Genotypes. *Crop breeding, genetics and cytology of Crop Science*, 44, 1156-1162.

Njau P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R and 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 (2), 022-027.

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

Pretorius, Z. A., Singh, R. P., Wagoire, W. W. and Payne, T. S. (2000). Detection of virulence to wheat stem rust gene *Sr*31 in *Puccinia graminis* f. sp. *tritici* in Uganda. *Phytopathology,* 84, 203.

Ram Din, M., Khan, M., Qasim, M., Jehan, S. and Iqbal Khan, M. M, (2003). Induced mutation studies in three wheat (*Triticume aestivum L*.) varieties for some morphological and agronomical characters, *Asian Journal of Plant Science*, 2(17-24), 1179-1182.

Roelfs, A. P. (1978). *Estimated losses caused by rust in small grain cereals in the Unite States, 1918-76.* USDA-ARS.

Rouse, M.N. and Jin, Y. (2011). Stem rust resistance in A-genome diploid relatives of wheat. *Plant Disease* 95: 941-944.

Saari, E. E. and Prescott, J. M. (1985). World distribution in relation to economic losses. *"The Cereal Rusts Vol. II. Diseases, Distribution, Epidemiology, and Control"* (Roelfs, A. P., and Bushnell, W.R. (eds) (pp. 259-98). Academic Press, Orland.

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., Bhavani, S., Njau, P., Herrera – Foessel, S, Pawan, Singh, K., Singh, S. and Govindan, V. (2011). The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production. *Annual Review of Phytopatholothy*, 49, 465 – 482.

Temesgen, K., Bekele G., Balcha, Y. and Ayele, B. (1995). Status of wheat rusts in the major producing regions of Ethiopia. In: Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, October 2-6, 1994. *Breeding for disease resistance with emphasis on durability,* Danial, D. L. (edition) (pp. 180-184).

CHAPTER TWO
LITERATURE REVIEW

2.1 Wheat production

Wheat growing is practiced in 82 countries worldwide with the developing countries producing more than 100,000 hectares of wheat annually (Saari and Prescott, 1985). In the world the top most wheat producing countries include China of 16.8%, India 14.0%, U.S.A 9.1%, France 5.9% and Russian Federation 5.5% in total (FAOSTAT, 2015). Bread wheat is the second most important cereal crop in Kenya after maize (GOK, 1997) in terms of human consumption. The current average annual production is about 0.3 million tonnes which is far short of Kenya's domestic demand of about 0.9 tonnes. This makes Kenya to be a net importer of wheat of close to two-thirds of its requirements to the people (USDA, 2013). The limitations on high production are usually due to abiotic and biotic factors particularly rust diseases which have contributed to heavy crop loss in the country. Stem rust has been the most destructive disease, with the virulent race of *Ug99* in Kenya hence the major Kenyan germplasm available are known to be susceptible or partially susceptible (Njau *et al*., 2009).

2.2 Importance of wheat

Wheat is one of the major crops that is central to achieving development in agriculture and the second most important grain after maize in Kenya (CBS 2003). It is mainly used as a human food and livestock feed (USDA-FAS, 2011). It is nutritious and can be easily processed into various types of food such as biscuits, noodles, bread, pancakes, pan breads (*chapattis*) for home-use baking and other confectionary products (USDA-FAS, 2011). Bran from flour milling is used in livestock feed. The germ is a 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 fiber (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 and polymers while starch has been used to replace some cosmetics and pharmaceutical products (ACPFG, 2008).

2.3 Overview of wheat stem rust

Stem rust is a devastating disease which causes a great loss to wheat production in many parts of the world since the emergence of western civilization (Peterson, 2001). The pathogen is an obligate biotroph, heteroecious in its life cycle and heterothallic in mating type (Alexopoulos *et al*., 1996). It bears many physiological races that are generated by mutation

(Roelfs, 1985). The ecological conditions prevailing in wheat growing areas in Kenya favor the development of the disease. The wheat-growing areas are situated in highland regions ranging from 1,800 to 3,000 meters above sea level. These areas are the hot spot for the evolution and survival of new rust races (Saari and Prescott, 1985). The growing season usually collides with the favorable conditions of pathogen sporulation in which the temperature usually range from 18 to 30°C. The race *Ug99* was first identified in Uganda in 1998 and which was discovered by Wagoire *et al*., 1998 and identified in 1999 (Pretorius *et al*., 2000). *Ug99* is the only known race of stem rust that has virulence for gene *Sr31* known to be located in the translocation1BL.1RS (Mago *et al*., 2004) from rye (*Secale cereale*). It was designated as TTKS by Wanyera *et al.* (2006) using the North American nomenclature system (Roelfs and Martens, 1988) and more recently it was designated as TTKSK after a fifth set of differentials was added to further expand the characterization to indicate virulence to *Sr36* (Jin *et al*., 2008). The race *Ug99* carries virulence to gene *Sr31* and virulence to most genes of wheat origin, as well as to gene *Sr38* that was introduced into wheat from *Triticum ventricosum* (Jin *et al*., 2007). This virulence combination have accounted for the wide-spread *Ug99* susceptibility in wheat varieties worldwide. A variant of *Ug99* that is virulent to *Sr24* (TTKST) was detected in 2006 in Kenya which anticipated that mutation toward more complex virulence would occur as the fungal population size increased and selection pressure is placed on the population by resistant varieties (Jin *et al*., 2008). Important commercial varieties in Kenya have been rendered susceptible (Jin *et al.*, 2008) due to the re-emergence of a new virulent race TTTSK (*Ug99+Sr36*) in Eastern Africa region (Pretorius *et al.,* 2000).

2.4 Stem rust resistance genes

Use of resistant cultivars of wheat has always been effective control method of stem rust disease worldwide. In 1999, a new race of stem rust pathogen was detected in Uganda that was virulent against the traditional wheat resistance genes including *Sr31* and *Sr38* (Pretorius *et al*., 2000). This race was denominated TTKS or *Ug99* which was found in Kenya in 2003 and in 2007 in Yemen (Wanyera *et al*., 2006). This virulent race was postulated that it could soon migrate to the Middle East and Central Asia attack many of the current cultivars realized worldwide (Singh *et al*., 2006). The effective resistance genes determined were *Sr7a, 13, 22, 24, 25, 26, 27, 28, 29, 32, 33, 35, 37, 39, 40* and *44*; and the ineffective resistance genes were *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 11, 15, 17, 21, 30* and *38* (Wanyera *et al*., 2005). Among the effective genes, *Sr13, 22, 24, 26, 29* and *36* may have some immediate value while *Sr36* has conferred resistance to *Ug99* (McIntosh *et al*., 1995).

Once only the *Sr2* gene was known to confer slow rusting APR; now, four more genes—*Sr55*, *Sr56*, *Sr57*, and *Sr58*—have been characterized and additional quantitative trait loci identified. Cloning of some rust resistance genes opens new perspectives on rust control in the future through the development of multiple resistance gene cassettes. However, at present, disease-surveillance-based chemical control, large-scale deployment of new varieties with multiple race-specific genes or adequate levels of APR, and reducing the cultivation of susceptible varieties in rust hot-spot areas remains the best stem rust management strategy (Ravi *et al.*, 2015).

2.5 Economic importance of stem rust

During 2007 cropping season, areas where the disease was not controlled led to 100% loss of the crop regardless of the variety (Wanyera, 2008). The amount of losses caused by rust is influenced by the degree or severity of infection. Major yield losses to stem rust that occurred in Kenya were when *Ug99* was first detected in the year 2007 crop season. The rust fungus is easily dispersed by wind, with regard to that it has rapidly spread from the East African region to North Africa, Middle East and West-South Asia (Ali *et al*., 2009). Growing of wheat in narrow genetic base coupled with vertical resistance has led to high wheat yield losses ranging from 70-100% especially among highly susceptible cultivars (Wanyera, 2008; Njau *et al*., 2009). The disease usually causes cereal yield losses in several ways. The fungus absorbs nutrients from the plant tissues that would be used for grain development in a healthy plant. As pustules break through the epidermal tissue, the plant finds it difficult to control transpiration, so its metabolism becomes less efficient. Desiccation or infection by other fungi and bacteria also can occur. Interference with the vascular tissues results in shriveled grains, light in weight thereby lowering grain quality (USDA, 2004). On the other hand rusts lower the crop's forage value and predispose plants to other diseases. Rusted plants are less palatable and are toxic to livestock and severe disease also causes straw breakage, resulting in loss of spikes during combine harvesting (Wiese, 1991). Stem rust can cause losses of 50% in one month when conditions for its development are favorable and 100% loss on susceptible cultivars in heavy infestations (Roelfs *et al*., 1992).

2.6 Rust Pathogen Host Range

Cereal and grass host ranges of *Puccinia graminis* are many of which the primary hosts include *Triticum aestivum* L. and *T. turgidum* L. and the closely related grasses that exist with a wide range of specific and nonspecific resistance. The resistance is expressed in terms of

reduction in number of lesions, size of the sporulating area, increase in the length of the latent period and reduced length of the sporulating period (Roelfs *et al*., 1992). *Hordeum vulgare* and other species of Aegilops are stem rust hosts. *Berberis vulgaris* is the main alternate host for stem rust and a major source of new combinations of genes for virulence and aggressiveness in the pathogen (Groth and Roelfs, 1982) which has made breeding for resistance difficult.

2.7 Biological aspect of the pathogen

Rust fungi are obligate parasites thus require living host tissue for growth and reproduction hence in their absence they survive as spores. In most rust fungi only teliospores are adapted to survive apart from a living host plant for a few months under field conditions (Schumann and Leonard, 2000). Stem rust fungus is heteroecious thus require two unrelated host plants to complete its life cycle (Alexopoulos *et al*., 1996), wheat being the primary host and barberry the alternative host. It is also a macrocyclic, producing all the five spores' stages, basidiospores, pycniospores, aeciospores, urediniospores and teliospores. The barberry which was introduced along with cereals to North America and Europe played a significant role in the early epidemics and production of new races of stem rust. Eradication of barberry plants have largely been enhanced worldwide and their importance as a source of inoculum and new races in the regions is insignificant (Harder *et al*., 2001). *Berberis holstii* being a close relative to common barberry has been identified in several major wheat growing-areas, for example, Mau-Narok and at the slopes of Mount Kenya. Therefore, mycelium or uredinia on volunteer wheat appears to be the main source of inoculum, because the existence of stem rust sexual cycle has not been reported in Kenya (Kurt, 2001). Stem rust pathotypes usually occur and are probably formed by mutation or somatic hybridization.

2.9 Environmental conditions with respect to disease cycle

Exposure of each new and susceptible wheat variety to spores of the fungus which is the primary inoculum leads to the start and development of the stem rust disease cycle. Different regions in which the wheat variety is grown determines the source of the first spores that infect the variety (Schumann and Leonard, 2000). In warm climates, wheat is planted in late fall and harvested in early summer. The first spores to infect the young plants in the fall are urediniospores and usually come from infected volunteer wheat plants which sprout from seeds that may have spilled in the field while harvesting (Schumann and Leonard, 2000). These volunteer wheat plants can become infected from spores produced on late-maturing wheat plants still in the field and can serve as carriers of the disease through summer to the next fall-

sown crop of wheat (Schumann and Leonard, 2000). In regions with temperate climates, wheat is usually planted either in the fall or the spring seasons.

Development of stem rust fungus is favored by heavy dews, high humidity and warm temperatures. The optimal temperature for infection in wheat by stem rust ranges from 15-30^{o}C (Roelfs *et al*., 1992). Sunlight and hours of leaf wetness, resulting from rain or dew are usually required for infection to occur. Urediniospores are usually produced 7-14 days after infection hence as a result of the temperature requirements stem rust usually appears in June and July in the central and northern Great Plains, respectively (Roelfs.,1989). Late maturing cultivars usually are affected more severely than early maturing cultivars.

2.9 Symptoms of stem rust

The stem rust life cycle is very complex but 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 Leonard and Szabo 2005, the symptoms start to appear on the wheat as the main host and other grass hosts including barley after seven to fifteen days of infection where the oval pustules of powdery brick-red urediniospores break through the epidermis. The pustules frequently occur on the leaf sheaths and stems (Agrios, 2005) then later in the season the pustules of black teliospores begin to appear in infected host species (Roelfs, *et al*., 1992). Pycnia usually occur in spring season on the upper leaf surfaces in barberry plants which are often in small clusters and exude pycniospores in sticky honeydew. After five to ten days, cylindrical shaped structures filled with orange- yellow powdery aeciospores break through the lower leaf surface and sometimes elongate to extend up to 5mm from the leaf surface (Agrios, 2005).

2.10 Epidemiology of stem rust

Stem rust is a known disease of wheat, barley, triticale and oats occurring almost everywhere these crops are grown (Stubbs *et al*., 1986) and being an obligate parasite is unable to complete its life cycle in the absence of a living plant host. Stem rust urediniospores can be dispersed through three modes i.e. long-distance dispersal by a single event as well as assisted dispersal, stepwise range expansion and extinction and recolonization (Singh *et al*., 2008). Dispersal by a single event is rare and it includes the movement of the urediniospores across whole continents. Stem rust spores have moved up to 8000 km from the south of Africa to Australia (Brown and Hovmoller, 2002). Through this way, spores are able to withstand a high range of environmental pressures and can travel for long distance (Singh *et al*., 2008). Human

beings are associated with the assisted means of dispersal of spores since the spores mostly are carried and transported on clothing as well as through the trade of infected wheat (Singh *et al*., 2008). Stepwise range expansion mostly spreads the spores at the slightly smaller scale of countries and regions. The current expansion of the stem rust strain *Ug99* is an example of stepwise range expansion.

The strain first originated in Uganda in 1999, then migrated into the Middle East, and spread to Asia (Singh *et al*., 2004). Extinction and recolonization occurs through smaller distances and happens on land that is too stressful for the spores to survive (Singh *et al*., 2008). The fungus persists on successive crops including wheat and barley, as they are planted at different times of the year depending mainly on the rainfall patterns. Uredinia on volunteer wheat and barley are the most important source of inoculum in tropical highlands (Saari and Prescott, 1985). Stem rust epidemics are particularly frequent at the middle altitude range in Kenya (Kurt, 2001). This is probably due to the influence of the wheat and barley cultivated on the southern Rift Valley which supports an endemic rust fungal population, which is thought to be responsible for the inoculum exchange within Rift Valley between Kenya and Tanzania (Saari and Prescott, 1985).

2.11 Life cycle of stem rust disease

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 *Berberis vulgaris* or the late-maturing wheat plants still in the field (Schumann and Leonard, 2000). Under favorable conditions the fungus produces thick-walled two-celled teliospores near the end of the growing season of the gramineous host. Each teliospore cell contains two haploid nuclei when first formed, but karyogamy occurs early in teliospore maturation. Teliospore stalks remain intact and the spores are not dispersed from the telial pustule until suitable conditions where upon they will germinate in synchrony with the onset of bud break and new leaf growth in the alternate host of *Berberis spp* (Roelfs and Groth, 1988). Meiosis begins shortly after karyogamy but is suspended at diplonema of the first meiotic division during the period of telispore dormancy (Boehm *et al*., 1992). The teliospore produces a hyphal protrusion called a promycelium of which on completion of meiosis, four haploid nuclei are separated from each other in the promycelium by three transverse septa. A structure called sterigma forms on each promycelium cell and the haploid nucleus migrates through the sterigma into the newly forming

basidiospore as it expands at the tip of the sterigma (Roelfs, 1985). Mitosis results in two identical haploid nuclei per mature basidiospore. Mature basidiospores are ejected from the sterigmata and carried by air currents to infect alternative hosts of *Barberry vulgaris*. Due to thick cuticle on the leaf surface basidiospore germ tubes are unable to penetrate directly thus infection results in formation of flask-shaped pycnia on the upper surface of the leaf. Small, thin-walled pycniospores form within the pycnium and exude from the tip of the pycnium in a drop of pycnial nectar. The nectar is attractive to insects that, along with rain splashing serve to disseminate pycniospores among pycnia (Agrios, 2005). Pycniospores, which serve as the male gametes, consist mainly of a single haploid nucleus with little surrounding cytoplasm. Flexuous hyphae, which extend out of the flask-shaped pycnia, serve as the female gametes. Two mating types commonly represented by + and – have been identified and appear to be under monogenic control (Roelfs, 1985). When a pycniospore of one mating type contacts a flexuous hypha in a pycnium of the other mating type, fusion occurs and the haploid nucleus from the pycniospore migrates through a fusion tube into the flexuous hypha and through the monokaryotic hyphae until it reaches the cells of the protoaecium at the base of the pycnium (Schumann and Leonard, 2000). With nuclear division and paired association of + and – mating type nuclei the dikaryotic state is established. New growth commences and a cup-shaped, dikaryotic aecium is formed below the pycnium, eventually rupturing the lower epidermis of the leaf. Chains of single-celled dikaryotic aeciospores are produced which can infect the gramineous host (Roelfs, 1985). When an aeciospore successfully infects its gramineous host, the fungus produces a dense mat of hyphae beneath the host epidermis (Roelfs *et al*., 1992). Sporophores grow from the mat and produce masses of single-celled dikaryotic urediniospores that rupture the host epidermis producing a pustule known as a uredinium (Roelfs, 1978). Urediniospores are dispersed by wind and can reinfect the gramineous host. This completes the life cycle (Leonard and Szabo, 2005).

2.12 Major production constraints of wheat

Due to global warming, and potential climate abnormalities associated with it, crops typically encounter an increased number of abiotic and biotic stress combinations, which severely affect their growth and yield **(**Ramegowda and Senthil-Kumar, 2015). Among the abiotic factors include drought, nutrient deficiencies, and water logging (Peters *et al*., 2014). Among the biotic stresses include pests, grass weeds and diseases.

2.12.1 Abiotic factors

Drought is one of the major abiotic constraints on wheat production in drought prone areas of Kenya and parts of the world. In such areas, moisture stress is an important yield limiting factor for crop production. Moreover, because of degradation and poor vegetation cover, soils in semi-arid and arid areas have low fertility, with poor water holding capacity (CIMMYT, 1997; Torkamani, 2005). Nutrient deficiency is also one of the important abiotic stresses in wheat production in the country. It is probably the most widely spread problem in Africa due to the limited use of chemical fertilizers and lack of crop rotation (FAO, 2002). According to Schneider and Anderson (2010), soil fertility related constraints such as expensive or limited access to nitrogen fertilizer, nitrogen deficiency, and soil fertility depletion were present in over 40% of wheat growing areas in sub-Saharan Africa, and accounted for 20% of the total yield gap. In Kenya, soil depletion is a problem in some wheat growing areas, which led to stagnant crop yields despite the use of modern inputs such as enhanced seed and some fertilizers. Water logging is another important abiotic factor that threatens wheat production, mostly in the highlands above 1400 masl in Kenya. It impedes the performances of cereal crops during the main rainy season in the highlands of Kenya (FAO, 2002). In such areas, early planting is not possible, which in turn reduces the length of the growing cycle and consequently the yield.

2.12.2 Biotic factors

Diseases are among the most important yield limiting factors in wheat production. Wheat in Kenya is attacked by a number of diseases that reduce the quality and quantity of grain. Among the major wheat diseases are the wheat rusts: stem rust (*Puccinia graminis* f.sp. *tritici*), leaf rust (*Puccinia triticina)*, and stripe rust (*Puccinia striiformis* f.sp. *tritici*). Of the three rusts, stem rust is widely distributed throughout the major wheat growing regions of Kenya and may cause severe food shortages when it occurs in epidemic proportions (Singh *et al.*, 2008). Yield losses from stem rust can reach up to 100% on susceptible cultivars (Park, 2007; Hodson, 2013). In addition to diseases, grass weeds are among major wheat production constraints in Kenya (*http://www.kalro.org/sites/default/files/Wheat-Handbook-2016.pdf*). Continuous production of wheat especially in the Kenya highlands and repeated application of broad-leaf herbicides have increased the problem of grass weeds (Rezene and Yohannes, 2003).

2.13 Management of stem rust Disease

Several methods are available to control wheat stem rust. Attempts have been made to minimize or control stem rust through different cultural methods and chemical measures. Fungicides have been widely investigated for use in control of stem rust (Roelfs, 1985). The use of early maturing cultivars, early planting and destruction of volunteer wheat and other susceptible grasses can be used to control stem rust. However, all these approaches are of little importance and breeding for stem rust resistance remains the most effective and practical method of stem rust management (Singh, 1998). Generally, it is essential to understand epidemiology of stem rust to start any control measure, especially one involving cultural or chemical control (Singh *et al.*, 2002).

2.13.1 Host Resistance to stem rust

Genetic host resistance is the most desirable method of disease control because it is the most effective, least expensive and environmentally friendly approach (Expert Panel, 2005). According to the panel, developing and deploying cultivars with new sources of stem rust resistance effective against *Ug99* will take about 10 years. The quickest way to reduce the risk of stem rust is to identify very susceptible cultivars and discourage their production in areas prone to stem rust epidemics. However, acquiring and maintaining adequate resistance is difficult because of genetic plasticity of the stem rust pathogen (McIntosh *et al*., 1995).

2.13.2 Seedling resistance to stem rust

Seedling resistance is controlled by one or a few genes that confer highly effective resistance during the entire life of the wheat plant (Roelfs *et al*., 1992). Seedling resistance genes are active during the adult plant stage and they are classified into race-specific resistance types (Lagudah, 2010). With respect to that, fifty five stem rust race-specific resistance genes based on seedling resistance test have been identified (Singh *et al*., 2011). However, these seedling resistance genes are often broken down due to new and various races of the rusts pathogen (Chen and Moore, 2002).

Currently, it is not possible to accurately predict the durability of these resistance genes since some seedling genes are overcome by new races before being deployed in commercial cultivars. However, other genes like *Sr31*, the seedling stage resistance gene overcome by *Ug99*, have been durable and provided useful resistance globally for several decades. Most known stem rust resistance genes in wheat have been tested in seedling and adult plants for

reaction to *Ug99* (Jin *et al*., 2007). Results indicated that seventeen of the forty six genes were effective in seedling and adult plants to *Ug99* while twenty five were ineffective and four were inconclusive. However, variants of *Ug99* with virulence to additional *Sr* genes have been detected (Jin *et al*., 2009), which emphasizes the need to aggressively pursue identification of novel stem rust resistance genes that provide effective seedling stage resistance.

2.13.3 Adult plant resistance (APR) to stem rust.

Plants with adult-plant resistance are susceptible at seedlings stage but become resistant as they mature. Generally, they have intermediate levels of stem rust in the field thus comparing to a very susceptible cultivar with no adult plant resistance (Jin *et al*., 2007). Resistant gene *Sr*2 was detected in several highly resistant old Kenyan cultivars, including ‘‘Kenya Plume’’ (Singh and McIntosh, 1986) and CIMMYT- derived semi-dwarf wheat ‘‘Pavon 76,’’ ‘‘Parula,’’ ‘‘Kiritati,’’ and ‘‘Kingbird’’. Pavon 76 and Kiritati genotypes were resistant since the initiation of rigorous screening in 2005 at Njoro, Kenya with maximum disease scores of 20MR- MS was done. Kingbird a new advanced line released is at present the best known source of adult plant resistance in semi-dwarf wheat with maximum score recorded to be 5 MR-MS during the same period (Njau *et al*., 2010). Because these wheat are susceptible at seedlings with race Ug99, their resistance is speculated to be based on multiple additive genes where *Sr*2 is an important component (Singh *et al*., 2004). Moderate and high levels of adult plant resistance would be useful wherever wheat is prone to stem rust epidemics and even low levels of adult plant resistance may be sufficient to prevent losses in areas that are marginal for stem rust development.

2.14 Mutation Breeding for Stem Rust Resistance

Genetic variability is of prime importance for the improvement of many crop species, including wheat, and nearly all crop improvement programs depend on genetic diversity in the available germplasm (Akfirat *et al*., 2013). Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms (Akfirat *et al*., 2013). Exploitation of induced genetic diversity is a useful strategy that has shown improvement of all major food crops and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability (Parry *et al*., 2009).

The use of physical mutagens, like X-rays, gamma and chemical mutagens for inducing variation, has well established in the years, whereby more than 2500 varieties derived from mutagenesis programs have been released, as listed in the IAEA/FAO mutant variety database, including: 205 wheat lines, 534 rice lines, and 71 maize lines (http://wwwinfocris.iaea.org/MVD/). These induced mutations have helped to develop many agronomical important traits such as shorter growing period, yield traits, increased tolerance to abiotic and biotic stresses in major crops such as wheat, maize (Till *et al*., 2004), rice (Suzuki *et al*., 2008), barley (Talame *et al*., 2004), cotton, peanuts and beans (Kenzhebayeva *et al*., 2013). The mutants developed in wheat had great potential for direct release and to include them in cross breeding programme (Sakin *et al*., 2005). Of these include *Triticum durum* (Sakin and Yildirim, 2004) and hexaploid bread wheat (Slade *et al*., 2005).

2.15 Cultural method

Moisture on leaves and excessive foliar factor nitrogen favor infections by rust fungi thus the farmers should consider the factor in spacing, row orientation and fertilizer schedules. Most changes occurring in production activities have effects on the stem rust (Roelfs, 1985). Irrigation practices in summer wheat growing areas are being enhanced which may increase the survival of infected volunteer plants. No-till or minimum tillage also increases the probability that rust fungi may successfully overwinter in the protective layer of stubble from the previous crop. Use of earlier-maturing wheat varieties has helped reduce the threat of stem rust epidemics (Roelfs *et al*., 1992) and modern wheat varieties in the central Great plain region USA usually mature about two weeks earlier than older varieties hence this limits the length of time for stem rust epidemics to develop and as well as the numbers of urediniospores that can contribute to epidemics farther in the north. Planting at the onset of rainfall and growing of early maturing cultivars tend to escape heavy losses due to stem rust infection (Agrios, 1988) which is no longer feasible in Kenya because there has been no fixed sowing dates and low adherence to growing recommended cultivars, hence enabling the stem rust.

2.16 Chemical control

High costs of fungicide application as well as potential environmental hazards have made the use of chemical control method on cereal diseases undesirable. However fungicides are used world-wide to maintain production levels in wheat cultivars lacking adequate levels of resistance (Ireta and Gilchrist, 1994). Chemicals have so far played a minor role in stem rust control (Knott,1989) due to the effectiveness of host resistance, the rate of disease increase,

for wheat stem rust under ideal conditions and the relatively low economic return per hectare of wheat in comparison to the cost of fungicide application (Rowell, 1985). Large scale farmers in Kenya spray their wheat crop with fungicides to protect it against stem rust. This is costing them about US$10 million annually (Expert Panel, 2005). In Kenya and other developing countries it is not economical to spray large areas with low yielding and low priced situations since stem rust epidemics are difficult to predict considering fluctuation of weather conditions (KARI, 2005).

2.17 References

Akfirat F. S. and Uncuoglu A. A. (2013). Genetic diversity of winter wheat (*Triticum aestivum* L.) revealed by SSR markers. *Biochemical Genetics*, 51, 223–229.

Alexopoulos, C. J., Mims C. W., and Blackwell., M. (1996). *Introductory mycology*. Wiley, New York, New York, USA

Ali, S., Shah, S. J. A. and Rahman, H. (2009). Multi-location variability in Pakistan for partial resistance in wheat to *Puccinia striiformis* f.sp. *tritici. Phytopatholothy of Mediterranean,* 48, 269-279.

Agrios G. N. (1988) *Plant Pathology*. 3rd Edition. London, New York, Academic Press.

Agrios, G. N. (2005). *Plant Pathology*. 5rd Edition. Academinc Press, Inc. San Diego.

Australian Centre for Plant Functional Genomics (ACPFG) (2008). The Biology of *Triticum aestivum* L. em Thell. (Bread wheat). htAtp://www.acpfg.com.

Boehm, E. W. A., Wenstrom, J. C., McLaughlin, D. J., Szabo, L. J., Roelfs, A.P. and Bushnell, W. R. (1992). An ultrastructural pachytene karyotype for *Puccinia graminis* f. sp. *tritici. Canadian Journal of Botany,* 70, 401–413.

Brown, J. K. M. and Hovmoller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, 297, 537–41.

Central bureau of statistics (CBS*)*, Kenya. (2003). *National Economic survey 2003 Kenya*.

Chen, X. M. and Moore, M. K. (2002). Epidemics and races of *Puccinia striiformis* in North America in 2001. *Phytopathology*, 92, 14 –15.

Expert Panel on the Stem Rust Outbreak in Eastern Africa (2005). Sounding the alarm on global stem rust: *An assessment of race Ug99 in Kenya and Ethiopia and potential for impact in neighbouring regions and beyond*. D.F CIMMYT, México.

Food and Agricultural Organization, (2002). Bread wheat improvement and production. *Plant production and protection Series* No. 30, Rome Italy

Food and Agriculture Organization database statistics (2015). http://faostat3.fao.org.

CIMMYT, (1997). *International Maize and Wheat improvement centre and ICARDA* 2000. *Conservation tillage; A viable option for sustainable agriculture in Central Asia*, almaly, kazakhstan and Allepo Syria

Government of Kenya (1997) The Eighth National Development Plan for Kenya, *Government of Kenya Printers*, Nairobi (1997).

Groth, J. V and Roelfs A. P. (1982). The effect of sexual and asexual reproduction on race abundance in cereal rust fungus populations. *Phytopathology*, 72, 1503-1507.

Harder, D. E., Baum, B. R. and McCallum, B. D. (2001). Virulence in populations of *Puccinia graminis* f. sp. *tritici* in Canada from 1952 to 1998: *A non-parametric analysis of Concetration Botany*, 79, 556-569.

Hodson, D. (2013). Localized stem rust epidemic in southern Ethiopia extreme caution and vigilance needed in East Africa and Middle East region. *A global wheat rust monitoring system.* Available at: http://rusttracker.cimmyt.org/? p=5473 Accessed 12 February 2014.

Ireta, M. J. and Gilchrist, S. L. (1994). Fusarium head scab of wheat (*Fusarium graminearu* Schwabe). *Wheat Special Report*. No. 21b. D.F: CIMMYT, Mexico.

International Food Policy Research Institute, IFPRI Discussion paper 00910. (2009). *Combating stem and leaf rust of wheat*. www.ifpri.org/millionsfed.

Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M., Njau, P., Fetch, T., Pretorius, Z. A. and 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 Disease,* 91, 1096-1099.

Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R. and Fetch, T. (2008). Detection of virulence to resistance gene *Sr* 24 within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease,* 92, 923-926.

Jin, Y., Szabo, L. J., Rouse, M. N., Fetch, T. J., Pretorius, Z. A., Wanyera, R. and Njau, P. (2009). Detection of virulence to resistance gene Sr36 within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. *Plant Disease,* 93, 367-370.

Kenya Agriculture and Research Institute, (2005). *Effect of a new race on wheat production/ use of fungicides and its cost to large versus small scale farmers, situation of current cultivars KARI Njoro*.

Kenzhebayeva, S., Alybayeva, R., Atabayeva, S. and Doktrbai, G. (2013). Developing salt tolerant lines of spring wheat using mutation techniques. *Proceeding International conference. Life Science and Biological Engineering.* (pp. 2376-2318). Japan.

Knott, D. R. (1989). The Wheat Rusts-Breeding for resistance, Springer Verslag, Berlin Heidelberg, 201pp.

Kurt, J. L. (2001). *Black Stem Rust Biology and Threat to Wheat Growers.* USDA, ARS Cereal Disease Laboratory @http://www.cdl.umn.edu.

Lagudah, E. S. (2010). Molecular genetics of non - specific rust resistance race in wheat. (pp 183-196). *Borlaug Global Rust Initiative, Proceeding of Technical Workshop,* (verified on May 30 - 31, 2010) St. Petersburg, Russia.

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

Mago, R., Spielmeyer, W., Lawrence, G. J., Ellis, J. G. and Pryor, A. J (2004). Resistance genes for rye stem rust (*SrR*) and barley powdery mildew (*Mla*) are located in syntenic regions on short arm of chromosome. *Genome,* 47, 112-121.

McIntosh, R. A., Wellings, C. R. and Park, R. F (1995) '*Wheat rusts: an altas of resistance genes.*' (CSIRO Australia, Kluwer Academic Publishers: Dordrecht)

Njau P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R and 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 (2), 022-027.

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

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

Parry, M. A. J., Madgwick, P. J., Bayon, C., Tearall, K., Lopez, A. H., Baudo, M., Rakszegi, M., Hamada, W., Yasin, A. A., Quabbou, H., Labhilili, M. and Phillip, A. L. (2009). Mutation discovery for crop improvements. *Journal Expert of Botany*, 60 (10), 2817-2825.

Peters, K., Breitsameter, L. and Gerowitt, B. (2014). Impact of climate change on weeds in agriculture: a review. *Agricultural Sustainability and Development.* 34 707–721. 10.1007/s13593-014-0245-2

Peterson, P. D. (2001). The campaign to eradicate the common barberry in the United States. In Ancient Enemy to Modern Foe. (eds). *Stem Rust of Wheat* (pp 16-50) APS Press, St. Paul, M.N. P.D. Peterson

Pretorius, Z. A., Singh, R. P., Wagoire, W. W. and Payne, T. S. (2000). Detection of virulence to wheat stem rust gene *Sr*31 in *Puccinia graminis* f. sp. *tritici* in Uganda. *Phytopathology,* 84, 203.

Ramegowda, V. and Senthil-Kumar, M. (2015). The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. *Journal of Plant Physiology.* 176 47–54. 10.1016/j.jplph.2014.11.008

Ravi P. S., David P. H., Yue J., Evans S. L., Michael A. A., Sridhar B., Matthew N. R., Zacharias A. P., Les J. S., Julio Huerta-Espino, Bhoja R. B., Caixia L., and Mogens S. H. (2015*) Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic Control*

Rezene, F. and L. Yohannes. 2003. Control of *Snowdenia polystachya* in large scale wheat production: Herbicide resistance in context. p.79-88. *In*: Bedada, G. (ed.) *Proceedings of the Agronomy Workshop*. 20-21 March 2000. Bale Agricultural Development Enterprise (BADE), Melkassa, Ethiopia.

Roelfs, A. P. (1978). *Estimated losses caused by rust in small grain cereals in the United States, 1918-76.* USDA-ARS.

Roelfs, A. P. (1985). Wheat and rye stem rust. In Roelfs A.P, and Bushnell, W.R., (eds). *The Cereal Rusts Diseases, Distribution, Epidemiology, and Control* (Pp. 3-37). Academic Press, Orlando.

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

Roelfs, A. P., and Groth, J. V. (1988). *Puccinia graminis* f.sp. *tritici,* black stem rust of *Triticum* spp. In G.S. Sidhu, (eds). *Advances in Plant Pathology,Vol. VI, Genetics of Pathogenic Fungi* (Pp. 345-361). Academic Press, London.

Roelfs, A.P. 1989. Epidemiology of the cereal rusts in North America. *Canadian Journal of Plant Pathology* 11:86-90.

Roelfs, A. P., Singh, R. P. and Saari, E. E. (1992). *Rust Diseases of Wheat: Concepts and Methods of Disease Management* (pp 81). D.F. CIMMTY, Mexico.

Rowell, J. B (1985). Controlled infection by *Puccinia graminis* f.sp. *tritici* under artificial conditions. In: *The Cereal Rusts Vol.I Origins, specificity, structure, and physiology*, W.R. Bushnell and A.P. Roelfs (eds) (Pp 292-332). Academic Press, Orlando, FL.USA.

Saari, E. E. and Prescott, J. M. (1985). World distribution in relation to economic losses. *"The Cereal Rusts Vol. II. Diseases, Distribution, Epidemiology, and Control"* (Roelfs, A. P., and Bushnell, W.R. (eds) (pp. 259-98). Academic Press, Orland.

Sakin, M. A. and Yildirim, A. (2004). Induced mutations for yield and its components in durum wheat (*Triticum durum*). *Food, Agriculture and Environment,* 2 (1), 285-290.

Sakin, M. A., Yildirim A. and Gokmen, S. (2005). Determining some yield and some quality characteristics of mutants induced from a durum wheat (*Triticum durum*) cultivar. *Turkey Journal of Agriculture,* 29, 61-67

Schneider, K. and L. Anderson. (2010). *Yield Gap and Productivity Potential in Ethiopian Agriculture: Staple Grains and Pulses*. EPAR Brief No. 98. Prepared for the farmer productivity team of the Bill and Melinda Gates Foundation.

Schumann, G. L and Leonard, K. J. (2000). *The Plant Health Instructor*, DOI:10.1094/PHI-I-2000-0721-01.

Singh, R.S. (1998). *Plant disease*. Seventhed. Oxford and IBH Publishing Co.Pu.LTD, New Delhi. Pp 371-400.

Singh, R.P., Huerta-Espino, J. and Roelfs, A.P. (2002). The Wheat Rusts. pp.554. *In:* B.C. Curtis, S. Rajaram, H. Gomez Macpherson (eds.). *Bread Wheat Improvement and Production*. Food and Agriculture Organization (FAO) of the United Nations. Rome, Italy.

Singh, R. P., William, H. M., Huerta-Espino, J. and Rosewarne, G. (2004). Wheat rust in Asia: Meeting the challenges with old and new technologies. In ''New Directions for Diverse Planet. *Proceedings of the 4th International Crop Science Congress''* http://www.Cropscience.org.au./icsc2004/symposia/3/7/141_singhrp.htm. September 26–October 1, 2004. Brisbane, Australia.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M., Wanyera, R., Njau, P. and Ward, R. W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources,* 1, 54.

Singh, R. P, Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S. A and Ward, R.W (2008). Will Stem Rust Destroy the World's Wheat Crop? *Advance Agronomy*, 98, 271-309.

Singh, R. P., Hodson, D. P., Huerta – Espino, J, Jin, Y., Bhavani, S., Njau, P., Herrera – Foessel, S, Pawan, Singh, K., Singh, S. and Govindan, V. (2011). The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production. *Annual Review of Phytopatholothy*, 49, 465 – 482.

Slade, A. J., Fuerstenberg, S. I., Loeffler, D., Steine, M .N and Facciotti, D. (2005). A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. *Nature of Biotechnology*, 23, 75-81.

Stubbs, R. W., Prescott, J. M., Saari, E. E. and Dubin, H. J. (1986). *Cereal Disease Methodology Manual,* (CIMMTY), Mexico.

Suzuki, A., Eiguchi, M., Kumamaru, T., Satoh, H., Matsusaka, H., Moriguchi, K., Nagato, Y. and Kurata, N. (2008). MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. *Molecular Genetics and Genomics*, 279, 213-223.

Talame, V., Bovina, R., Sanguineti, M. C., Tuberosa, R., Lundqvist, U and, Salvi, S. (2008) TILL More, a resource for the discovery of chemically induced mutants in barley. *Plant Biotechnology Journal,* 6, 477-485.

Till, B. J., Reynolds, S. H. and Weil, C. (2004). Discovery of induced point mutations in maize genes by TILLING. BMC *Plant Biology,* 4, 12.

Torkamani J. (2005). Using a whole-farm modelling approach to assess prospects of technologies under uncertainty. *Agricultural systems.* 85, (2), 138-154

USDA, ARS Cereal Disease Laboratory. (2004). *Barberry testing at the Cereal Disease Laboratory*. http://www.cdl.umn.edu.

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

United States Department of Agriculture (USDA) (2013). *"Kenya Corn, Wheat and Rice Grain and Feed Annual Report"*.

Wagoire, W. W., Stolen, O. and 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. and Singh, R. (2005). The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. *Plant Disease,* 89.

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

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

Wiese, M. V. (1991). *Compendium of wheat diseases*. Second edition. *The American Phytopathological Society Press*, United States of America.

CHAPTER THREE

EVALUATION OF STEM RUST SEEDLING RESISTANCE IN KENYAN BREAD WHEAT (*Triticum aestivum* L.) MUTANT LINES

3.1 Abstract

Race TTKSK (*Ug99*) of stem rust is a serious threat to wheat production worldwide because of its wide virulence on many cultivars and its rapid spread over countries. The objective of this study was to determine resistance to *Puccinia graminis* f.sp *tritici* races of TTKSK and TTKTK in the seedling stage among the wheat mutant lines. Duma, Kwale and NJBWII mutant lines were used to evaluate sources of resistance to the race TTKSK and TTKTK since the parents are susceptible. In an attempt to identify stem rust resistance lines effective against the two races at seedling stage, sixty three mutant lines and six checks (NJBWII, Kwale, Duma, Cacuke, Robin and Kingbird) were evaluated in the greenhouse. A high frequency (53.6% and 88.4%) of mutant lines were resistance to the TTKTK and TTKSK races respectively at seedling stage with low infection types ranging from flecks (;) to resistant (2+). Additionally, frequency for susceptibility was (46.38%) for TTKTK and (11.59%) for TTKSK on the evaluated genotypes. Some genotypes like Duma 200gry (1124) and NJBWII 100gry (50) were resistant and susceptible respectively to the races. Resistance in these genotypes may be due to uncharacterized resistance genes or gene combinations that could not be resolved with the collection of races used. The information presented, when combined with previous characterization of stem rust resistance genes in the genotypes will be useful for plant breeders in rationalizing germplasm enhancement programs.

3.2 Introduction

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* historically has been one of the most ` important diseases in the world. In 1999, a new strain known as *Ug99* (race TTKSK), was able to cause disease on previously resistant wheat cultivars carrying stem rust resistance gene *Sr31* in Uganda. In 2006 and 2007, new variants of *Ug99* emerged and infected wheat varieties carrying *Sr31* (race TTKSK), *Sr24* (race TTKST) and *Sr36* (race TTTSK) which were identified in Kenya (Jin *et al*., 2009). Previously, these genes conferred major resistance genes that were used in breeding programs for control of stem rust. Due to movement and spreading of new races of stem rust worldwide in wheat production regions, there is need for identification and transfer of novel sources of resistance genes to the susceptible varieties that have high yield potential.

Mutation breeding is one of the tools being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007). Mutation induction offers significant increase in crop production (Kharkwal and Shu, 2009) and the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution.

Seedling resistance protects the plant against virulent pathogen isolate during their entire growing period and it is race specific controlled by major genes. However, when used extensively over a long period of time, new races of *Ug99* usually overcomes it leading to susceptibility of the released germplasm (Rajaram *et al*., 1988). The qualitative resistance is classified into race-specific or vertical, seedling resistance, monogenic, hypersensitive (Lowe *et al*., 2011). Race specific resistance is usually governed by a hypersensitive response, controlled by major genes which are often led by a boom and bust cycle (Priyamvada and Tiwari, 2011).

The seedling resistance genes are usually effective at the seedling stages, and they are characterized by the gene-for-gene interaction model (Flor, 1971). These resistance genes are also active during the adult plant stage, and they are classified into race-specific resistance types (Lagudah, 2010). Through different studies, fifty five stem rust race-specific resistance genes based on seedling resistance test have been identified (McIntosh *et al*., 2010; Singh *et al*., 2011). However, the seedling resistance genes are often broken down due to new and various races of the rusts pathogen that are evolving and mutating in the wheat field areas (Chen and Moore, 2002).

3.3 Materials and Method

3.3.1 Wheat genotypes

Sixty three mutant lines used in this experiment were developed from three selected Kenyan wheat varieties including NjoroBWII/NJBWII, Kwale and Duma. The three parental cultivars had been previously screened for stem rust resistance in the International Screening Nursery at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Kenya. The three parental wheat cultivar seeds were sent to International Atomic Energy Agency in Vienna, Austria and subjected to gamma irradiation at three levels of 100, 200 and 400 gry to get the sixty three mutant lines. Three parental cultivars of NjoroBWII/NJBWII, Kwale, and Duma, the resistant wheat cultivar Kingbird (Njau *et al*., 2010), and the two susceptible cultivars Robin and Cacuke were also included in the experiments.

3.3.2 Pathogen isolates

Two *Puccinia graminis* f.sp *tritici* races (TTKTK and TTKSK) were used to test the 69 wheat genotypes in the greenhouse. The races were obtained from stem rust samples collected at trap nursery of stem rust resistance screening nursery (SRRSN), KALRO, Njoro.

3.3.3 Seedling resistance test

Ten seeds of each genotype were planted in 10 cm wide square pots filled with a vermiculite potting mix and placed in a plastic tray with each pot in a fixed position. Seedlings were applied upon the fourth day of emergence with 0.15g/pot of CAN fertilizer. The urediniospores were collected from the wheat screening trap nursery. Afterwards, they were chopped into small sizes where the epidermal sheath was removed. The epidermal sheath containing spores were suspended in Tween 20 liquid oil. Using hemocytometer, the diluted urediniospores were counted at an approximate concentration of 6×10^6 spores/ml. Nine days old seedlings were inoculated with a single pustule of TTKTK urediniospores on the leaves. The inoculated seedlings were kept in a dew chamber for 16-20 hr dark at 18-22°C and relative humidity of 95% before transferring to a growth and sporulation chamber in the greenhouse adjusted at 18-24°C. The procedure above was also applied to the other experimental set using TTKSK urediniospores separately. Disease infection type (IT) was observed 15 days after inoculation following the procedure (Stakman *et al*., 1962), where "0" means no disease and the genotype is resistant while "4" shows the highly susceptible genotype. The infection types "; or flecks", "1", "1+", "2",are resistant or combinations indicated low infection types (resistance), while, the infection types "3-", "3", "3+" and "4" indicated susceptibility (Figure 3.1). The infection

types readings indicated: (0) No uredinia or other macroscopic sign of infection, (; or flecks) No uredinia, but hypersensitive necrotic or chlorotic flecks present, (1) Small uredinia surrounded by necrosis, (2) Small to medium uredinia often surrounded by chlorosis; green islands may be surrounded by chlorotic or necrotic, (3) Medium sized uredinia that may be associated with chlorosis, (4) Large uredinia without chlorosis. The variations were refined by modifying characters as follows: -, uredinia somewhat smaller than normal for the infection type; +, uredinia somewhat larger than normal for the infection type. The experiment was repeated thrice per the races tested and only the genotypes that produced similar infection types in the three experiments were considered. When there was infection type 0 (immune reaction) in the three tests, the test was repeated to exclude the possibility of disease escape.

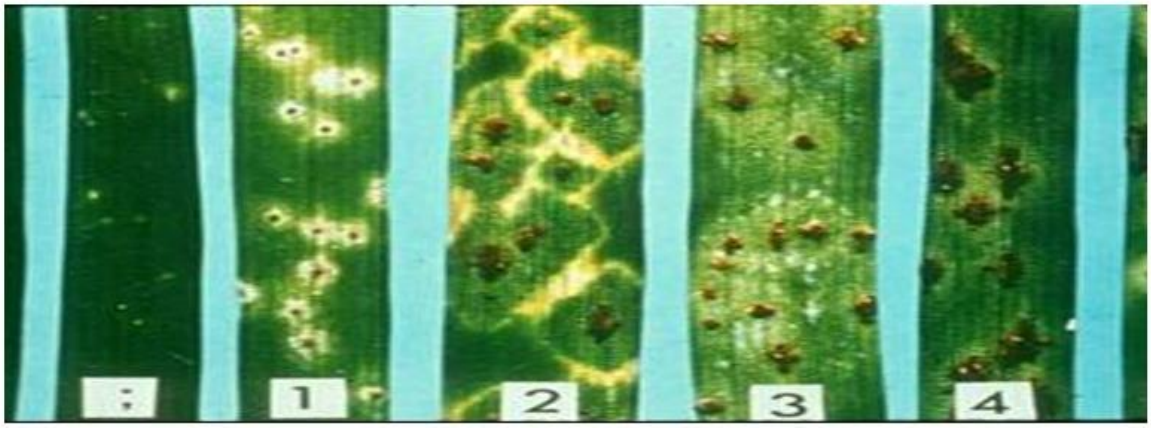

Figure 3:1: Stakman's Infection Type Scale

3.4 Data analysis

The average infection types for the 10 seedlings per pot were recorded for data analysis. Sixty nine genotypes were grouped according to the infection types recorded in the greenhouse during the season and used to draw graphs of frequency distribution of the infection types among the genotypes and percentage of genotypes exhibiting resistance and susceptibility in respect to the pathogens.

3.5 Results

Of the total 63 genotypes assessed, 37 were resistant to TTKTK race while 61 were resistant to TTKSK races. In general the infection type coding resistance was ranging from (; -flecks) to (2+ (resistant) whereby the maximum infection type mostly displayed was 2+ on the primary leaves of the seedling. Thirty two genotypes were susceptible, 28 had a maximum infection type of 3 and the remaining four mostly the checks genotypes were noted with score of 4 for TTKTK race. With respect to TTKSK eight genotypes were susceptible, six genotypes had infection type of 3 and two had infection type of 4 (Table 3.1). However, some genotypes were noted having two combinations of disease scores, NJBWII 200gry (641) had a combination infection type (1, 2) to TTKTK race. Kwale 200gry(1777) and Kwale 200gry(1875) had infection type (1+, 2) while NJBWII 100gry(288), Duma 200gry(1099) and Duma 200gry(1124) had infection type (1+ 2) to TTKSK race (Table 3.1). A high frequency (53.6% and 88.4%) of mutant lines were resistance to the TTKTK and TTKSK races respectively at seedling stage with low infection types ranging from (flecks) to (2+). Additionally, frequency for susceptibility was (46.38%) for TTKTK and (11.59%) for TTKSK on the evaluated genotypes (Figure 3.2).

The infection types frequency distribution presented in (Figure. 3.3) depicts a continuous variation for the two races. The frequencies of the genotypes categorized as resistant and susceptible in their reaction to the two races varied markedly depending on the race (Figure.3.3). The results presented in (Figure. 3.3) shows that 36 genotypes had resistance “1” to “1+” when inoculated with TTKSK while 8 genotypes were resistant to TTKTK. Out of the total genotypes, twenty eight and six genotypes were susceptible to TTKTK and TTKSK races respectively. This shows that TTKTK was most virulent to the genotypes tested in the study (Figure. 3.3). Among the mutant lines, Duma 200gry (1124), Kwale 100gry (1468) and NJBWII 200gry (608) were examples of resistant lines while NJBWII 400gry (798) and Duma parent were among the susceptible (Figure 3.4).

Table 3:1: Infection types of Bread wheat (*Triticum aestivum*) mutant lines exhibiting resistant and susceptible reaction to *Puccinia graminis* f. sp. *tritici* races, TTKTK and TTKSK at the seedling stage.

	***Puccinia graminis* f.sp tritici races**	
Genotypes	**TTKTK**	**TTKSK**
NJBWII Parent and the mutant lines		
NJBWII PARENT	3	2+
NJBWII 100 GRY(50)	3	1+
NJBWII 100GRY(57)	3	2
NJBWII 100 GRY(140)	3	2+
NJBWII 100 GRY(288)	3	1+ 2
NJBWII 100 GRY(382)	;	1+
NJBWII 100GRY(404)	2	1+
NJBWII 100GRY(415)	3	; , 1
NJBWII 200GRY(602)	2+	1+
NJBWII 200GRY(608)	2+	2
NJBWII 200 GRY(612)	3	1+
NJBWII 200 GRY(634)	2+	1+
NJBWII 200 GRY(641)	1, 2	1+
NJBWII 200 GRY(660)	2	2+
NJBWII 200 GRY(662)	2+	1
NJBWII 400 GRY(675)	3	2+
NJBWII 400 GRY(776)	2	1+
NJBWII 400 GRY(798)	3	3
NJBWII 400 GRY(849)	2+	2
NJBWII 400 GRY(908)	2+	1+
NJBWII 400 GRY(915)	3	1
NJBWII 400 GRY(930)	3	1
Duma parent and the mutant lines		
DUMA PARENT	4	4
DUMA 100 GRY(987)	;	1+
DUMA 100 GRY(992)	1+	1
DUMA 100 GRY(993)	3	2+
DUMA 100 GRY(995)	1	1+
DUMA 100 GRY(996)	2	3
DUMA 100 GRY(997)	3	2+
DUMA 100 GRY(1010)	3	2+
DUMA 200 GRY(1026)	3	2+
DUMA 200 GRY(1030)	2+	1+
DUMA 200 GRY(1033)	2	1+
DUMA 200 GRY(1099)	3	1+ 2
DUMA 200 GRY(1103)	1+	1+
DUMA 200 GRY(1124)	;	1+ 2
DUMA 200 GRY(1145)	2	1+

DUMA 400 GRY(1299)	3	2+
DUMA 400 GRY(1295)	2+	3
DUMA 400 GRY(1304)	2+	2
DUMA 400 GRY(1437)	4	3
DUMA 400 GRY(1349)	3	3
DUMA 400 GRY(1368)	2	1+
DUMA 400 GRY(1403)	2+	1+
Kwale parent and the mutant lines		
KWALE PARENT	3	2+
KWALE 100 GRY(1468)	1	1+
KWALE 100 GRY(1470)	3	1
KWALE 100 GRY(1483)	3	1
KWALE 100 GRY(1492)	3	2
KWALE 100 GRY(1499)	2+	1
KWALE 100 GRY(1502)	; ,1	1+
KWALE 100 GRY(1556)	3	2
KWALE 200 GRY(1621)	3	1
KWALE 200 GRY(1715)	3	1
KWALE 200 GRY(1731)	2	1
KWALE 200 GRY(1750)	; , 1	1
KWALE 200 GRY(1768)	3	2+
KWALE 200 GRY(1777)	3	1 2
KWALE 200 GRY(1818)	3	2
KWALE 400 GRY(1875)	2+	1 2
KWALE 400 GRY(1877)	2	1+
KWALE 400 GRY(1895)	1+	2+
KWALE 400 GRY(1907)	1+	1
KWALE 400 GRY(1949)	2+	1
KWALE 400 GRY(1961)	2+	1+
KWALE 400 GRY(1964)	2+	1
Resistant and Susceptible* checks**		
KINGBIRD**	;	; ,1
CACUKE*	4	4
ROBIN*	4	3

Infection type (IT) was based on the scale described by Stakman *et al*. (1962) with ITs flecks (;) 1,1+,2, 2+ considered resistant and 3, 4 considered susceptible. Positive (+) = larger uredinia than the normal size.

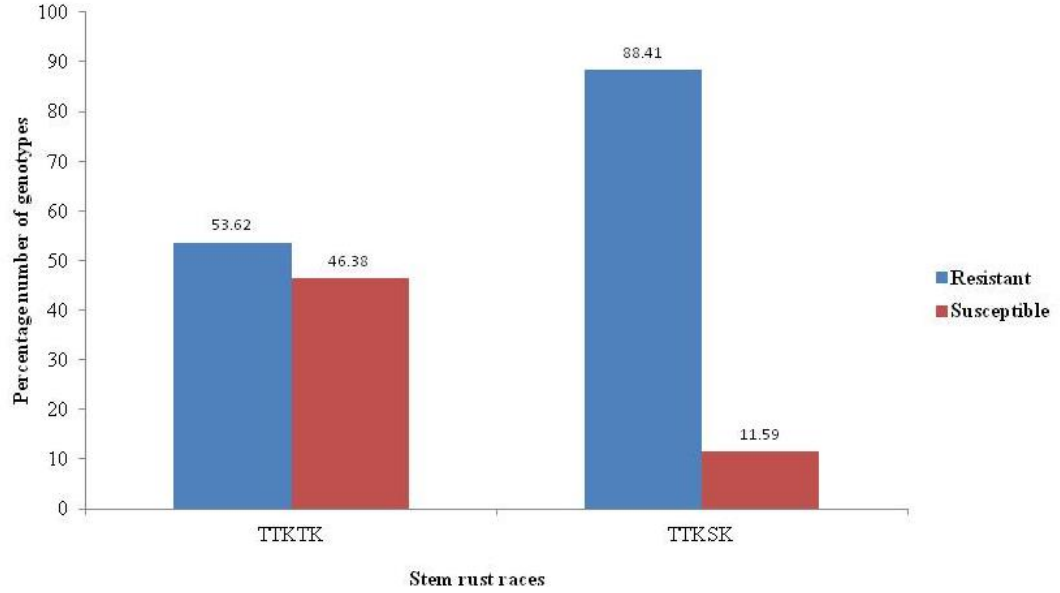

Figure 3:2: Percentage of bread wheat (*Triticum aestivum*) mutant lines exhibiting resistant and susceptible reaction to *Puccinia graminis* f. sp. *tritici* races TTKTK and TTKSK.

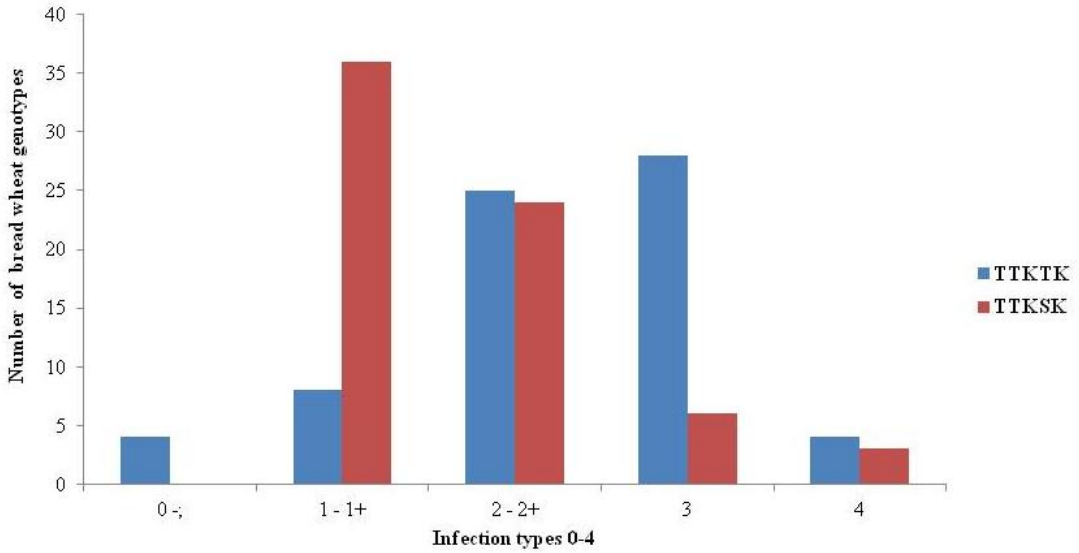

Figure 3.3: Frequency distribution of infection types (ITs) of 69 wheat (*Triticum aestivum*) genotypes evaluated at the seedling stage with two stem rust races.

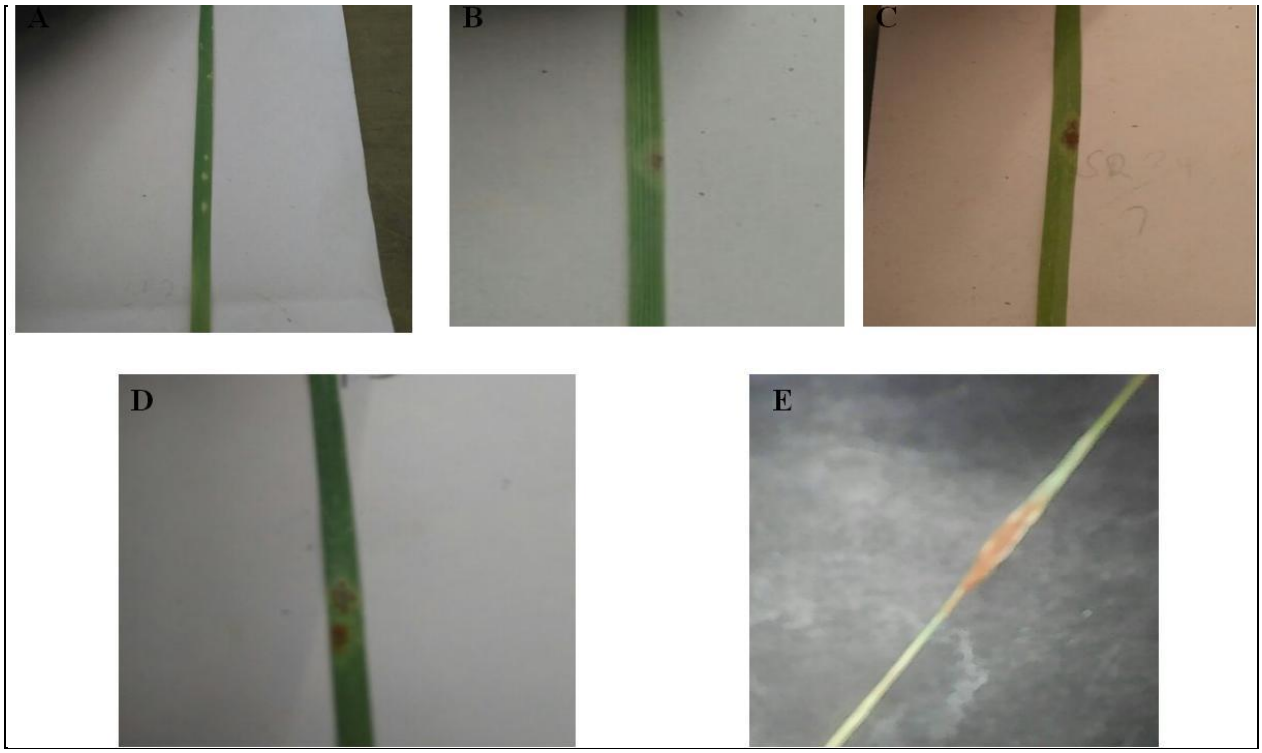

Figure 3:4: Some the genotypes with various infection types A: Duma 200gry (1124) (; or Flecks - Resistant), B: Kwale 100gry (1468) (1- Resistant), C: NJBWII 200gry (608)(2– Resistant), D: NJBWII 400gry(798) (3– Susceptible), E: Duma parent (4 – Susceptible).

3.6 Discussion

Two virulent races (TTKTK and TTKSK) were inoculated on the test genotypes at seedling stage in the greenhouse in which there was no complete resistance (0) observed. Moreover, most of the mutant lines in the current study evaluated against the two races of stem rust were noted having infection types from flecks (;) to resistant (2+). A great protection against stem rust is determined by specific genes and usually expressed at seedling stage. In regard to non-specific genes, they are usually assumed to be quantitative.

There was variation in resistance spectrum observed among the tested genotypes. According to this study, the mutant lines evaluated showed a broader resistance spectrum compared to their parents Duma, Kwale and NjoroBWII/NJBWII. This implies that after irradiation some genes in the chromosomes of the mutant lines may have been successfully altered during the process. The parents (Duma, Kwale and NJBWII) were among the old varieties bred in the late 90s and were part of the first varieties noted to be susceptible to the new *Ug99* stem rust race (Njau *et al*., 2009). In additional, this might have been due to the fact that the varieties are postulated to carry stem rust resistance ineffective genes against *Ug99* e.g. *Sr31* gene which was broken down by the new *Ug99* stem rust race in the late 90s.

Olivera *et al*., 2012a found sources of TTKSK resistance from emmer wheat and the infection types ranged from 2 to 2+. Similarly, the current test genotypes showed results of infection types (2 to 2+) where 34.78% of the genotypes exhibited that range of resistance to TTKSK race. On the other hand, the present study results were also in agreement with (Worku, 2014) studied on resistance to TTKSK and TTTSK races of *Puccinia graminis* f.sp. *tritici* in Ethiopian tetraploid wheat accessions and found most of the accessions resistant to TTKSK race.

In the current study it was proven that *Sr* tmp gene from Robin is ineffective to the TTKTK race since the variety was highly susceptible when tested in the greenhouse. With the evaluated genotypes, 46.38% were susceptible to the race while 53.62% were resistant. For the susceptible genotypes, the race was virulent to the genes in the test genotypes. This showed that the genes were ineffective.

Consequently, the high percentage of resistance showed that most lines have major genes. The low infection types scored on these genotypes could be either due to one or more of the *Sr*-genes or a combination that had similar infection type patterns. On the other hand, the significant proportion of the tested genotypes showing resistance or susceptibility may due to gene alteration during irradiation. As a result, this requires additional molecular work and race analysis with a wider virulence spectrum than the present races to determine the genes that are

responsible for the low or high infection types displayed by the genotypes against some of the races.

3.7 Conclusion

The results of this study demonstrated that the lines that showed resistance could serve as a source of resistance to race TTKSK and TTKTK. The lines could be exposed to other races to ascertain their level of resistance. Further genetic analysis is required to confirm the present effective genes in the resistance lines after gamma-rays radiation since alteration may have occurred to the genes in the chromosomes. This could bring a background on which genes can be incorporated to the susceptible varieties that had been broken down by the present races.

3.8 References

Adamu, A. K., and Aliyu, H. (2007). Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). *Scientific World Journal*, 2(4): 9-12.

Chen, X. M. and Moore, M. K. (2002). Epidemics and races of *Puccinia striiformis* in North America in 2001. *Phytopathology*, 92, 14 –15.

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

Jin, Y., Szabo, L. J., Rouse, M. N., Fetch, T. J., Pretorius, Z. A., Wanyera, R. and Njau, P. (2009). Detection of virulence to resistance gene Sr36 within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. *Plant Disease,* 93, 367-370.

Kharkwal, M.C. and Shu, Q.Y. (2009). *The Role of Induced Mutations in World Food Security*. *In*: Induced Plant Mutations in the Genomics Era, Shu, Q.Y. (Ed.). Food and Agriculture Organization of the United Nations, Rome, Italy, pp: 33-38.

Lagudah, E. S. (2010). Molecular genetics of non - specific rust resistance race in wheat. (pp 183-196). *Borlaug Global Rust Initiative, Proceeding of Technical Workshop,* (verified on May 30 - 31, 2010) St. Petersburg, Russia.

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

McIntosh, R.A., Dubcovsky, J., Rogers, W.J., Morris, C., Appels, R. and Xi, X.C. (2010), "Catalogue of gene symbols. KOMUGI Integrated Wheat Science Database". *http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp;jsessionid=689B192F53CED7CB561A9DC624FB9518.lb1,%202010*

Njau P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R and 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 (2), 022-027.

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

Olivera, P.D., Badebo, A., Xu, S., S., Klindworth, D.L. and Jin, Y. (2012). Resistance to race TTKSK of *Puccinia graminis* f. sp. *tritici* in emmer wheat. *Crop Science* 52: 2234-2242.

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

Rajaram S, Singh RP, Torres E. (1988). Current CIMMYT approaches in breeding wheat for rust resistance. In: Simmonds NW, Rajaram S, editors. *Breeding Strategies for resistance to the Rust of Wheat*. CIMMYT, Mexico, D. pp.101-118

Singh, R. P., Hodson, D. P., Huerta – Espino, J, Jin, Y., Bhavani, S., Njau, P., Herrera – Foessel, S, Pawan, Singh, K., Singh, S. and Govindan, V. (2011). The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production. *Annual Review of Phytopatholothy*, 49, 465 – 482.

Stakman, E. C, Steward D. M and Loegering W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici*. U.S. *Department of Agriculture and Agricultural. Resource Services*. E-617.

Worku, D. (2014). Resistance to TTKSK and TTTSK Races of *Puccinia graminis* f.sp. *tritici* in Ethiopian Tetraploid Wheat Accessions. *Journal of Biology, Agriculture and Healthcare*. Vol.4, No.8, 2014

CHAPTER FOUR

ADULT PLANT RESISTANCE OF WHEAT (*Triticum aestivum* L.) MUTANT LINES TO STEM RUST (*Puccinia graminis* f.sp *tritici*) ACROSS THREE DIFFERENT LOCATIONS

4.1 Abstract

Stem rust (*Puccinia graminis* f. sp *tritici*) is a destructive disease of wheat (*Triticum aestivum* L.) making it a major challenge to wheat production in Kenya as well as other wheat growing countries. Due to this, mutation breeding has been as a source of increasing variability and confers specific improvement to the Kenyan varieties without significantly altering its phenotype. The objective of this study was to determine adult plant resistance of wheat mutant lines to stem rust across three different locations. The study area was in three locations, Nakuru County (Njoro and Mau Narok) and Meru County (Timau) during 2015-2016 cropping season. Sixty three mutant lines and six checks (NJBWII, Duma and Kwale, Kingbird, Robin and Cacuke) were evaluated under field conditions with three replications in an alpha lattice (23 rows by 3 columns) design. Mean for area under disease progress curve and coefficient of infection revealed that Duma200gry (1026), Duma200gry (1124) were best disease performers. The calculated variance (*Si*) distinguished stable genotypes in terms of disease and yield which included Duma100gry (995) and Kwale100gry (1483), respectively. There was positive effect of dosage 400gry on the mutant lines in terms of disease, yield and 1000 kernel weight, mostly with the Duma mutant lines. The mean grain yield for the genotypes ranged from 5.5 to 14.1 t ha^{-1}. Genotype, location and genotype by location interaction for the area under disease progress curve, coefficient of infection and yield were significant at $P< 0.01$ and $P<0.001$. There was a negative correlation displayed between yield and disease components. R-Square values revealed 0.1508 and 0.3911 of the variation in yield was contributed by the disease severity and area under disease progress curve, respectively. Considering the best lines both in disease and yield can be taken to further screening in breeding programs.

4.2 Introduction

Ug99 (TTKSK) race and its variants have been resulting to huge wheat yield losses which can go up to 100% in most of Kenya wheat growing areas and worldwide (Wanyera *et al.*, 2006, 2008; Singh *et al*., 2006, 2008; Admassu *et al*., 2008; Njau *et al*., 2009). Though in Kenya, wheat breeding programmes have attempted to develop resistant varieties, virulence of the pathogen has been reported on most of the varieties at both seedling stage and adult plant resistance stages (Njau *et al*., 2009). Due to this, mutation induction is one of the techniques that has been employed to create variation within wheat varieties (Maluszynki *et al*., 2003; Parry *et al*., 2009). Some of these variations have been achieved using physical mutagens, like X-rays, gamma-rays, neutrons and chemical mutagens.

Wheat (*Triticum aestivum* L.) is one of the major food crops in the world. FAO (2012) estimated that the world wheat production had risen to 700 million tonnes in the year 2011 from 553.92 million tonnes in 2003/2004, 607 million in 2007 and 655.7 million tonnes in 2010. In Kenya, wheat is second important crop after maize with an annual production of 0.2 million tonnes in 2009, 0.25 million tonnes in 2010, 0.2 million tonnes in 2011 and 0.25 million tonnes in 2012. This production cannot meet the demand which has been growing at 5% per annum in the recent years to 0.9 million tonnes in 2012 (FAO, 2012). To fill the gap, Kenya imports about 0.65 million tonnes of wheat annually. However, wheat production can be increased by addressing the current constraints facing the small-scale farmers, especially small-scale who have limited resources and lack access to production technologies.

Biotic factors like diseases, weed and pests are the major constraints to wheat production in Kenya. They may destroy between 31% and 42% of all crops annually (Park, 2007). Important wheat diseases include; rusts, smuts, bunts, leaf blight, powdery mildew and head scab (Priyamvada *et al.*, 2011). 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) to wheat crop in the world. Leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) can cause up to 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 attack previously resistant cultivars. It can move long distances by wind and develop rapidly under optimal environmental conditions (Priyamvada *et al.*, 2011). This study was

aimed at identifying possible elite wheat mutant lines containing resistance to stem rust that can be advanced to further levels in breeding programs.

4.3 Materials and Method

4.3.1 Genotypes

Sixty three mutant lines used in this experiment were developed from three selected Kenyan wheat varieties including NjoroBWII, Kwale and Duma. The three parental cultivars had been previously screened for stem rust resistance in the International Screening Nursery at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro Kenya. The three parental wheat cultivar seeds were sent to International Atomic Energy Agency in Vienna, Austria and subjected to gamma irradiation at three levels of 100, 200 and 400 gry to get the sixty three mutant lines. Three parental cultivars of NjoroBWII/NJBWII, Kwale, and Duma, the resistant wheat cultivar Kingbird (Njau *et al*., 2010), and the two susceptible cultivars Robin and Cacuke were also included in the experiments.

4.3.2 Description of study area

The study was conducted during the 2015 cropping season across three locations in Kenya namely KALRO, Njoro and Mau Narok in Nakuru county and Timau in Meru county. Njoro station is located at 0 20′S, 35º 56′E and 2185 m elevation, with daily average temperature ranging from 9.7ºC (night) and 23.5ºC (day). Variations of daily temperatures in this area is approximately + 2ºC occurring mostly during the day hours and receives on average 939 mm of precipitation annually. The soil type is predominantly Mollic Andosols. Mau-Narok is situated 0º 39′S, 35º57′ E at 2,900 MASL and annual rainfall of 1,200 to 1,400 mm, minimum and maximum temperatures ranging 6 to 14ºC and 22 to 26ºC, respectively. Timau is situated 0º 5'S, 37º 20'E at 2640 MASL and annual rainfall of 896 mm, minimum and maximum temperatures of 5°C and 23°C respectively. These sites fall within agro ecological zone III (Jaetzold *et al*., 2010). These areas have been described as 'hot-spot' for stem rust epidemics (Saari and Prescott, 1985). These microclimates have strong positive effect on the frequency of stem rust epidemics in the highlands of Kenya where most of the wheat is produced.

4.3.3 Land preparation and experimental design

Land preparation was done to a fine tilth to allow uniform crop establishment. The first plough was done two months prior to planting using mould board plough. Second land preparation was carried out using disc harrow one week to planting. Planting was done by hand

and each line was planted in plot of two rows of 1m length by 0.2m width at spacing of 0.4m and at a seed rate of 125kg ha^{-1}. Each entry was separated by 0.3m and 0.5m wide alleyways within and between the blocks, respectively. Mixtures of susceptible wheat cultivars was planted around the trial plot and in the middle of the 0.5m alleyway on both sides of plots to facilitate uniform inoculum build up and serve as spreader. The experimental design used in the three locations was alpha-lattice (23 rows × 3 columns) design with three replications. DAP fertilizer was used during planting at the rate of 50 kg ha^{-1}. Buctril MC (225g L^{-1} Bromoxynil octanoate and 225g L^{-1}MCPA Ethylhexylester), a post emergence herbicide was sprayed at tillering stage at the rate of 7ml L^{-1} of water to control broad-leaved weeds. Insect pest was controlled using Bulldock Duo (225 g L^{-1} Beta-Cyfluthrin) where it was sprayed at the rate of 10 ml L^{-1} of water. The trial was top dressed using urea fertilizer at a rate of 30kg N ha^{-1} at jointing stage. Hand weeding was done twice at stem elongation and booting stages to eradicate grasses.

4.3.4 Screening for Adult Plant Resistance and agronomic traits under field conditions

Adult plant stage assessment was done in the field under natural inoculation in which the spreader rows and field trials across sites acted as natural infection. Assessment 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 into five categories according to Roelfs *et al.* (1992); R = resistant, MR = moderately resistant, MS = moderately susceptible, MSS = moderately susceptible to susceptible and S = susceptible and overlapping responses between two categories were denoted using a dash (—) between the categories for example MR/MS (Figure. 4.1 A). The stem rust severity was determined by use of the modified Cobb's scale where the severities were ranging from 5%-100% (Figure.4.1 B) (Peterson *et al*., 1948).

The number of effective tillers at harvest was determined by taking five samples of wheat per plot randomly and the number of tillers was counted. The height of the plant was taken during harvesting; five samples of the crop were taken from each plot and measured from the base of the plant to the base of a spike using a one-meter ruler. The length of the spike was taken at the end of maturity where five samples were taken from each plot. One-meter ruler was used in measuring the spike length. To determine the number of seeds per spike, five plants per plot were randomly selected. One spike per plant was randomly picked and the number of

seeds determined by counting manually. Thousand kernel weight (TKW) from each plot was counted and weighed using an electronic weighing balance.

Figure 4:1: (A) Roelfs Field Disease Response to Infection Scale (Roelfs *et al.*, (1992)

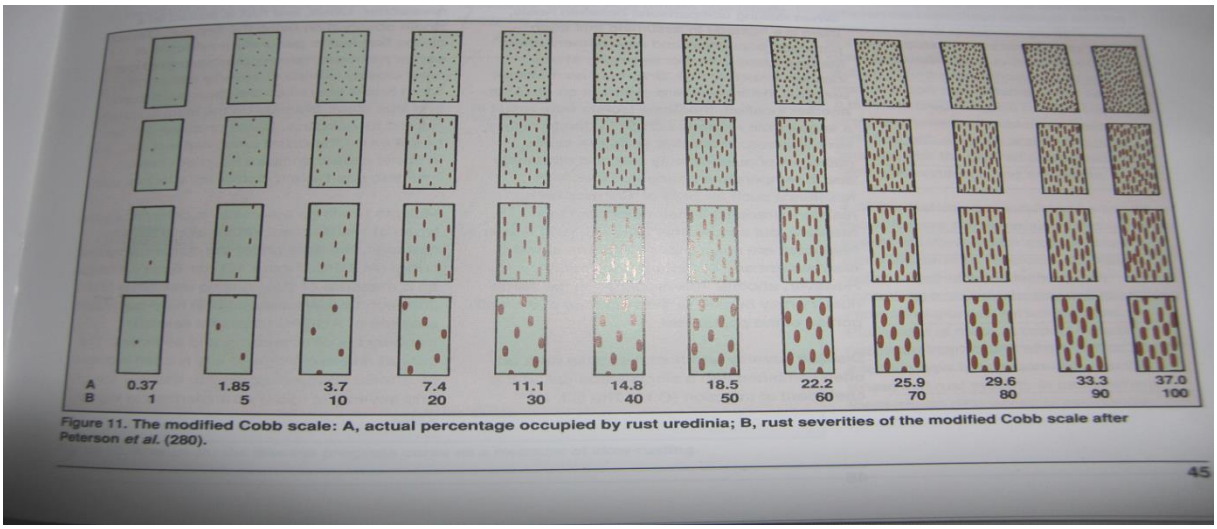

Figure 4:2: (B) The Modified Cobb Scale a; actual percentage occupied by rust urediniospores; b; rust severities of the modified Cobb scale after Peterson *et al.*, (1948).

4.4 Data analysis

The integral model for area under the disease progress curve (AUDPC) function was used to calculate the mean disease severity. AUDPC values were calculated for each plot using the Wilcoxson *et al*., 1975 formula:

AUDPC $= \sum_{I-1}^{n-1} 0.5(Xi + 1 + Xi)\ (ti + 1 - ti)$

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

4.4.1 Statistical analysis

Analysis of variance for area under disease progress curve for disease severity, coefficient of infection and thousand kernel weight across the sites was performed using the Statistical Analysis Software (SAS) version 9.1 procedural PROC GLM (SAS, 2002) with genotypes as the fixed effects while location, replicates and blocks within replicates considered random. The following statistical model was used;

$Y_{ijkl} = \mu + G_i + L_j + R_k + B_{l(k)} + GL_{ij} + \sum_{ijkl}$

Where; Y_{ijkl}= observations; μ= mean of the experiment; G_i= effect of the i^{th} genotype; L_j= effect of j^{th} location; R_k= effect of the k^{th} replicate (superblock); $B_{(l(k))}$= effect of the l^{th} incomplete block within the k^{th} replicate; GL_{ij} = effect of i^{th} genotype in j^{th} location;ε_{ijk}= experimental error. The disease severity scores were transformed using square root(x+1) and means transformed back to original score. The least significant difference was determined at (P<0.05).

4.4.2 Correlation and Regression Analysis

Correlation analysis was conducted to quantify the degree to which the disease level was related to traits associated with wheat yield. Linear regression analysis was also conducted to find the best equation and equation line that had predicted the best traits from disease level. These analyses were performed using SAS program.

4.4.3 Genotype by environment interaction (GEI), disease resistance and stability analysis

A combined analysis of variance procedure was used to identify the existence of disease resistance, genotype*environment interaction (GEI) and stability from replicated multi-location trials. According to Lin *et al.*, 1986 analysis of static stability, genotypic variance (S_i^2) was computed to determine disease resistance and yield stability. The analysis followed the equation;

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

Where, X_{ij}= disease value (AUDPC) 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.

4.5 Results

4.5.1 Analysis of variance and Genotype by Location (GL) Interaction effects.

Of the total variance of coefficient of infection (CI), the site main effect accounted for 30.60%, genotype 23.45% and the site by genotype interaction 22.63%. In regard to AUDPC variation, site was 42.88%, genotype 20.57% and the interaction 16.52% (Table 4.1). For yield and 1000 kernel weight, site main effect was 76.99% and 77.79%, genotype was 7.78% and 4.64% and the interaction accounted for 11.85% and 9.86% of the total variation, respectively (Table 4.1). These results show that CI, AUDPC, TKW and yield were significantly affected by changes in environment.

4.5.2 Mean performance of site, genotypes and genotype by location (GL) interaction regarding to area under disease progress curve (AUDPC) and coefficient of infection (CI)

There was a significant difference among the sixty nine genotypes regarding AUDPC and Coefficient of infection (CI) values (Table 4.1) on the three sites (Mau Narok, Njoro and Timau). Variation in disease was also noted on the test sites during that cropping season. Timau had the lowest disease pressure as evidenced by its mean values of CI (3.04) and AUDPC (6.36), followed by Mau Narok with CI and AUDPC values of 3.79 and 14.15, respectively (Figure 4.2). Njoro had the highest disease pressure with AUDPC and CI values of 14.72 and 5.32, respectively (Figure 4.2). Among the genotypes, the highest mean values for CI were scored on Robin, *Cacuke*, Duma parent, Kwale100gry (1483), NJBWII 200gry (602) and NJBWII 100gry (50) of 7.6, 7.4 and 6.7, 5.0, 5.0, 4.8 respectively across the sites (Table 4.2). The lowest mean values for CI among the genotypes were Duma200gry (1124) and Kingbird (resistant check) in the three sites with 2.6 and 2.8 respectively. The highest AUDPC mean values were recorded on Robin, *Cacuke*, Duma parent, Kwale100gry (1483), Kwale100gry (1556) and NJBWII 100gry (50) and NJBWII parent of 25.5, 22.6, 19.5, 14.8, 14, 13.5, 13.5 respectively (Table 4.2). The lowest mean values for AUDPC among the genotypes in the three sites were recorded on Duma200gry (1026), Duma200gry (1124), Duma400gry (1304), NJBWII100gry (404) and Duma200gry (1030) of 8.8, 8.8, 9.1, 9.3 and 9.3 respectively (Table 4.2).

Table 4:1: Summary for means squares of disease parameters, thousand kernel weight and grain yield of 69 wheat (*Triticum aestivum* L.) genotypes evaluated across three locations in Kenya during 2015-2016 cropping season.

Source of variance	d.f	CI	AUDPC	TKW	Yield t/ha
Site	2	276.0396779***	4514.357504***	0.02600441***	9086.71453***
Rep	2	1.7254750	15.000306	0.00001517	17.87741***
Block(Rep)	6	0.8025722	11.497370	0.00003440**	7.02101**
Genotype	68	6.2734997***	63.690222***	0.00004561***	25.26314***
Genotype×Site	136	3.0883381***	25.578582***	0.00004848***	20.56680***
Error	406	0.994303	9.84922	0.00001213	1.84834
Total		1820.652174	21056.48699	0.06685043	23604.45741
CV%		24.60087	26.71610	10.40681	14.00653
R^2		0.778360	0.810093	0.926344	0.968208

CI; coefficient of infection, AUDPC; area under disease progress curve, TKW; thousand kernel weight. ** represents significance at (P<0.01), *** represents significance at (P< 0.001).

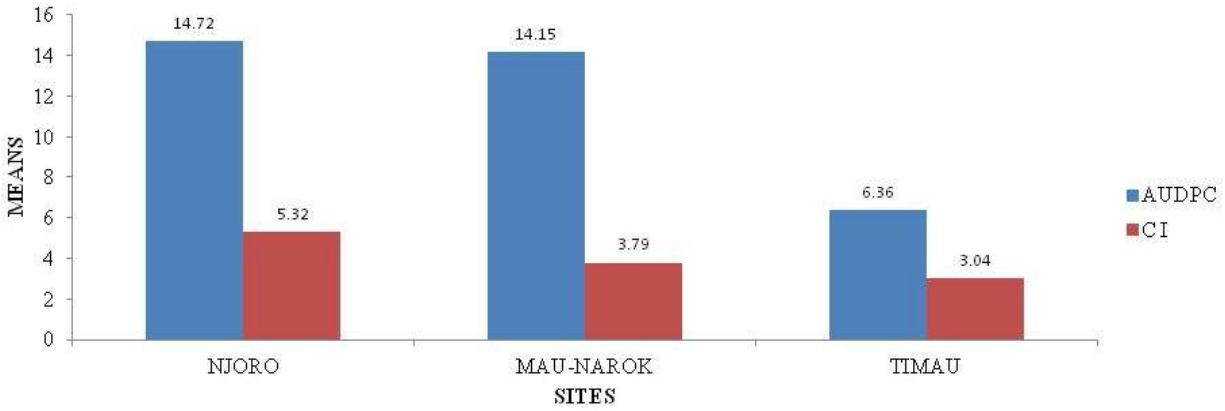

Figure 4.2: Mean performance of the genotypes for AUDPC and CI among the three evaluated sites

Table 4:2: Means of area under disease progress curve and coefficient of infection for the evaluated genotypes in Kenya

	AUPDC				C I			
	Njoro	Mau Narok	Timau	Mean	Njoro	Mau Narok	Timau	Mean
NJBWII100GRY(50)	15.90	18.27	6.27	13.5	6.40	4.87	3.10	4.8

NJBWII100GRY(57)	11.80	11.70	7.03	10.2	4.10	3.00	3.37	3.5
NJBWII100GRY(140)	11.87	12.63	9.83	11.4	3.90	3.47	4.27	3.8
NJBWII100GRY(288)	14.03	13.53	3.67	10.4	5.40	3.67	2.13	3.7
NJBWII100GRY(382)	12.93	11.57	5.47	10.0	4.40	3.00	3.10	3.5
NJBWII100GRY(404)	9.93	9.40	8.63	9.3	4.23	2.83	3.47	3.5
NJBWII100GRY(415)	12.67	14.47	7.43	11.5	4.53	3.87	3.30	3.9
NJBWII200GRY(602)	16.13	18.70	4.03	13.0	6.87	5.53	2.47	5.0
NJBWII200GRY(608)	14.17	12.77	5.47	10.8	6.17	3.60	2.73	4.2
NJBWII200GRY(612)	16.13	15.40	3.07	11.5	6.17	3.87	2.07	4.0
NJBWII200GRY(634)	15.30	15.70	8.03	13.0	6.13	3.90	3.23	4.4
NJBWII200GRY(641)	14.27	14.63	7.10	12.0	4.20	3.67	3.47	3.8
NJBWII200GRY(660)	14.73	13.50	5.90	11.4	6.37	3.90	2.60	4.3
NJBWII200GRY(662)	16.60	14.30	4.80	11.9	6.60	4.07	2.47	4.4
NJBWII400GRY(675)	16.00	17.60	3.67	12.4	6.17	4.87	2.40	4.5
NJBWII400GRY(776)	13.20	9.67	7.87	10.2	4.70	1.57	3.63	3.3
NJBWII400GRY(798)	11.53	13.70	5.10	10.1	4.57	3.60	2.47	3.5
NJBWII400GRY(849)	13.90	10.17	7.60	10.6	5.00	2.67	3.10	3.6
NJBWII400GRY(908)	13.40	11.60	3.67	9.6	4.33	2.93	2.00	3.1
NJBWII400GRY(915)	12.63	11.53	9.83	11.3	4.03	2.53	4.10	3.6
NJBWII400GRY(930)	14.07	14.83	4.10	11.0	5.00	3.93	2.40	3.8
DUMA100GRY(987)	10.53	10.03	9.47	10.0	3.27	3.00	4.47	3.6
DUMA100GRY(992)	12.40	9.90	10.13	10.8	4.27	2.47	4.23	3.7
DUMA100GRY(993)	11.53	10.03	7.27	9.6	3.93	3.37	3.37	3.6
DUMA100GRY(995)	10.40	10.63	10.10	10.4	3.20	2.80	4.30	3.4
DUMA100GRY(996)	11.93	12.17	6.73	10.3	3.80	3.37	3.77	3.6
DUMA100GRY(997)	11.77	12.83	8.37	11.0	3.73	3.60	3.80	3.7
DUMA100GRY(1010)	11.00	11.20	6.83	9.7	4.23	2.87	3.27	3.5
DUMA200GRY(1026)	11.57	10.70	4.10	8.8	3.63	2.90	2.60	3.0
DUMA200GRY(1030)	11.53	13.37	3.07	9.3	5.23	2.93	1.80	3.3
DUMA200GRY(1033)	10.50	11.83	7.83	10.1	3.27	3.00	3.57	3.3
DUMA200GRY(1099)	13.33	10.90	9.23	11.2	4.23	2.97	3.73	3.6
DUMA200GRY(1103)	12.67	12.17	8.57	11.1	4.13	2.80	3.63	3.5
DUMA200GRY(1124)	11.93	10.40	4.10	8.8	3.47	2.33	2.10	2.6
DUMA200GRY(1145)	12.17	13.67	6.23	10.7	4.87	3.73	2.77	3.8
DUMA400GRY(1299)	14.90	11.87	6.27	11.0	4.40	3.13	3.37	3.6
DUMA400GRY(1295)	14.27	15.30	3.27	10.9	5.63	4.07	2.20	4.0
DUMA400GRY(1304)	13.00	7.17	7.10	9.1	5.70	2.33	3.40	3.8
DUMA400GRY(1437)	10.03	9.67	8.47	9.4	3.47	2.80	4.00	3.4
DUMA400GRY(1349)	15.33	13.30	5.90	11.5	5.70	3.53	3.27	4.2
DUMA400GRY(1368)	13.63	13.63	6.23	11.2	5.23	4.23	2.53	4.0
DUMA400GRY(1403)	14.37	11.23	10.30	12.0	4.90	3.77	4.77	4.5
KWALE100GRY(1468)	14.07	13.87	5.47	11.1	5.47	4.00	2.83	4.1
KWALE100GRY(1470)	17.80	16.27	4.87	13.0	6.13	4.23	2.67	4.3

KWALE100GRY(1483)	19.63	16.80	7.87	14.8	6.93	4.43	3.70	5.0
KWALE100GRY(1492)	15.90	13.67	7.70	12.4	5.00	3.90	3.30	4.1
KWALE100GRY(1499)	18.00	12.97	5.47	12.1	6.13	3.83	2.73	4.2
KWALE100GRY(1502)	13.23	16.00	4.27	11.2	5.23	4.30	2.20	3.9
KWALE100GRY(1556)	16.73	17.43	7.70	14.0	6.07	4.77	3.37	4.7
KWALE200GRY(1621)	12.67	14.97	3.67	10.4	5.13	3.90	2.00	3.7
KWALE200GRY(1715)	14.43	18.50	4.70	12.5	5.17	4.87	2.47	4.2
KWALE200GRY(1731)	14.43	20.00	4.87	13.1	5.10	5.57	2.00	4.2
KWALE200GRY(1750)	19.77	13.00	6.23	13.0	6.87	3.63	2.83	4.4
KWALE200GRY(1768)	16.13	16.87	4.43	12.5	5.70	4.23	2.00	4.0
KWALE200GRY(1777)	14.07	15.83	5.90	11.9	6.27	4.63	2.37	4.4
KWALE200GRY(1818)	17.40	16.63	3.87	12.6	6.77	4.67	2.47	4.6
KWALE400GRY(1875)	14.33	18.27	3.07	11.9	5.33	3.87	1.97	4.1
KWALE400GRY(1877)	13.57	16.70	6.67	12.3	4.87	4.33	3.53	4.2
KWALE400GRY(1895)	15.87	13.03	3.23	10.7	6.17	3.43	2.07	3.9
KWALE400GRY(1907)	15.57	12.90	5.47	11.3	5.93	3.27	2.67	4.0
KWALE400GRY(1949)	14.07	12.00	8.47	11.5	5.33	3.40	3.57	4.1
KWALE400GRY(1961)	13.83	10.63	5.20	9.9	5.13	2.60	3.13	3.6
KWALE400GRY(1964)	16.57	10.90	9.57	12.3	4.63	2.93	4.40	4.0
NJBWIIPARENT	16.70	16.27	7.43	13.5	7.43	4.13	3.40	5.0
DUMA PARENT	25.60	22.93	9.83	19.5	8.97	7.07	4.10	6.7
KWALEPARENT	15.67	16.23	5.47	12.5	6.83	4.27	2.73	4.6
KINGBIRD	16.47	12.20	2.63	10.4	5.07	2.07	1.37	2.8
CACUKE	28.83	28.53	10.47	22.6	9.67	8.17	4.37	7.4
ROBIN	34.17	35.93	6.50	25.5	10.00	10.00	2.73	7.6

4.5.4 Genotypic performance stability across the sites

Genotype stability was measured using area under disease progress curve and yield. In this case, Duma100gry (995) and Kwale100gry (1483) were the most stable genotypes among

the mutant lines and checks, respectively. Duma100gry (992), Duma100gry (995) and Duma100gry (1033) appeared the outstanding most stable genotypes on both AUDPC and yield evaluated across the sites (Table 4.3and 4.4).

Table 4:3: The stem rust (*Puccinia graminis* f. sp. *tritici*) Area Under Disease Progress Curve(AUDPC), and stability values for the wheat (*Triticum aestivum* L.) genotypes that proved better than the resistant and susceptible check as evaluated across three locations in Kenya

		AUDPC		
No	**GENOTYPES**	**MEAN**	S^2	**Stability rank**
1	DUMA100GRY(995)	10.4	0.1	1
2	DUMA100GRY(987)	10.0	0.3	2
3	NJBWII100GRY(404)	9.3	0.4	3
4	DUMA400GRY(1437)	9.4	0.7	4
5	DUMA100GRY(992)	10.8	1.9	5
6	NJBWII400GRY(915)	11.3	2.0	6
7	NJBWII100GRY(140)	11.4	2.1	7
8	DUMA200GRY(1033)	10.1	4.1	8
9	DUMA200GRY(1099)	11.2	4.3	9
10	DUMA400GRY(1403)	12.0	4.5	10
MEANS	**11.1**			
CV%	**26.7**			
$LSD_{0.05}$	**2.91**			

CV; coefficient of variation, LSD; Least significant difference

Table 4:4: The stem rust (*Puccini graminis* f. sp. *tritici*) Yield and stability values for the wheat (*Triticum aestivum* L.) genotypes that proved better than the resistant and susceptible check as evaluated across three locations in Kenya.

		YIELD		
No	**GENOTYPES**	**MEAN**	S^2	**Stability rank**
1	KWALE100GRY(1483)	6.1	5.3	1
2	DUMA200GRY(1033)	9.6	5.8	2
3	KWALE200GRY(1621)	5.5	9.6	3
4	DUMA100GRY(996)	10.2	10.1	4
5	DUMA200GRY(1124)	8.1	15.2	5
6	DUMA100GRY(995)	9.7	15.3	6
7	DUMA100GRY(992)	8.0	16.9	7
8	NJBWII100GRY(140)	10.9	18.6	8
9	KWALE400GRY(1964)	11.5	20.3	9
10	KWALE400GRY(1895)	7.6	22.5	10
MEANS	**9.71**			
CV%	**14.00**			
$LSD_{0.05}$	**1.26**			

CV; coefficient of variation, LSD; Least significant difference

4.5.4 Correlation and regression analysis

The Pearson correlation analysis showed that there was negative correlation between yield, 1000 kernel weight and disease parameters. Yield and 1000 kernel weight displayed negative correlation to AUDPC and CI respectively (Table 4.5).

The regression equation revealed that for every percent increase in disease severity and AUDPC there was yield loss of -1.1803 and -0.59, respectively. R- Square values computed for the genotypes revealed that 0.1508 and 0.3911 of the variation in yield was contributed by the disease severity and AUDPC, respectively (Figure 4.3 and 4.4).

Table 4:5: Correlation between yield, kernel weight and the disease parameters of the wheat genotypes evaluated for stem rust during 2015-2016 main season.

Variables	Kernel weight	Yield	AUDPC	C I
Kernel weight	-	0.85768***	-0.63528***	-0.43051***
Yield		-	-0.62588	-0.44027***
AUDPC			-	0.8445***
C I				-

******* Negative relationship between the variables at (P<0.001) significant difference, AUDPC; area under disease progress curve, CI; coefficient of infection.

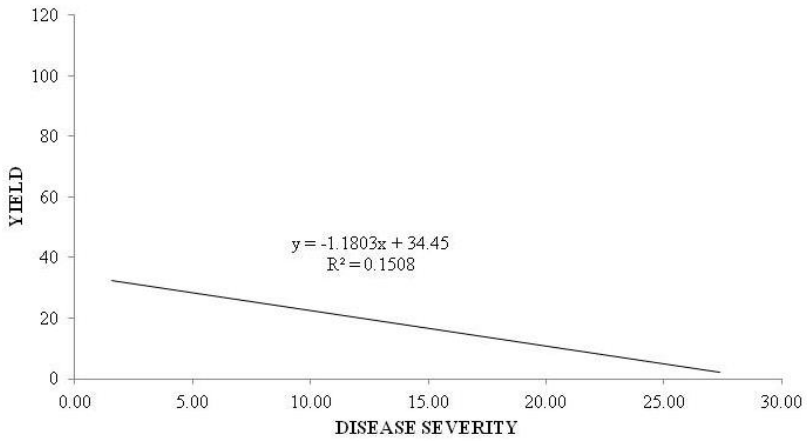

Figure 4:3: Association of yield with disease severity of the evaluated wheat genotypes across three sites

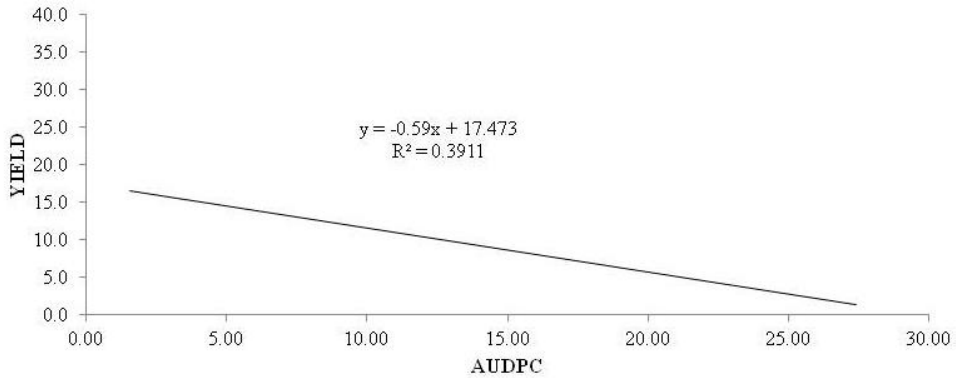

Figure 4:4: Association of yield with AUDPC of the evaluated wheat genotypes across three sites

4.6 Discussion

There was a wide variation on disease responses among the evaluated wheat genotypes as evidence by the area under progress curve and coefficient of infection mean values. Some of these disease means for the mutant lines corresponded closely to the means of their parents. Similar variations in stem rust response among wheat genotypes had previously been reported (Tabassum, 2011; Macharia and Wanyera, 2012). Singh *et al*. (2011) reported significant genetic variability in disease response among various genotypes in different environments. Present results showed that some mutants exhibited high disease mean values hence highly susceptible; for instance, Kwale 100gry (1483) and NJBWII 100gry (50). Some were noted with low disease mean values hence moderately resistant like Duma 200gry (1124), Duma 200gry (1026) and Duma 400gry (1304). These variations in the infection type were observed across the three sites. These genotypes which were noted having moderate resistance response could be probably carrying minor genes.

In regard to sites, Njoro and Mau Narok were noted having high mean values on AUDPC and CI as compared to Timau by the evaluated genotypes. This indicated maximum disease pressure was reached during the cropping season. This was also evident by the high infection noted on the susceptible spreader showing that high disease pressure was reached across the three sites. This might be due to their difference in geographical location, crop management practices and type of cultivars grown. Moreover, the high disease record in these study areas may be due to the conduciveness of the environmental conditions, cultivation of susceptible cultivars and possibly occurrence of high spore load in the atmosphere in the regions.

There was variability of yield that existed across the evaluated genotypes and locations. This could be attributed to diverse genetic backgrounds of the genotypes, climatic conditions of location, disease pressure in that particular area and genotype*location (GL) interaction effects. The present study noted greater effect of improved yield mainly on Timau location and mutants with dosage of 400 gry. For instant, Duma400gry (1295) and Duma400gry (1299) had the highest mean values (14.14 and 13.24) for yield compared to their Duma parent (9.88) mean values across the sites.

There was effect of improved yield noted on the evaluated genotypes despite high or low levels of stem rust disease attack; for instance, Duma400gry (1295) and Duma400gry (1299) had the highest mean values (14.14 and 13.24) compared to their Duma parent (9.88) mean values across the sites. Contrary, Kwale200gry (1621), Kwale100gry (1483) and Kwale100gry (1492) had the least yield means (5.54, 6.13 and 6.93) which did not deviate

much from their Kwale parent (8.9) (data not shown). This shows that after irradiation chromosomes carrying genes for those traits may have been altered in the process. Besides, the parents (Duma, Kwale and NJBWII) were among the old varieties bred in the late 90s and were part of the first varieties noted to be susceptible to the new *Ug99* stem rust race (Njau *et al*., 2009) and consequently low yield.

Nonetheless, Timau had the highest yield mean values while Njoro had the least, thus the difference in yield among the sites could be attributed to both environmental conditions and difference in stem rust infection. Timau had low disease pressure and subsequent high yield from the evaluated genotypes. Previous studies showed that the high disease scores obtained led to low yield in Njoro since stem rust has a high prevalence in this region. Environmental conditions such as temperature and moisture considerably affect disease expressions and consequently of yield. The existence of such variation enables the breeder to select both high yielding and disease resistant genotypes across different sites. Moreover, gamma rays in particular are important physical mutagen which is well known with their effects on the plant growth and development by inducing physiological, morphological and cytological changes in cells and tissues. Singh *et al.,* (2008) reported that stem rust reduces grain yield of wheat cultivars, which is due to the injury on the photosynthetic surface of the plant (Berghaus and Reisener 1985). This leads to more energy expenditure impacted on plant defense mechanisms rather than for growth and grain formation (Boyle and Walters, 2005; Walters and Heil, 2007).

As regards to Roelfs, (1985) findings, the fungus also reduces the food and water supply within the plants. For spore formation and production, the fungus needs food and water that would otherwise be used in the formation of well developed kernels. Additionally, the fungal pustules could cause loss of water by evaporation through numerous ruptures of the plant epidermis. Reduction in kernel weight of the genotypes could be attributed to heavy stem rust disease pressure experienced in that cropping season. Due to this, the heavily rusted plants had low yield, poor grain quality with shriveled seeds like “Robin”. Studies by Nzuve *et al.* (2012), stem rust significantly reduce kernel weight in wheat when there is severe attack in the field. Presently, it was obvious that the affected genotypes had shriveled seeds resulting to reduced kernel weight due to high disease attack especially to the prone area in Njoro. During grain filling the fungus (rust) usually competes for photosynthesis resulting to reduced number and size of seeds on the plants (Agrios, 1988). This led to a conclusion that the significant effect of stem rust on 1000 kernel weight of the genotypes was brought about by its effect on photosynthesis and grain filling.

According to Francis and Kannenburg (1978) stability analysis for type one concept of stability indicated that genotypes are considered stable if they are among environment variance are small. In this case the genotypes like Duma100gry (992), Duma100gry (995) and Duma100gry (1033) were considered stable. These genotypes are useful to farmers because they would give consistent varieties that can withstand unpredictable and transient environmental fluctuations. In addition, the preference of breeder and farmers is high yielding and stable varieties, hence yield stability and adaptability is an important concept in wheat breeding. Nevertheless, the best yield is always accompanied with minimal disease of which most pathologists work on to get best lines.

A strong negative correlation between the disease parameters, yield and 1000 kernel weight was noted. This showed that there was a negative impact where an increase in disease resulted to decrease in yield and vice versa. There was a weak relationship between the disease and yield consequently the variation in yield was assumed not only due to disease response but also due to location and genotype*location interaction variability.

4.6 Conclusion

The study concluded that mutation breeding is necessary to enhance genetic variability in Kenyan wheat varieties so that to achieve durable and broad spectrum resistance in disease and best yield. Results of this study revealed existence of variation in stem rust resistance and grain yield among the evaluated the mutant lines. Moreover isolation of mutants with multiple traits was also involved which would be ideal to grow under different environmental conditions. With respect to disease and yield performance, mutant lines Duma 200gry (1124), Duma200gry (1026) and Duma400gry (1304) portrayed moderate resistance while Duma400gry (1295) and Duma400gry (1299) performed best (disease and yield) in the locations thus they are recommended for further screening in the breeding programs. Therefore, the national wheat breeding stations to can carry out more mutagenesis work to develop new varieties that could be readily adapted on different locations with varying agro-climatic and growing conditions and low available resources.

4.7 References

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

Agrios, G. N. (1988) *Plant Pathology*, 3rd Edition. London, New York, Academic Press.

Berghaus, R. and Reisener, H. J. (1985) Changes in photosynthesis of wheat plants infected with wheat stem rust (*Puccinia graminis* f.sp. *tritici*). *Journal of Phytopathology* 112:165–172.

Boehm, E. W. A., Wenstrom, J. C., McLaughlin, D. J., Szabo, L. J., Roelfs, A.P. and Bushnell, W. R. (1992). An ultrastructural pachytene karyotype for *Puccinia graminis* f. sp. *tritici. Canadian Journal of Botany,* 70, 401–413.

Boyle, C. and Walters, D., (2005). Induction of systemic protection against rust infection in broad bean by saccharin: Effects on plant growth and development. *New Phytopathology*. 167, 607 and 612.

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

Francis, T. R., and 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.

Jaetzold, R., Schmidt, H., Hornetz, B., and Shisanya, C. (2010). *Farm management Handbook* Vol.II, 2nd Edition. Part B Central Kenya. Subpart B1a: Southern Rift Valley.

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

Macharia, J. K. and Wanyera, R. (2012). Effect 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.

Maluszynski, M. and Szarejko, I. (2003). Induced mutations in the green and gene Revolutions. In: Tuberosa R., Phillips R.L., Gale M. (eds.), *Proceedings of the International Congress* (pp. 403-425) *"In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution.*

McIntosh, R. A., Hart, G. E., Devos, K. M., Gale, M. D., and 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.

Njau P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R and 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 (2), 022-027.

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

Nzuve, F.M., Bhavani, S., Tusiime, G., Njau, P. and 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.

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

Parry, M. A. J., Madgwick, P. J., Bayon, C., Tearall, K., Lopez, A. H., Baudo, M., Rakszegi, M., Hamada, W., Yasin, A. A., Quabbou, H., Labhilili, M. and Phillip, A. L. (2009). Mutation discovery for crop improvements. *Journal Expert of Botany*, 60 (10), 2817-2825.

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

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

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

Roelfs, A. P. (1985). Wheat and rye stem rust. In Roelfs A.P, and Bushnell, W.R., (eds). *The Cereal Rusts Diseases, Distribution, Epidemiology, and Control* (Pp. 3-37). Academic Press, Orlando.

Roelfs, A. P., Singh, R. P. and Saari, E. E.(1992). *Rust Diseases of Wheat: Concepts and Methods of Disease Management* (pp 81). D.F. CIMMTY, Mexico.

Saari, E. E. and Prescott, J. M. (1985). World distribution in relation to economic losses. *"The Cereal Rusts Vol. II. Diseases, Distribution, Epidemiology, and Control"* (Roelfs, A. P., and Bushnell, W.R. (eds) (pp. 259-98). Academic Press, Orland.

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

Singh, R. P., William, H. M., Huerta-Espino, J. and Rosewarne, G. (2004). Wheat rust in Asia: Meeting the challenges with old and new technologies. In ''New Directions for Diverse Planet. *Proceedings of the 4th International Crop Science Congress''*

http://www.Cropscience.org.au./icsc2004/symposia/3/7/141_singhrp.htm. September 26–October 1, 2004. Brisbane, Australia.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M., Wanyera, R., Njau, P. and Ward, R. W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources,* 1, 54.

Singh, R. P, Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S. A and Ward, R.W (2008). Will Stem Rust Destroy the World's Wheat Crop? *Advance Agronomy,* 98, 271-309.

Singh, R. P., Hodson, D. P., Huerta – Espino, J, Jin, Y., Bhavani, S., Njau, P., Herrera – Foessel, S, Pawan, Singh, K., Singh, S. and Govindan, V. (2011). The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production. *Annual Review of Phytopatholothy,* 49, 465 – 482.

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.

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

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

Wilcoxson, R. D., Skovmand, B. and Atif, A. H. (1975).“Evaluation of wheat cultivars ability to retard development of stem rust.” *Annuals of Applied Biology,* 80, 275-2181.

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

CHAPTER FIVE

VARIABILITY IN STEM RUST SEVERITY OF THE WHEAT MUTANT LINES ACROSS AGRO-ECOLOGICAL ZONES IN KENYA

5.1 Abstract

Stem rust severity and host response variability are affected by the interaction of the host, pathogen and the climatic conditions. The objective of this study was to evaluate the variability in stem rust severity of the wheat mutant lines across different locations. In the current study, mutation breeding was used to create variation in stem rust races resistance. Wheat varieties seeds (Njoro BWII), Kwale and Duma-M_0) were sent to Vienna in Austria for irradiation at the International Atomic Energy Agency at dosage of 100, 200 and 400 grays (gry). The mutant lines M_1-M_6 were planted in the stem rust screening blocks at Kenya Agriculture and Livestock Research Organization (KALRO)-FCRC, Njoro. Afterwards, they were evaluated, selected and harvested based on the quantitave traits like disease, height, maturity and yield. In 2015-2016 cropping season, sixty three selected M_7 lines together with six checks were screened for stem rust at three sites in alpha-lattice design with three replications. High significant level of stem rust severity variability ($P<0.001$) occurred across locations and among the tested genotypes. Host infection types produced by the tested genotypes were between Resistant Moderate Resistant (RMR) and Moderate Susceptible-Susceptible (MMS), except for *Cacuke*, Robin and Duma Parent, where they had Susceptible (S) infection type. Variability in the host response could be attributed to the various races expression together with different climatic conditions during that season. Therefore, it is imperative to consider mutation breeding for continual screening of these mutant lines for effective resistant genes as the present races have become more virulent to the released lines.

5.2 Introduction

Stem rust caused by the fungus *Puccinia graminis* has acquired much attention due to the danger it displays to the global wheat productions. Since *Ug99* and the variants detection, its variants have been discovered in Eastern and Southern African countries, Yemen, Iran, and Egypt. So far, eight races belonging to the *Ug99* lineage are known including TTKSK, TTKSF, TTKST, TTSK, TTKSP, PTKSK, PTKST and TTKSF+ (Singh *et al.,* 2015). According to Singh *et al.* (2008), variability in virulence at varying locations has also been reported for stem rust races. This has led to increased pathogen monitoring activities which has resulted in identification of other races in Africa and Asia with additional virulence to commercially important resistance genes (Singh *et al.,* 2015).

In Kenya, wheat is the second most significant crop after maize and contributes extensively to food security (Singh *et al*., 2006). Upon dependence greatly on rain-fed agriculture and climatic conditions found at altitudes of 1,500 masl, yield in Kenya is low as compared to the world's top grower China, which produces 120 million metric tonnes a year (Aquino *et al.,* 2002). Wheat requires optimum amount of rainfall with fair distribution during the growth period. In some highlands of Kenya such as Timau and Mau-narok, rainfall distribution is bimodal, main rainy season and short rainy season which ranges between 600-2000 mm per annum. The crop is mostly cultivated in both seasons especially in Njoro area. Though the seasons differ, the crop growth stage overlaps in most cases. This creates favorable condition for stem rust urediniospores to be moved from one season to another and leads to the development of epidemics in wheat growing areas (Schumann and Leonard, 2000). Some of the major wheat growing areas in Kenya include: Rift-valley and central regions of Uashin Gishu, Narok, Marakwet, Elgeyo marakwet, Londiani, Molo, Nakuru and Timau areas. In view of the variability across location in climatic conditions and race population in terms of virulence, the host response resulting from host-pathogen interaction in the field needs to be studied at different locations.

Through resistance, it will be possible to understand the interaction of the evaluated genotypes and races and also the variability across locations (Xi *et al.,* 2003). Variability in disease severity and host infection types across different environments could provide an overall guide reflecting the field resistance expression as varied across locations. Similarly, it could provide an idea about the relative variability in race population, coupled with environmental conditions, when the same set of genotypes is tested.

Although varieties that could offer some resistance have been developed, the rust pathogen keeps mutating into virulent forms. Therefore, there is need to deploy varieties that offer durable and broad-spectrum resistance (Admassu *et al.,* 2012). Identification and promotion of new stem rust resistant varieties that will significantly enhance yield potential than current varieties in conjunction with other desirable traits would be the best strategy (Rouse and Jin, 2011). This will ensure their fast adoption and thus succeed in replacing the existing susceptible varieties. This is an achievable objective as most of the current popular varieties were developed during early to mid 1990s and yield potential of new spring wheat germplasm has progressed significantly since then (Njau *et al*., 2009).

Testing of new wheat lines with adequate resistance to *Ug99* in various countries has indicated that new wheat varieties with higher yields than current varieties can be a reality. High emphasis is currently being given to seed multiplication of these wheat lines (CIMMYT, 2005). Due to this, it is important to employ more breeding programmes such as mutagenesis being one of them to check on the disease resistance as well as yield potential of the breeding lines at every stage (Parry *et al*., 2009). This will ensure that novel sources of resistance to the emerging strains of the pathogen as well as good yield potential are identified, gathered and utilized.

Variability in disease severity and host infection types across different environments could provide an overall guide reflecting the field resistance expression varying across locations. Similarly, it could provide an idea about the relative variability in races population, coupled with environmental conditions, when the same set of genotypes is tested. The objective of the study was to determine the variability of stem rust disease severity of the wheat mutant lines across different agro-ecological zones in Kenya.

5.3 Materials and Method

5.3.1 Research material

Sixty three mutant lines in the experiment were developed from three selected Kenyan wheat varieties (Njoro BWII, Kwale and Duma). The three parental cultivars had been previously screened for stem rust resistance in the International Screening Nursery at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Kenya. The three parental wheat cultivar seeds were sent to International Atomic Energy Agency in Vienna, Austria and subjected to gamma irradiation at three levels of 100, 200 and 400 gry to get the sixty three mutant lines. Three parental cultivars of NjoroBWII/NJBWII, Kwale, and Duma, the resistant wheat cultivar Kingbird (Njau *et al*., 2010), and the two susceptible cultivars Robin and Cacuke were also included in the experiments.

5.3.2 Area of study

The study was conducted during the 2015 cropping season across three locations in Kenya namely Kenya Agricultural and Livestock Organization (KALRO), Njoro; Mau Narok in Nakuru county and Timau in Meru county. Njoro station is located at 0 20′S, 35° 56′E and 2185 m elevation, with daily average temperature ranging from 9.7ºC (night) and 23.5ºC (day). Variations of daily temperatures in this area is approximately + 2ºC occurring mostly during the day hours, and receives on average 939 mm of precipitation annually. The soil type is predominantly Mollic Andosols. Mau-narok is situated 0° 39′S, 35°57′ E at 2,900 MASL and annual rainfall of 1,200 to 1,400 mm, minimum and maximum temperatures ranging from 6 to 14ºC and 22 to 26ºC, respectively. Timau is situated 0° 5'S, 37° 20'E at 2640 MASL and annual rainfall of 896 mm, minimum and maximum temperatures of 5°C and 23°C respectively. The sites fall within agro ecological zone III (Jaetzold *et al*., 2010). These areas have been described as 'hot-spot' for stem rust epidemics (Saari and Prescott, 1985). These microclimates have strong positive effect on the frequency of stem rust epidemics in the highlands of Kenya where most of the wheat is produced.

5.3.3 Experimental procedure and design

Land preparation was done to a fine tilth to allow uniform crop establishment. The first plough was done two months prior to planting using mould board plough. Second land preparation was carried out using disc harrow one week to planting. The experimental design used in the three locations was alpha-lattice (23 rows × 3 columns) design with three replications.

5.3.4 Planting and Field Management

Planting was done by hand and each line was planted in plot of two rows of 1m length by 0.2m width at spacing of 0.4m and at a seed rate of 125kg ha^{-1}. Each entry was separated by 0.3m and 0.5m wide alleyways within and between the blocks, respectively. Mixtures of susceptible wheat cultivars was planted around the trial plot and in the middle of the 0.5m alleyway on both sides of plots to facilitate uniform inoculum build up and serve as spreader. DAP fertilizer was used during planting at the rate of 50 kg ha^{-1}. Buctril MC (225g L^{-1} Bromoxynil octanoate and 225g L^{-1}MCPA Ethylhexylester), a post emergence herbicide was sprayed at tillering stage at the rate of 7ml L^{-1} of water to control broad-leaved weeds. Insect pests were controlled using Bulldock Duo (225 g L^{-1} Beta-Cyfluthrin) where it was sprayed at the rate of 10 ml L^{-1} of water. The trial was top dressed with urea at a rate of 30kg N ha^{-1} at jointing stage. Hand weeding was done twice at stem elongation and booting stages to eradicate grasses. Adult plant stage assessment was done in the field under natural inoculation in which the field trials across sites acted as natural infection.

5.4 Data collection

5.4.1 Screening for stem rust severity and host infection type in the field

Stem rust severity estimate which is the proportion of the stem of a plant affected by the disease was assessed at weekly interval from the time of disease symptom appearance to physiological maturity of the crop. Severity was recorded ranging from 5%−100% using the modified Cobb's scale (Peterson *et al*., 1948) by assessing randomly the plants per plot. The average stem rust severity from the plants of each plot was used for analysis. During disease assessment, the growth stage of the crop was recorded to observe onset and progress of the disease in relation to wheat phenology. Crop growth stage was assessed based on the decimalized key developed by Zadoks *et al*. (1974).

The infection type was assessed on plant growth stage from milk to early dough stage (Zadok's growth stage 75 to 85) (Zadoks *et al*., 1974) of grain development. The plant response to infection was classified into five categories according to Roelfs *et al.* (1992); R = resistant, MR = moderately resistant, MS = moderately susceptible, MSS= moderately susceptible susceptible and S = susceptible and overlapping responses between two categories was denoted using a dash (−) between the categories.

5.5 Data Analysis

Total disease severity data of the genotypes across the sites was statistically analyzed using Statistical Analysis System (SAS) Version 9.2 software (SAS, 2002). ANOVA was performed using general linear model (GLM) of SAS procedure and means separated using Least Significant Difference (LSD).

5.6 Results

5.6.1 Host infection types among the tested wheat genotypes

Different wheat genotypes showed varying degree of resistance and susceptibility across the three sites. Out of the total evaluated genotypes, there were no genotypes that were noted to be immune or completely resistant to the races across the sites (Figure 5.1). However, in Timau and Mau-Narok one genotype (1.5%) and two genotypes (2.96%) were noted having RMR and MR reactions, respectively Figure 5.1. For M (MR/MS) reaction, in Njoro there were eight (11.63%) genotypes, five (7.3%) genotypes in Mau-narok and six (8.7%) genotypes in Timau (Figure 5.1). For MS, MSS and S category, the genotypes noted to have the reactions were twenty four (34.4%) in Njoro, thirteen (18.8%) in Mau-Narok and sixty two (89.96%) in Timau, (thirty four (49.3%) in Njoro, fourty six (66.7%) in Mau-Narok and none (0%) in Timau and (three (4.3%) in Njoro, three (4.3%) in Mau-narok and none (0%) in Timau respectively (Figure 5.1).

Examples of some genotypes which exhibited the reactions above include; Kingbird (check) which exhibited resistant to moderately resistant response (RMR) in Timau (Figure. 5.2 A), while in Mau-Narok it displayed MR reaction. Duma 200 gry(1124) displayed MR reaction in Mau-Narok. Duma parent Figure.5.2 B, Robin and Cacuke that were used as checks (Figure.5. 2 C and D) were susceptible in Njoro and Mau-Narok. For MR/MS reaction in Njoro, there were eight genotypes, for example Duma 200 gry (1124) (Figure.5.2 E) Mau-Narok had five genotypes and Timau five genotypes. The other genotypes recorded low response of moderately susceptible (MS), for example NJBWII 100 gry (404) (Figure.5.2 F) which was mostly in Timau.

5.6.2 Stem rust disease severity among the tested genotypes

Out of sixty nine genotypes, eight (11.6%) genotypes were noted having severity score of < 5 in Timau only (Figure 5.3). Ten genotypes (14.5%) were noted having severity scores of >10-20 in Njoro, forty eight (69.6%) in Mau-Narok and fifty six (81.2%) in Timau (Figure 5.3). In the category of 25-45 severity scores, there were thirty nine (57.9%) genotypes in

Njoro, eighteen (26.1%) and five (7.3%) genotypes in Mau-Narok and Timau respectively (Figure 5.3). With respect to 50-75 severity category, there were sixteen (23.2%) genotypes in Njoro, two (2.9%) genotypes in Mau-Narok and none was noted in Timau (Figure 5.3). In severity category of 80-100, three (4.4%) genotypes were noted in Njoro, two (2.95%) genotypes in Mau-Narok and none in Timau (Figure 5.3).

Considerably, less disease variation was observed on the most mutant lines compared to their parents. For instance, in NJBWII genotypes, most mutants severity scores for example, NJBWII 100gry (404) had lower severity scores (20%, 10% and 15%) compared to the parent scores of (65%, 25% and 15%) in Njoro, Mau-Narok and Timau respectively (Table 5.1). For Kwale genotypes, mutants like Kwale100gry (1483) had (55%, 25% and 20%) and Kwale200gry (1818) had (55%, 30% and 10%) severity scores which did not deviate much from their parent scores (55%, 30% and 15%) in Njoro, Mau-Narok and Timau respectively (Table 5.1). Duma mutant lines had much lower severity scores for instant, Duma200gry (1124) had (20%, 10% and 10%) and Duma 200gry (1033) had (20%, 10% and 15%) compared to the parent scores of (80%, 65% and 30%) in Njoro, Mau-Narok and Timau respectively (Table 5.1).

In addition, the tested genotypes in Njoro and Mau-Narok were highly significant in terms of stem rust severity at ($P<0.001$) while Timau was significant at ($P<0.01$) (Table 5.2). Of the total variance of the sites, genotypes and genotype-site interaction effects accounted for 17.52%, 0.27% and 0.16% of the total variation respectively (Table 5.3).

Mean severity scores revealed that Njoro had the highest disease severity followed closely by Mau-Narok and Timau had the least disease severity scores (Figure 5.4). In addition, *Cacuke* and Robin displayed highest disease severity especially in Njoro and Mau-Narok than Timau while Kingbird displayed low severity across the sites (Figure 5.5). Significantly, among the mutant lines for example, Duma 200gry (1124) and Duma 200gry (1026) were noted to have low stem rust disease severity while Kwale 100gry (1483) and NJBWII 100gry (50) exhibited high severity scores (Figure 5.5).

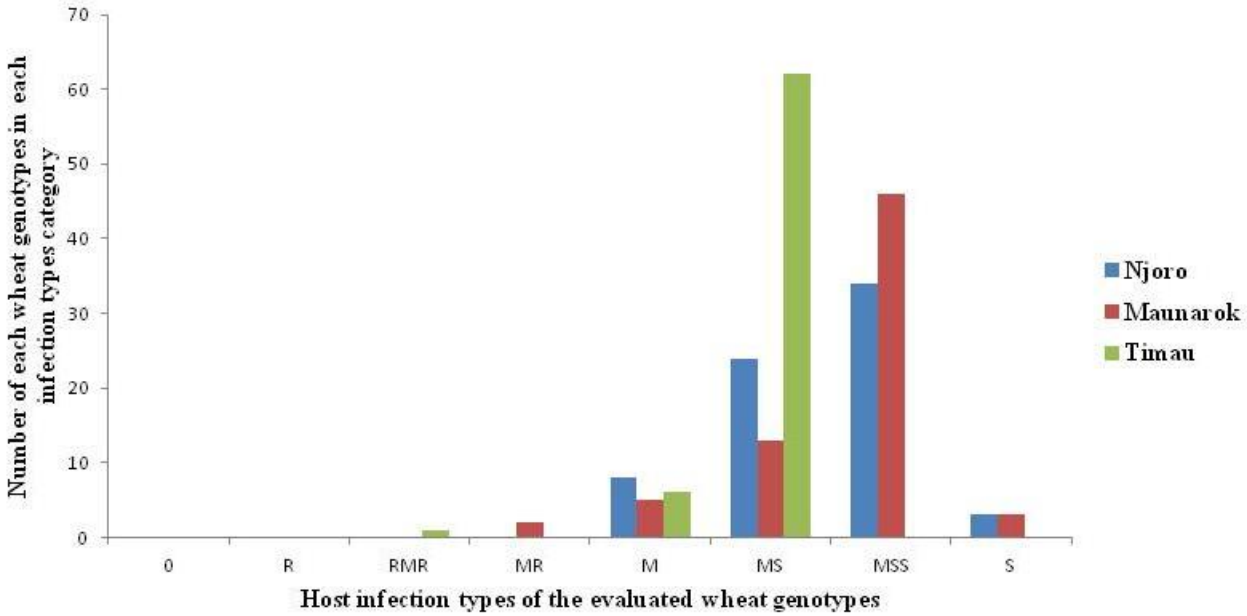

Figure 5:1: Number of wheat genotypes in different categories of stem rust reactions at Njoro, Mau-Narok and Timau sites in 2015 -2016 cropping season.

Figure 5:2: A: Kingbird (5% Resistant moderate resistant (RMR); B: Duma variety (60% Susceptible (S); C: Cacuke (100% Susceptible); D: Robin (90% Susceptible); E: Duma 200gry (1124) (10% Moderate resistant- moderate susceptible (MR/MS or M); F: NjoroBWII 100gry (404) (15% Moderate susceptible (MS).

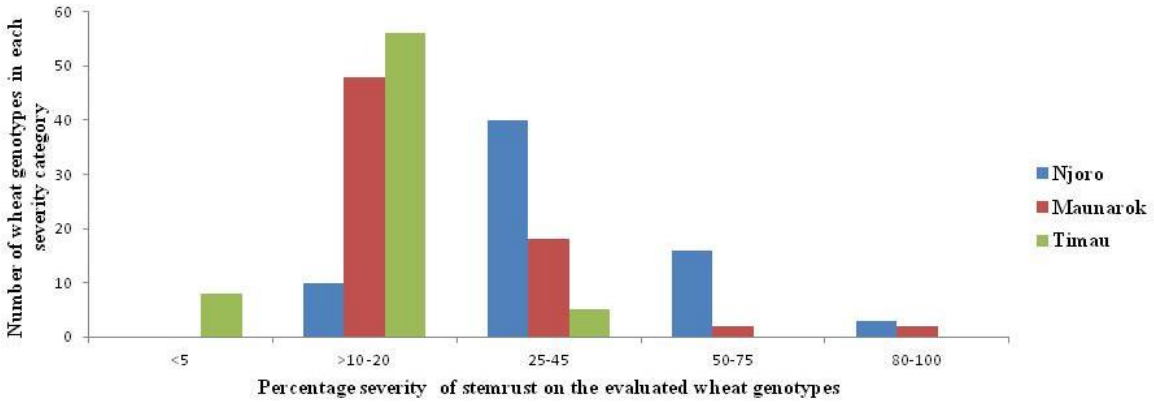

Figure 5:3: Wheat genotypes evaluated in Njoro, Mau-Narok and Timau and the stem rust severity categories.

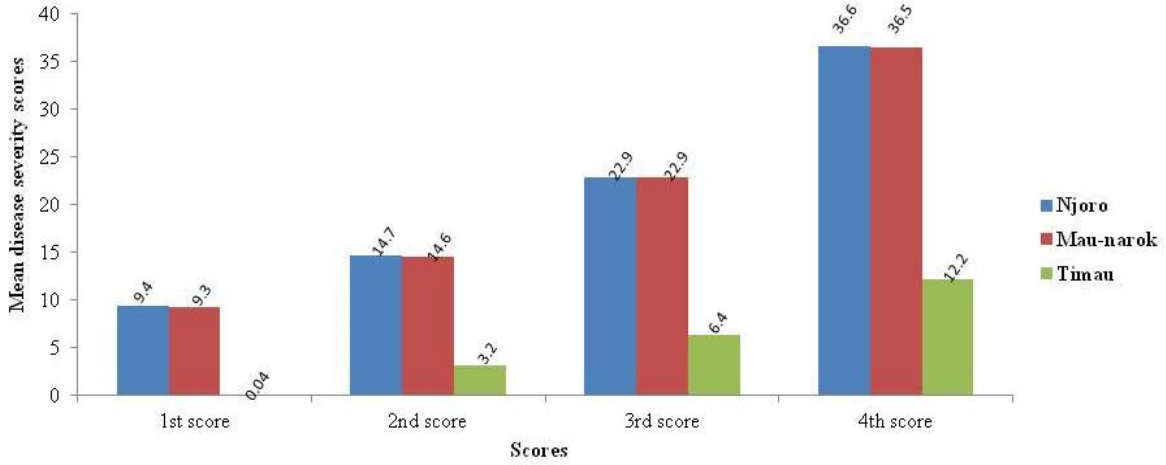

Figure 5:4: Mean performance of the genotypes on four taken severity scores during the 2015-2016 cropping season across the three sites.

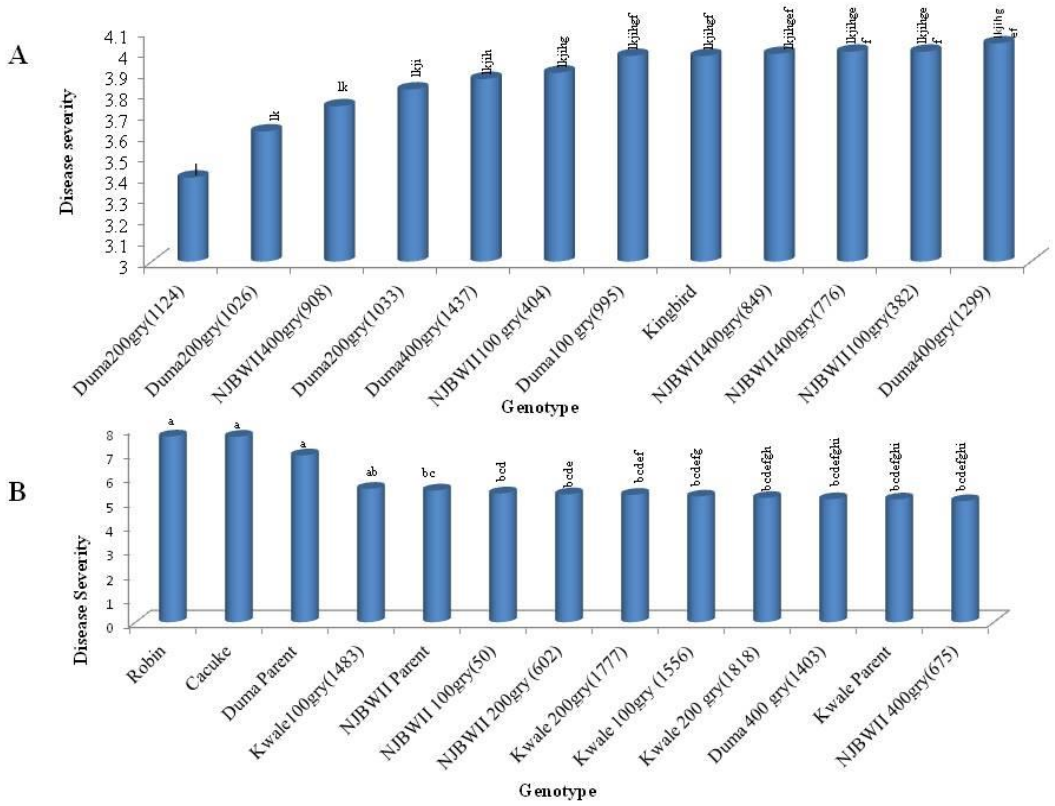

Figure 5:5: Disease severity performance of some genotypes during 2015-2016 cropping season. Significantly, the genotypes selected showed Moderate Resistant (A) and Susceptible(B)

Table 5:1: Summary of observed final disease severity scores and infection types of the evaluated genotypes across the locations.

Genotypes	Njoro		Mau-narok		Timau	
	Final disease severity	Host infection types	Final disease severity	Host infection types	Final disease severity	Host infection types
NJBWIIPARENT(CHECK)	**65**	**MSS**	**25**	**MSS**	**15**	**MS**
NJBWII100GRY(50)	50	MSS	30	MSS	15	MS
NJBWII100GRY(57)	20	MS	15	MSS	15	MS
NJBWII100GRY(140)	30	MS	15	MSS	20	MS
NJBWII100GRY(288)	40	MS	20	MSS	5	MS
NJBWII100GRY(382)	25	MS	15	MSS	15	MS
NJBWII100GRY(404)	20	MS	10	MS	15	MS
NJBWII100GRY(415)	30	M	20	MSS	15	MS
NJBWII200GRY(602)	55	MSS	40	MSS	10	MS
NJBWII200GRY(608)	50	MS	15	MSS	10	MS
NJBWII200GRY(612)	50	MS	20	MSS	5	MS
NJBWII200GRY(634)	50	MS	20	MSS	15	MS
NJBWII200GRY(641)	25	M	20	MSS	15	MS
NJBWII200GRY(660)	50	MSS	20	MSS	10	MS
NJBWII200GRY(662)	55	MSS	20	MSS	10	MS
NJBWII400GRY(675)	50	MSS	30	MSS	10	MS
NJBWII400GRY(776)	30	MS	10	MS	20	MS
NJBWII400GRY(798)	30	MS	20	MS	10	MS
NJBWII400GRY(849)	30	MS	10	MSS	15	MS
NJBWII400GRY(908)	25	MS	15	M	10	M
NJBWII400GRY(915)	25	MS	15	MS	20	MS
NJBWII400GRY(930)	30	MSS	15	MSS	10	MS
DUMA PARENT(CHECK)	**80**	**S**	**65**	**S**	**30**	**MS**
DUMA100GRY(987)	20	M	10	MSS	25	MS
DUMA100GRY(992)	25	MS	10	MSS	10	MS
DUMA100GRY(993)	25	MS	15	MSS	15	MS
DUMA100GRY(995)	15	MS	10	MS	10	MS
DUMA100GRY(996)	20	MS	15	MSS	20	MS
DUMA100GRY(997)	20	MSS	20	MSS	20	MS
DUMA100GRY(1010)	25	MS	15	MS	15	MS
DUMA200GRY(1026)	20	MS	15	M	10	MS
DUMA200GRY(1030)	35	MS	15	M	5	M
DUMA200GRY(1033)	20	M	10	MS	15	MS
DUMA200GRY(1099)	30	M	15	MS	20	MS
DUMA200GRY(1103)	30	M	15	M	20	M
DUMA200GRY(1124)	20	M	10	MR	10	M
DUMA200GRY(1145)	30	MS	20	MS	10	MS
DUMA400GRY(1299)	25	MSS	15	MSS	15	MS
DUMA400GRY(1295)	40	MSS	20	MSS	5	MS
DUMA400GRY(1304)	40	MS	10	MS	15	MS

DUMA400GRY(1437)	15	MSS	10	MS	20	MS
DUMA400GRY(1349)	40	MSS	15	MSS	15	MS
DUMA400GRY(1368)	35	MS	20	MS	10	MS
DUMA400GRY(1403)	30	MSS	20	MSS	30	MS
KWALEPARENT(CHECK	**55**	**MSS**	**25**	**MSS**	**15**	**MS**
KWALE100GRY(1468)	40	MSS	20	MSS	10	MS
KWALE100GRY(1470)	50	MSS	25	MSS	10	MS
KWALE100GRY(1483)	55	MSS	25	MSS	20	MS
KWALE100GRY(1492)	30	MSS	20	MSS	15	MS
KWALE100GRY(1499)	45	MSS	20	MS	10	MS
KWALE100GRY(1502)	40	MSS	30	MSS	5	MS
KWALE100GRY(1556)	40	MSS	30	MSS	15	MS
KWALE200GRY(1621)	30	MSS	20	MSS	10	MS
KWALE200GRY(1715)	35	MSS	35	MSS	10	MS
KWALE200GRY(1731)	30	MSS	40	MSS	10	MS
KWALE200GRY(1750)	50	MSS	20	MSS	10	MS
KWALE200GRY(1768)	40	MSS	25	MSS	10	MS
KWALE200GRY(1777)	50	MSS	30	MSS	10	MS
KWALE200GRY(1818)	55	MSS	30	MSS	10	MS
KWALE400GRY(1875)	35	MSS	30	MSS	5	M
KWALE400GRY(1877)	30	MSS	25	MSS	15	MS
KWALE400GRY(1895)	50	MSS	20	M	5	M
KWALE400GRY(1907)	40	MSS	15	MSS	10	MS
KWALE400GRY(1949)	35	MSS	15	MSS	15	MS
KWALE400GRY(1961)	30	MSS	10	MSS	15	MS
KWALE400GRY(1964)	30	MS	10	MSS	10	MS
KINGBIRD(CHECK)*	**40**	**M**	**25**	**MR**	**5**	**RMR**
CACUKE(CHECK)	**95**	**S**	**85**	**S**	**30**	**MS**
ROBIN(CHECK)	**100**	**S**	**100**	**S**	**30**	**MS**

R- resistant; MR- moderate resistant; M-moderately resistant/moderately susceptible; RMR- resistant-moderate resistant; MS- moderate susceptible; MSS-moderate susceptible-susceptible

Table 5:2: Means squares for severity of each location among the evaluated 69 wheat (*Triticum aestivum* L.) genotypes in Kenya during 2015-2016 cropping season.

		Njoro	Mau-narok	Timau
Source of variation	df	----------Severity----------		
Genotype	68	4.6856280***	4.5132239***	1.8205769**
Rep	2	2.1617874	0.0223671	0.4249758
Block(rep)	6	3.1029469	2.7822222	1.9353623
Error	130	0.5896314	1.5562200	1.0574708
Total		418.2160386	525.9458937	273.7325604
CV%		12.91122	28.80097	29.68003
R^2		0.816717	0.615343	0.497790

, * represents significance at (P<0.01) and (P<0.001) respectively.

Table 5:3: Summary for means squares for disease severity of 69 wheat (*Triticum aestivum* L.) genotypes evaluated across three locations in Kenya during 2015-2016 cropping season.

Source of variance	d.f	Disease severity
Site	2	328.637536***
Rep	2	0.764492
Block(Rep)	6	0.898439
Genotype	68	5.058182***
Genotype×Site	136	2.919595***
Error	406	1.137078
Total		1875.169565
CV%		23.27661
R^2		0.7538.7

*** represents significance at (P<0.001)

5.7 Discussion

Generally, there was a wide range of virulence expressed by the stem rust races on the genotypes across the evaluated agro-ecological sites. Variability for stem rust races had been reported earlier across locations with different climatic conditions in Kenya (Singh *et al.*, 2008). Similarly, the epidemiological components of *Puccinia graminis* are known to be affected by warm and moist weather conditions which were evident in the tested environments (Wanyera *et al*., 2008). This suggests that the variation across locations in the expression of resistance genes in a given set of genotypes could be as a result of variability in the existing races along with the climatic conditions and geographic features of the area, which affect the stem rust infection process (Ali *et al*., 2009). In that regard, the variability of genotypes responses to the stem rust in the present study could be due to the variation in climatic changes of these areas along with the variability in the prevalent races.

Previously, Wanyera *et al*., (2006) research found that the levels of stem rust infection varied with environment as well as varieties/lines that were being evaluated in that location. In the study, the mutants were evaluated in different environmental conditions to test whether they may vary with locations with reference race virulence and disease pressure. Difference in disease severity and infection reactions was also noted across the sites and among the evaluated genotypes. During cropping season, the sites showed varying difference in stem rust severity where Njoro and Mau-Narok was noted with high mean values while low mean values in Timau (Figure 4). Cheruiyot *et al*. (2015) reported Mau-Narok as the most stem rust prone area compared to Njoro. In the present study, Njoro was noted to be most prone to the disease. This could be attributed to the high initial stem rust pressure during that season. In addition, this might be due to their difference in geographical location, crop management practices and type of cultivars grown. Considerably, variation in the stem rust severity of the mutant lines and experimental checks tested was noted ranging from 5 to 100%. This showed that there was significant variation in the tested sites and genotypes with regard to severity variability hence the tested sites had enough disease pressure to assess the level of resistance or susceptibility in the tested genotypes (Table 2 and 3).

Stem rust urediniospore stage is usually favored by hot days (25-30ºC) and mild nights (15-20ºC) and wet leaves from rain or dew. The minimum, optimum and maximum temperatures for spore germination are 2, 15 to 24 and 30ºC, respectively (Roelfs *et al*., 1992) and for sporulation 5, 30 and 40ºC, respectively (Rowell, 1984). Njoro and Mau-Narok usually experience these climatic conditions hence favored the high disease pressure on the tested genotypes. Safavi (2012) studied on slow rusting resistance in Iranian barley cultivars to

Puccinia striiformis f. sp. *Hordei* and proposed that lines with disease values of 1-30%, 31-50% and 51-70% were regarded as possessing high, moderate and low levels of rusting resistance, respectively.

In present study, the severity values of genotypes were in agreement with the findings where in Njoro, thirty three genotypes had low levels of disease severity (1-30%), while twenty six genotypes had moderate levels of severity of 35-50%, in Mau-Narok there were sixty three genotypes in (1-30%), three genotypes in the range (35-50%) and two genotypes in (55-70%) category while in Timau most of the genotypes were in the range of 1-20%, an indication of low disease pressure in the region. The variation in the disease severity could be as a result of the difference in genetic background of the genotypes, geographical features of the test sites and probably crop management practices and type of cultivars grown during the cropping seasons.

5.8 Conclusion

Based on the present study finding, the mutants showed much disease variability in the tested sites compared to the parents. As a result, Duma mutant lines including Duma 200gry(1026), Duma 200gry (1033) and Duma 200gry (1124) that showed levels of resistance that could be used directly for crop improvement either by providing sources of resistance for breeding programmes or by correcting a fault in the parental cultivar. The tested sites were stem rust hotspots suitable for evaluation of genotypes.

Further studies are needed to understand the variability in the pathogen virulence profile across these locations and the detailed effects of climatic conditions on resistance genes of these varieties.

5.9 References

Admassu, B., Friedt , W. and Ordon, F. (2012). Stem rust seedling resistance genes in Ethiopian wheat cultivars and breeding lines. *African Crop Science Journal* 20: 149-161.

Ali, S., Shah, S. J. A. and Rahman, H. (2009). Multi-location variability in Pakistan for partial resistance in wheat to *Puccinia striiformis* f.sp. *tritici. Phytopatholothy of Mediterranean,* 48, 269-279.

Aquino, P., Carrion, F., and Calvo, R. (2002). Selected wheat statistics. *In ''CIMMYT 2000–2001 World Wheat Overview and Outlook: Developing No-Till Packages for Small Scale Farmers''* (J. Ekboir, ed.), pp. 52–62. CIMMYT, Mexico, D.F.

Cheruiyot D., Okwiri P. P. O., Njau, P. N., Arama, P. F and Sridhar, B. (2015). Evaluation of advanced wheat (*Triticum aestivum L*.) lines for stem rust (*Puccinia graminis*) resistance and yield. *International Journal of Agronomy and Agricultural Research*, 6, 57-70.

CIMMYT. (2005). Sounding the Alarm on Global Stem Rust: *An Assessment of Race Ug99 in Kenya and Ethiopia and the Potential for Impact in Neighboring Countries and Beyond*. Mexico City, Mexico.

Jaetzold, R., Schmidt, H., Hornetz, B., and Shisanya, C. (2010). *Farm management Handbook* Vol.II, 2nd Edition. Part B Central Kenya. Subpart B1a: Southern Rift Valley.

Njau P. N., Wanyera, R., Macharia, G. K., Macharia, J., Singh, R and 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 (2), 022-027.

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

Parry, M. A. J., Madgwick, P. J., Bayon, C., Tearall, K., Lopez, A. H., Baudo, M., Rakszegi, M., Hamada, W., Yasin, A. A., Quabbou, H., Labhilili, M. and Phillip, A. L. (2009). Mutation discovery for crop improvements. *Journal Expert of Botany*, 60 (10), 2817-2825.

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

Roelfs, A. P., Singh, R. P. and Saari, E. E. (1992). *Rust Diseases of Wheat: Concepts and Methods of Disease Management* (pp 81). D.F. CIMMTY, Mexico.

Rouse, M.N. and Jin, Y. (2011). Stem rust resistance in A-genome diploid relatives of wheat. *Plant Disease* 95: 941-944.

Rowell, J. B., (1984). Controlled infection by *Puccinia graminis* f. sp. *tritici* under artificial condition. pp. 291-331. *In*: Bushnel, W. R. and Roelfs, A. P. (eds). *The Cereal Rusts*. Academic Press, Inc., London.

Saari, E. E. and Prescott, J. M. (1985). World distribution in relation to economic losses. *"The Cereal Rusts Vol. II. Diseases, Distribution, Epidemiology, and Control"* (Roelfs, A. P., and Bushnell, W.R. (eds) (pp. 259-98). Academic Press, Orland.

Safavi, S.A. (2012) Evaluation of slow rusting parameters in thirty-seven promising wheat lines to yellow rust. Tech Journal Eng Applied Science 2:324–329.

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

Schumann, G. L and Leonard, K. J. (2000). *The Plant Health Instructor*, DOI:10.1094/PHI-I-2000-0721-01.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M., Wanyera, R., Njau, P. and Ward, R. W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race *Ug99* (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources,* 1, 54.

Singh, R. P, Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S. A and Ward, R.W (2008). Will Stem Rust Destroy the World's Wheat Crop? *Advance Agronomy,* 98, 271-309.

Singh R. P., Hodson D. P., Jin Y., Lagudah E. S., Ayliffe M. A., Bhavani S. (2015). Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. *Phytopathology* 105, 872–884. 10.1094/PHYTO-01-15-0030

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

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

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

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- Screening of genotypes in the greenhouse permitted evaluation of genotypes under specific race infection and uniform disease pressure. There was variation in seedling infection type ranging from fleck to susceptible. High frequency (53.6% and 88.4%) of mutant lines were resistance to the TTKTK and TTKSK races respectively at seedling stage with low infection types ranging from flecks to 2+.
- Field experiment also showed variation in adult plant resistance to a wide range of the existing stem rust races. Mutant lines Duma 200gry (1124), Duma200gry (1026) and Duma400gry (1304) portrayed moderate resistance while Duma400gry (1295) and Duma400gry (1299) performed best (disease and yield) across the locations thus they are recommended for further screening in the breeding programmes.
- With regard to disease (area under disease progress curve and coefficient of infection) and yield, Kwale400gry (1875), NJBWII200gry (602) and Duma 400gry (1299) respectively were stable and broadly adapted across the three sites.

Recommendations

- Further genetic studies are required to confirm the presence of effective genes in the resistant mutant lines evaluated in the greenhouse. After confirmation of the genes against the tested races, efforts should be made to incorporate these effective genes from mutant lines into adapted backgrounds.
- Taking into account, field experiment revealed some of potential genotypes, among the genotypes were Duma 200gry (1124) and Duma 400gry (1299). 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. The results showed that Njoro and Mau-Narok were the most discriminative and therefore a more appropriate site for evaluating wheat genotypes under different stem rust races and disease pressure.

APPENDICES

Appendix 1

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Evaluation of Stem Rust (*Puccinia graminis* f.sp *tritici*) Seedling Resistance in Kenyan Bread Wheat (*Triticum aestivum* L.) Mutant Lines

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Abstract Race TTKSK (*Ug99*) of stem rust is a serious threat to wheat production worldwide because of its wide virulence on many cultivars and its rapid spread over countries. The objective of this study was to determine resistance to *Puccinia graminis* f.sp. *tritici* races of TTKTK and TTKSK at seedling stage among the wheat mutant lines. Duma, Kwale and NJBWII mutant lines were used since the parents are susceptible. Sixty three mutant lines along with six checks of NJBWII, Kwale, Duma, Cacuke, Robin and Kingbird were evaluated in the greenhouse at Kenya Agriculture and Livestock Organization, Njoro. A high frequency of mutant lines, 53.6% and 88.4% were resistant to TTKTK and TTKSK respectively, with low infection types ranging from ";" to "2+". In addition, frequency of susceptibility was 46.38% for TTKTK and 11.59% for TTKSK on the evaluated genotypes. Resistance in these genotypes may be due to uncharacterized resistance genes or gene combinations that could not be resolved with the collection of races used. The information presented, when combined with the previous characterization of stem rust resistance genes will be useful for plant breeders in rationalizing germplasm enhancement programs.

Keywords: *stem rust, seedling stage resistance, mutation*

Appendix 2

a) SAS ANOVA Procedure for Field Disease and Yield

```
data wheat;
input SITE   $ GENOTYPE   REP    BLOCK  TILLERS      SPK_LENGTH   HEIGHT SEED_SPIKE
      KLWHT  YIELD  AUDPC  CI;
cards;
NJORO  1      1      2      12     11.30  77     43     0.022  4.79   18.0   6.4
NJORO  1      2      1      9      10.70  78     45     0.023  4.47   13.9   6.4
,       ,   ,   ,   ,      ,      ,   ,    ,      ,        ,      ,
MAUNAROK      1      1      2      5      8.10   73.70  23     0.023  4.75   16.8   4.6
MAUNAROK      1      2      1      5      8.20   68.30  23     0.020  5.13   18.5   5.0
,           ,   ,   ,   ,     ,         ,      ,    ,        ,      ,     ,
TIMAU  1      1      2      7      12.20  87     51     0.046  16.11  8.9    4.1
,       ,   ,   ,   ,      ,      ,   ,    ,        ,      ,   ,
TIMAU  69     3      2      9      12.20  103    43     0.048  21.59  5.3    2.2
;
proc glm;
class site rep block genotype;
model  klwht yield audpc ci=site rep block(rep) genotype genotype*site/ss3;
means site genotype genotype*site/lsd;
test H=genotype E=genotype*site;
random site rep block(rep) genotype*site;
run;
```

b) SAS ANOVA Output for Field Disease and Yield

The SAS System 12:05 Thursday, January 18, 2017 40

The GLM Procedure

Class Level Information

Class	Levels	Values
SITE	3	MAUNAROK NJORO TIMAU
REP	3	1 2 3
BLOCK	3	1 2 3
GENOTYPE	69	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 67 68 69

Number of Observations Read 621

Number of Observations Used 621

Dependent Variable: KLWHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	214	0.06192651	0.00028938	23.86	<.0001
Error	406	0.00492392	0.00001213		
Corrected Total	620	0.06685043			

R-Square	Coeff Var	Root MSE	KLWHT Mean

0.926344	10.40681	0.003483	0.033464	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SITE	2	0.05200881	0.02600441	2144.18	<.0001
REP	2	0.00003035	0.00001517	1.25	0.2873
BLOCK(REP)	6	0.00020640	0.00003440	2.84	0.0102
GENOTYPE	68	0.00310123	0.00004561	3.76	<.0001
SITE*GENOTYPE	136	0.00659297	0.00004848	4.00	<.0001

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	214	22854.03001	106.79453	57.78	<.0001
Error	406	750.42740	1.84834		
Corrected Total	620	23604.45741			

R-Square	Coeff Var	Root MSE	YIELD Mean
0.968208	14.00653	1.359538	9.706457

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SITE	2	18173.42906	9086.71453	4916.14	<.0001
REP	2	35.75483	17.87741	9.67	<.0001
BLOCK(REP)	6	42.12604	7.02101	3.80	0.0011
GENOTYPE	68	1717.89342	25.26314	13.67	<.0001
SITE*GENOTYPE	136	2797.08492	20.56680	11.13	<.0001

Dependent Variable: AUDPC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	214	17057.70515	79.70890	8.09	<.0001
Error	406	3998.78184	9.84922		
Corrected Total	620	21056.48699			

R-Square	Coeff Var	Root MSE	AUDPC Mean

R-Square	Coeff Var	Root MSE	Yield Mean
0.810093	26.71610	3.138346	11.74702

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SITE	2	9028.715008	4514.357504	458.35	<.0001
REP	2	30.000612	15.000306	1.52	0.2193
BLOCK(REP)	6	68.984219	11.497370	1.17	0.3228
GENOTYPE	68	4330.935073	63.690222	6.47	<.0001
SITE*GENOTYPE	136	3478.687214	25.578582	2.60	<.0001

Dependent Variable: CI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	214	1413.345752	6.604419	6.58	<.0001
Error	406	407.306422	1.003218		
Corrected Total	620	1820.652174			

R-Square	Coeff Var	Root MSE	CI Mean
0.776285	24.72663	1.001608	4.050725

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SITE	2	557.1134300	278.5567150	277.66	<.0001
REP	2	3.1887923	1.5943961	1.59	0.2053
BLOCK(REP)	6	3.9647859	0.6607977	0.66	0.6831
GENOTYPE	68	426.9164768	6.2781835	6.26	<.0001
SITE*GENOTYPE	136	411.9799034	3.0292640	3.02	<.0001