# YIELD LOSSES RESULTING FROM A NEW RACE OF STEM RUST Ug99 (*Puccinia graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn.) IN BREAD WHEAT (*Triticum aestivum* L.) CARRYING SEEDLING/ADULT PLANT RESISTANCE GENES IN KENYA

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**A thesis submitted to the Graduate School in partial fulfilment of the requirements for the Degree of Master of Science in Agronomy (Crop Protection Option) of Egerton University**

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## DECLARATION AND RECOMMENDATION

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I declare that this thesis is my original work and has not been presented before to any University for any degree or other award.

Signed ..... Date 13/08/09

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### RECOMMENDATION

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

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# DEDICATION

This work is dedicated to our beloved late son, Wangai and daughter Wanjiku, my wife Muthoni, sons Macharia and Gichuhi for their prayers, support and encouragement during the study.

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Joseph Kinyoro Macharia

# ABSTRACT

Wheat stem rust control in Kenya has depended on the cultivation of resistant cultivars which have been effective over the past three decades. In 1999, a new race of *Puccinia graminis* Pers. f. sp. *tritici* Eriks & E. Henn., Ug99 (TTKS) virulent to all Kenyan commercial wheat cultivars was identified for the first time in East Africa. Identification and introgression of resistance genes into commercial cultivars is imperative. Study was carried out to assess the impact of race Ug99 on grain yield and yield components. Twenty wheat cultivars among them nine Kenyan commercial cultivars: Njoro Bw1, Njoro Bw2, Chozi, Duma, Kwale, Kenya Popo, Kenya Nyangumi, Kenya Kongoni and Kenya Fahari; plus eleven CIMMYT advanced breeding lines: 103141, 103067, 103108, 102091, 10101, 103247, 103247, 97, 6436, 6053 and 102012 were screened for seedling and adult plant resistance to the virulent race, Ug99. Greenhouse seedling test was done by growing clumps of plants in plastic pots. Seven-day old seedlings were inoculated with fresh urediniospores in water suspension using a small hand sprayer. After inoculation the seedlings were incubated for 16 hours in a dark dew chamber, kept moist by frequent spraying with water to ensure a high relative humidity above 80% and a temperature of 18-22°C. Thereafter, they were transferred to the greenhouse and incubated at 22-30°C. The infection types were assessed 14 days after inoculation. Field experiments were set up at Mau-Narok and KARI-Njoro, the cultivars were planted during 2006/7 cropping seasons. The treatment design consisted of a split plot arrangement with fungicide-protected and non-protected treatments as the main plot and the wheat cultivars as subplot in a randomized complete block design with three replications per site. Non-protected subplots were kept disease-free by four applications of foliar fungicide, AmistaXtra 280 SC (azoxystrobin 200 g/L + cyproconazole 80 g/L) at the rate of 1.0 L $ha^{-1}$ using a Knapsack sprayer at four growth stages: stem elongation (GS31, booting (GS45), heading (GS55) and flowering (GS65). The stem rust epidemic was created by artificially inoculating spreader rows which composed of a mixture of susceptible cultivars, Kenya Heroe and Ngamia. Disease severity notes and infection response were taken on whole plots using modified Cobb scale when 50% of the plants reached growth stages: heading (GS55), flowering (GS65), late milk (GS77) and soft dough (GS85). All the wheat cultivars and two breeding lines were susceptible, the remaining 103067, 102091, 10101, 103247, 103249, 97, 6436, 6053 and 102012 exhibited some level of seedling resistance to the Ug99 isolate. The field adult plant experiment showed variable resistance responses. Stem rust severities observed over the two experimental sites ranged from 2% for the most resistant line 102091 to 58% for the most susceptible cultivar Duma. Significant negative correlations between grain yield and stem rust severity were obtained across the sites. With the exception of Njoro Bw1, Chozi, Duma and Line 103141, the rest of the cultivars and breeding lines showed low area under the disease progress curve (AUDPC) values suggesting a polygenic mode of resistance. The susceptible control cultivar, Chozi had an average grain yield loss of 66.3%, while the average loss for all the cultivars ranged between 12.0% and 69.2%. The 1000-kernel weights were reduced by an average of 26.4% in Mau-Narok and 29.0% in KARI-Njoro. It was found that three Kenyan wheat cultivars, Njoro Bw2, Kenya Nyangumi and Kenya Kongoni had slow rusting traits and suffered less stem rust damage. These results identified four breeding lines (102091, 97, 6436 and 6053) which possessed satisfactory levels of resistance to race Ug99 of stem rust. The identified resistant breeding lines and slow-rusting cultivars should be exploited as a source of resistance by breeders for the improvement high yielding susceptible commercial cultivars.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| AUDPC | Area the under disease progress curve used as a measure of slow rusting |
| CIMMYT | International Centre for Maize and Wheat Improvement |
| FDS | Final Disease Severity |
| IT | Infection Type |
| IR | Infection Response |
| KARI | Kenya Agricultural Research Institute |
| LSD | Least Significant Difference |
| Non-prot | Non-protected |
| PBC | Pseudo-Black Chaff |
| Pgt | Puccinia graminis tritici |
| Prot | Fungicide-protected |
| r | Pearson coefficient of correlation (coefficient of correlation) |
| SAS | Statistical Analysis Systems |
| *Sr* | Stem rust resistance gene |
| TKW | Thousand kernel weight |
| TTKS | Stem rust race designated "Ug99" |
| UH | Upper Highland |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Bread wheat (*Triticum aestivum* L.) is an important cereal and food crop in terms of both harvested and production area in Kenya and runs second after maize (Republic of Kenya, 2004; KARI, 2005b). Wheat consumption in Kenya is increasing rapidly and stable production plays an important role in the Kenyan economy. The current annual production is estimated at 300,000 metric tonnes, which is far below the domestic requirements of over 700,000 metric tonnes per annum. The demand has been projected to reach 1 million metric tonnes by 2010 (Nyangito *et al.*, 2002; KARI, 2005b). However, this is unlikely to be satisfied partly due to pre-harvest losses caused by re-emerging diseases, insect pests and poor crop practices under resource constrained small scale farming conditions. A recent survey has revealed that stem rust disease caused by *Puccinia graminis* f. sp. *tritici* race Ug99 (TTKS) (Pretorius *et al.*, 2000) is causing enormous crop losses (Wanyera *et al.*, 2006). The impact on wheat production due to these losses has lead to changes in prices of wheat; consequently increasing commercial imports, which are already too high. For instance, during 2002 commercial imports of wheat was 54% (420,000 tons) of domestic demand (Nyangito *et al.*, 2002). To overcome this problem it is important to urgently identify sources of resistance against this stem rust race which is virulent to many wheat cultivars grown worldwide.

Wheat stem rust challenge has been addressed at Njoro, Kenya since 1927 (Green *et al.*, 1970). In recent times, high wheat stem rust incidences and severities have been recorded at high altitude farming areas, even though the fungus normally occurs in low to medium altitude areas (Wanyera *et al.*, 2006). Since there is a wheat crop in some part of the country during the year, this ensures significant pools of air-borne urediniospores spread by wind from one area to another throughout the year, and altitude is not a limiting factor. The magnitude of yield losses due to stem rust varies from low in moderately resistant to complete loss in susceptible cultivars under favourable weather conditions and high disease pressure. The Kenya highlands where most of the country's wheat is grown has climatic conditions which are most conducive for the establishment of Ug99 epidemics and has been designated as a stem rust 'hot-spot'.

Pretorius *et al.* (2000) reported the outbreak of a highly virulent race of stem rust of wheat caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks & E. Henn. in Uganda in 1999. The race is commonly known as Ug99 and designated TTKS based on North American system of nomenclature. The occurrence of Ug99 was subsequently detected in Kenya and in Ethiopia in 2005. It migrated to Sudan and crossed the red sea to Yemen in 2006, and its presence was confirmed in Iran in 2007 (Expert Panel on Stem Rust Outbreak in Eastern Africa, 2005; Singh *et al.*, 2006; Joshi *et al.*, 2008).

Although the existence of numerous physiological races of stem rust in Southern Africa and other regions is well documented, it is the emergence of race Ug99 (TTKS) that posses most danger because of its aggressiveness. The race has also been reported to overcome resistance genes *Sr*24, *Sr*36 and *Sr*38, besides the important resistance gene *Sr*31 initially transferred from rye to wheat (Jin and Singh, 2006). Most of the leading bread wheat cultivars and advanced breeding lines in Kenya are now susceptible; the present situation could turn into a disaster if rapid action is not taken to identify resistant cultivars. Furthermore, the high stem rust disease pressure in this region has reduced the life-span of most resistant commercial wheat cultivars to about 2 years, thus allowing farmers limited cultivar options. Therefore, stem rust poses a significant threat to wheat production and livelihoods, and unless quick intervention is undertaken the current epidemics are also likely to have serious global ramifications should the new race migrate to a new region.

Several studies have shown that both seedling and adult plant resistance genes are involved in the durable form of resistance to stem rust disease and that major genes mainly control this resistance (McIntosh, 1988; Knott, 1993; McIntosh *et al.*, 1995; Hare, 1997; Brown, 1997). Seedling resistance is generally known to be race-specific and susceptibility means that the major genes are absent or have broken down. Virtually all reports have considered seedling resistance during cultivar screening, while no studies currently are addressing adult plant resistance under field conditions (Hare, 1997). Results of seedling tests do not always give a reliable prediction of the level of resistance in mature plants in the field (Niks and Lindhout, 2006). Moreover, adult plant resistance cannot be identified in the seedling stage (Welty and Barker, 1992). There are, however, numerous genes for hypersensitivity that are only effective in

mature plants. Hence there is need to do investigations in both growth stages. The current farming systems in Kenya, where there is no regulation on the growing of susceptible cultivars and sowing periods, is increasing the spread of the disease due to 'green bridging' of wheat crops because wheat is grown virtually the whole year round. There is an urgent need to evaluate wheat cultivars and breeding lines to identify new sources of resistance that could enhance identification of resistant cultivars.

## 1.2 Statement of the Problem

The resurgence in 1999 of the new race of stem rust in East Africa after three decades, and the favourable environmental conditions in this region are responsible for the fast widespread and devastating stem rust epidemics that have occurred in major wheat growing areas since 2002. All the popular Kenyan commercial bread wheat cultivars are susceptible to race Ug99. This has been estimated to cause yield losses of over 70% (Expert Panel on the Stem Rust Outbreak in Eastern Africa, 2005). Consequently, this has lead to increased reliance on fungicides for control which is costly and environmentally unfriendly. There is need to identify other sources of resistance to be used in breeding programmes.

## 1.3 Justification

There is an urgent need to screen commercial bread wheat cultivars and advanced breeding lines for resistance to physiologic race Ug99 (TTKS) of *Puccinia graminis* f. sp. *tritici* to be used in breeding programmes. The occurrence of a virulent physiologic race of stem rust in eastern Africa region now poses a major threat to wheat production worldwide (Jin *et al.*, 2008). The ecological conditions in the Kenya highlands (1600 to 2900 metres above sea level) where most wheat is produced favour proliferation of stem rust races. This has highlighted the need for developing and deploying cultivars with effective resistance genes to enhance their longevity. Unfortunately, there is little published experimental work on epidemiology and virulence information, and precise data on actual yield losses caused by the new stem rust physiological race Ug99 (TTKS) in the farmers' fields. The results of this study will enhance knowledge on the level of vulnerability of popular commercial wheat cultivars to stem rust disease in Kenya. In addition, it will highlight the need to employ other effective management strategies to sustain wheat production.

Although the cereal-rust fungus is one of the most widely studied of all plant pathogens (Kurt, 2001a), relatively little work has been done to identify wheat cultivars resistant to the new physiological race Ug99 (TTKS) of stem rust (Singh *et al.*, 2006). Studies to quantify stem rust resistance expression, which is of utmost importance to Kenyan wheat farmers who function on very tight profit margins, will be of considerable importance. Furthermore, yield losses associated with this race have not been quantified under the Kenyan conditions. The results of this study will pave way towards effective management of stem rust disease, which is a threat to wheat production in Kenya. This will be a major contribution towards food self-sufficiency and alleviation of food insecurity which are among the objectives in the Kenya's agricultural policy as contained in the Strategy for Revitalising Agriculture 2004-2014 (Republic of Kenya, 2004).

## 1.4 Objectives

The broad objective of this study was to assess yield losses wheat cultivars and screen for their resistance to stem rust race Ug99. The specific objectives were:

1. To determine the loss on grain yield and yield components caused by race Ug99.
2. To identify race Ug99 resistant cultivars and recommend for use as source of resistance
3. To evaluate wheat cultivars for seedling and adult plant resistance to race Ug99.

## 1.5 Hypotheses

1. Race Ug99 infection has no effect on grain yield and yield components.
2. Wheat cultivars have no resistance to race Ug99 under greenhouse and field conditions.
3. Infection response of wheat cultivars to race Ug99 does not differ at seedling and adult plant growth stages.

## 1.6 Definition of Terms

| | |
|---|---|
| Adult plant resistance | Resistance expressed near or after heading. |
| Aggressiveness | Capacity of an isolate of a natural enemy to infect a host species or genotype to a higher degree than other isolates. |
| Avirulence | The property of a pathogen by which it cannot infect a host plant because of the effectiveness of one or more major genes for resistance. |

| | |
|---|---|
| Complex race | Race with a wide virulence spectrum. |
| Culture | A clone of a urediniospore that is maintained in the laboratory. |
| Differential set | A set of lines or cultivars by which races of a natural enemy can be characterized or distinguished. |
| Disease progress curve | Amount of disease (or its transformation) plotted against time. |
| Durable resistance | Resistance that remains effective for a long period in which it is applied at large scale in an environment conducive to the pathogen. |
| Field resistance | Resistance that is particularly clearly visible in the (experimental) field. |
| Forma specialis (f. sp.) | Unit within the pathogen that is distinguished by its host range. |
| Horizontal resistance | Non-specific host plant resistance controlled by polygenes. Protects plants by slowing down the spread of disease and the development of epidemics in the field. OR Resistance that is equally effective against all isolates evaluated. |
| Host (plant) | Plant species that is susceptible to a pathogen and serves both as source of nutrition and as living substrate. |
| Hypersensitivity | Resistance that is characterized by a chlorotic or necrotic spot (fleck) where a few cells died near the point of infection. No sporulation occurs. |
| Infection type | Nature of the reaction of the plant to an infection by a pathogen, which is expressed by a figure or letter according to a standardized scale. OR the visible symptoms of disease produced by interaction of host and pathogen in a specific environment.The term are synonymous to "reaction type". |
| Incidence | Degree of infection in a population of the host species that is expressed as the percentage infected or symptoms-showing individuals. |
| Incubate | To develop from the onset of the infection process to the production of the visible symptoms or signs. Certain environmental conditions are necessary to the plants in order to promote infection of those plants. |
| Inoculate | To apply spores of pathogen on or in plants in order to obtain an infection. |
| Inoculum | The spores or other propagules of the pathogen to which plants are exposed, and from which infection can take place. |

| | |
|---|---|
| Isolate | A sample of a pathogen that is stored alive or maintained in isolation on plants or in nutrient media or a clone derived from a single uredinium. |
| Latency period | Period elapsing from the moment of inoculation to the moment of becoming infectious (e.g. the onset of sporulation by the pathogen. |
| Pathogenicity | The capacity of a microbial organism to infect a plant species. |
| Partial resistance | Resistance that permits some sporulation. |
| Race | A group of genotypes within a pathogen species that is distinguished by its virulence and avirulence on a series of differential hosts. |
| Race-non-specific resistance | A resistance that is equally effective against all isolates evaluated. |
| Race-specific resistance | A resistance that is effective against only some isolates. |
| Residual resistance | The quantitative (mostly partial) resistance that surfaces when a virulent race appears that infects a formerly completely resistance cultivar. |
| Seedling resistance | Resistance expressed in the primary leaf often effective through the plant's life. |
| Slow-rusting | Occurs when cultivars displays a susceptible response, but with slower disease progress than in a susceptible check cultivar. |
| Tolerance | The theoretical ability of a plant to yield in spite of a high level of disease. |
| Vertical resistance | Host plant resistance to specific race of a pathogen; interaction of plant gene-conditioning resistant reaction with a pathogen gene for pathogenicity-conditioning avirulence. |
| Virulence | The capacity of a pathogen to infect a plant with one or more major genes for (hypersensitivity) resistance, because it does not possess any of the corresponding genes for avirulence. |
| Virulence spectrum | The series of genes for resistance to which a pathogen does not possess or express the corresponding avirulence alleles. |

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Importance and Distribution of Stem Rust

Stem rust, caused by *Puccinia graminis* Pers.: Pers. f. sp. *tritici* Ericks & E. Henn., is one of the most destructive diseases of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). In many wheat-growing areas, the disease has been controlled effectively through the widespread use of host resistance (Jin *et al.*, 2008). Similarly, the history of wheat production in Kenya has been mainly an effort of attempts to manage stem rust by resistant varieties (Green *et al.*, 1970). Wheat stem rust was recognized and rated the "greatest of plant diseases" by the early Romans (Wiese, 1991). The stem rust disease was introduced in North America during the early years of settlement by European immigrants (Harder *et al.*, 2001), and wheat was first grown in eastern Canada in 1605 and in western Canada about 1749 (Liu and Harder, 1996a; Eversmeyer and Kramer 2000). In Kenya, European settlers introduced wheat in the early 1900's in the highlands of the Rift Valley (Hassan *et al.*, 1993). The earliest wheat stem rust epidemic was reported in Kenya in 1908 (Green *et al.*, 1970).

Since the inception of the wheat breeding programme in Kenya until the mid 1970s stem rust was undoubtedly the most serious disease of wheat, and therefore was given a high research priority. Consequently, many resistant varieties were released after incorporation of genetic resistance gene *Sr*2. Due to the perception that stem rust disease had been controlled; therefore, breeding efforts were shifted to stripe rust disease (*Puccinia graminis* f. sp. *striiformis* Wested. Eriks.) that had become and, still is a major threat to wheat production (Danial, 1994). Besides Kenya, stem rust has been a major disease of many small grain cereals and forage grasses worldwide (Roelfs, 1988) and occurs primarily on wheat, but attacks barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.) and susceptible grasses (Green *et al.*, 1970; Zwer *et al.*, 1992; Park, 1997). The role of wild grasses as reservoirs of inoculum for wheat stem rust may not be significant in Eastern Africa region, no grass species were found which were susceptible to wheat stem rust pathogen (Harder *et al.*, 1972), but has been reported to be a serious problem for seed production of *Festuca* and *Lolium spp.* in temperate regions (Green and Martens, 1982; Kurt, 2001a).

The main alternate sexual host of stem rust is common barberry (*Berberis vulgaris* L.). It 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. Currently barberry plants have largely been eradicated worldwide and their importance as a source of inoculum and new races in these regions is insignificant (Harder *et al.*, 2001; Kurt, 2001a). However, the presence and functioning of common barberry in eastern Africa has not been documented (Green *et al.*, 1970: Owuoche *et al.*, 1994). But a close relative, *Berberis holstii* Engl., whose role in the epidemiology of wheat stem rust is not yet clear, has been identified in several major wheat growing-areas, for example, Mau-Narok and Narok districts, 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, 2001a).

In other regions, localized epidemics have continued to occur worldwide and have been reported to reduce yields by up to 50% in United States (Eversmeyer and Kramer, 2000). Growers reported yield reductions of 20 to 50%, and a few reported near total crop failures in Canada (Martens and McFadden, 1988; Park, 1996). Stem rust disease is largely under control worldwide except in Iran, Yemen, Sudan and Eastern Africa. For instance, in Ethiopia yield reductions of 60 to 100% have been reported, while in Kenya losses above 70% have been recorded under experimental conditions (Expert Panel on Stem Rust Outbreak in Eastern Africa, 2005; Singh *et al.*, 2006; Joshi *et al.*, 2008) ). The prevailing conducive climatic conditions, widespread agro-ecological zones, and continuous wheat cropping systems facilitate the stem rust pathogen populations to continuously evolve new virulent races that overcome the resistance of released wheat cultivars (Jaetzold and Schmidt, 1983). The major efforts in developing resistant cultivars revolve around search, incorporation and evaluation of resistance in new cultivars (Mclntosh *et al.*, 1995). The need is constantly demonstrated by the frequent appearance of numerous new pathogen races that are potentially dangerous (Kurt, 2001a).

In recent years some cultivars thought to have durable resistance genes such as Enkoy rusted in Ethiopia, while genes *Sr31* and *Sr24* broke-down in Uganda and Kenya (Pretorius *et al.*, 2000; Jin *et al.*, 2008). The recent evolution of race Ug99 of stem rust is responsible for the current breakdown of resistance in Kenyan commercial wheat cultivars with CIMMYT germplasm

background. Published data have shown that under favourable environmental conditions; hot days of 25-30°C, mild nights of 15-20°C with adequate moisture for night time dews, infection by the new race is capable of reducing the quality and quantity of the wheat grain within a few weeks. Although wheat stem rust has been under control for decades in Eastern Africa region, its resent re-emergence is now a threat and could wreak havoc to wheat production unless rapid action is taken.

## 2.2 General Taxonomy and Nomenclature

*Puccinia graminis* is a rust fungus in the phylum *Basidiomycota*, class: *Urediniomycetes*, order: *Uredinales*, family: *Pucciniaceae,* and genera: *Puccinia*, named after their noticeable sori that form a major group of plant pathogens. The rust fungi are generally obligate parasites and only survive on living plant hosts. In most areas where environmental conditions are favourable, the fungi reproduce almost exclusively through the asexual urediniospore cycle on the crop (Prescott *et al.,* 1986; Mclntosh *et al.*, 1995). The sexual cycle is not documented in Eastern Africa and therefore, stem rust pathogen is thought to mutate from avirulence to virulence on wheat cultivars possessing single major genes for resistance (Luig and Watson 1976; Rajaram *et al.*, 1988).

The classification of rust fungi into families and genera is based upon the morphological features of the teliospores. The species within the genera are distinguished on the basis of their host range and urediniospores characteristics (Stubbs *et al.,* 1986). In the genus *Puccinia*, host specificity is highly developed and a number of formae speciales based upon host range may be distinguished within each species. The pathogens are further divided into physiological races or biotypes that are identified on the basis of differential cultivars with single resistance genes (Green, 1981; Prescott *et al.*, 1986; Roelfs and Martens, 1988). The nomenclature is based on morphological features, host range and the distinct evolutionary histories of the subspecies. There is, however, no agreement regarding the morphologically based variety designations beyond the subspecies level.

Many workers prefer a forma specialis (f. sp.) nomenclature based on host specialization (Roelfs and Martens, 1988). The appropriate taxonomy then becomes *Puccinia graminis* subspecies

*graminis* or *graminicola,* followed by an f. sp. designation. However, in most current literature, the causal fungi are directly named and the subspecies designation is not included, for instance, *Puccinia graminis* f. sp. *tritici* (Mclntosh *et al.,* 1995). The form f. sp. is further subdivided into numerous races or pathotypes. These constitute a taxon below the forma speciales level. They are characterized by differences in physiological (pathogenic) reactions, resulting in different virulence/avirulence patterns on a selected set of differential resistance genotypes within a host genus (Luig and Watson, 1976). The differential set of Stakman *et al.* (1962) for *Puccinia graminis* f. sp. *tritici* has been used as a worldwide standard and still provides the basis for race classification in many countries (Park, 1996). The Modified International System of Nomenclature for *Puccinia graminis* f. sp. *tritici* (Roelfs and Martens, 1988) which uses enlarged common sets of differentials provide a useful means of comparing international virulence.

### 2.2.1 Etiology

Stem rust is a fungal disease of wheat (*Triticum aestivum* L.), caused by *Puccinia graminis* f. sp. *tritici.* It is a polycylic, heteroecious rust fungi that produces spermagonia and aecia on barberry and mahonia plants; uredia and telia on wheat. The presence of alternate hosts is important in disease epidemiology in providing local inoculum that initiate rust development in adjacent wheat crops and in acting as a source of new physiological races by hosting the sexual stage of the fungal life cycle (Wiese, 1991; McIntosh *et al.*, 1995; Kurt, 2001a). The recycling of the uredinal stage is the major means by which the fungus initiates and perpetuates an epidemic, besides the migration of urediniospores from other regions by wind (Martens *et al.*, 1983; Kurt, 2001b).

The development of the rust fungus in its host plants consists of several distinct stages (Liu and Harder, 1996b); germination of urediniospores, elongation of germ tubes, formation of appressoria, formation of substomatal vesicles, formation and growth of primary infection hyphae, formation of haustorium mother cells (HMCs) and haustoria, formation of secondary infection hyphae, HMCs, and subsequent colonization of host tissue (Liu and Harder, 1996b). *Puccinia graminis* f. sp. *tritici* fungus is believed to comprise of several clonal lineages, within which new race(s) from a distinct founding isolate via single-step mutations at loci governing pathogenicity arise.

### 2.2.2 Epidemiology

The fungus *Puccinia graminis* f. sp. *tritici* is widespread in occurrence except in very hot and dry regions. Most regions of the world have conditions that are more marginal for rust development (Prescott *et al.*, 1986; Roelfs *et al.*, 1992). Stem rust develops near 20°C and is seriously hampered below 15°C and above 40°C (Wiese, 1991; Kurt, 2001a). Conducive conditions such as source and timing of inoculum, availability of susceptible hosts, high temperature and humidity are required for stem rust development. Delayed crop maturity favours disease infection and development (Roelfs *et al.*, 1992), therefore, early maturing cultivars may sometimes escape heavy stem rust infection.

The occurrence and significance of the alternate hosts (*Berberis spp.* and *Mahonia spp.)* in temperate climates is well documented as a factor in disease epidemiology, but in the tropics, Kenya in particular, Barberry's function is not yet clear. Where full sexual cycle is involved new races arise through sexual recombination by shuffling of genes (Roelfs and Martens, 1988; Wiese, 1991). However, the full sexual cycle has not been reported in Kenya despite the wide existence of *Berberis hostii* Engl. (Plate 1), which is a close relative of the main alternate host, *Berberis vulgaris*. The uredinia on volunteer wheat and late planted crop are the most important sources of inoculum that perpetuate stem rust disease in Kenya. Little is known of the sources of variation of stem rust in East Africa. There is circumstantial evidence that some variation is due to somatic hybridization of existing virulence, followed by selection if a new race has a selection advantage (Harder *et al.*, 1972; Singh *et al.*, 2006). The moderate climates and the ability to grow wheat at anytime of the year create local endemic disease cycles in eastern Africa highlands; therefore, the fungus persists on volunteer wheat plants or on successive crops as they are sown at different times of the year depending mainly on rainfall patterns. Stem rust epidemics are particularly frequent at the middle altitude range in Kenya. This is probably due to the influence of the wheat cultivated south of the Rift Valley, which supports an endemic rust fungal population, which is thought to be responsible for the reservoir of the inoculum exchanged within Rift Valley between Kenya, Tanzania and South Africa (Green *et al.*, 1970; Harder *et al.*, 1972; Saari and Prescott, 1985*)*.



**Plate 1. Barberry plant (*Berberis holstii* Engl.) in Mau-Narok**

### 2.2.3 Symptoms

The symptoms on wheat appear first as long, narrow, elliptical blisters or pustules parallel with long axis on the stem, leaf or leaf sheath (Bushnell and Roelfs, 1984; Wiese, 1991). On the leaves uredinia generally penetrate to sporulate on both surfaces (Kurt, 2001b). In later stages of growth, blisters may appear on the neck and glumes of the wheat spike within a few days (Plate 2). The epidermis covering the pustules is ruptured irregularly and pushed back revealing a powdery mass of brick red-coloured urediniospores. The infected parts sometimes have invisible symptoms, but may feel rough to touch (Plate 3). The pustules called uredia vary in size from 1 to 3 mm wide by 10 mm long (Wiese, 1991). As infected plants mature the rusty colour of the pustules turns black and the fungus produces teliospores instead of urediniospores. The uredia

are transformed into black telia and more often uredia and telia do exist on wheat plants independently (Roelfs, 1984; Agrios, 1997).



**Plate 2. Susceptible cultivar Chozi**



**Plate 3. Resistant breeding line 102091**

### 2.2.4 Damages

The stem rust fungus attacks all the aerial parts of the wheat plant, but are most common on stem, leaf sheathes, upper and lower leaf surfaces. It also attacks wheat plants at various growth stages thus; seedling, vegetative, flowering and fruiting. Epidemics that occur during flowering are detrimental, but damage is greatest when the disease becomes severe before the grain is completely formed, because the damage to the conducting tissue results in fewer nutrients being transported to the grain (Wiese, 1991; Kurt, 2001a). Infected plants usually produce fewer tillers and set fewer seeds per head, and the kernels are smaller in size, generally shrivelled, weightless, and of poor milling quality and food value.

Overall the fungus reduces root growth, plant vigour and grain filling (Knott, 2000a). Under extreme situations, heavily infected plants may die (Plate 4), while heavy seedling infection may weaken the plants and predispose them to attack by other pathogens (Wiese, 1991; Agrios,

1997). Stem rust epidemics may reduce a healthy crop to a black tangle of broken stems and shrivelled grains with little feed value for livestock within a few weeks (Rowell, 1984; Prescott *et al.*, 1986). Cattle foraging on wheat straws of rusted crops usually find it unpalatable and occasionally develop allergic response to such fodder. Severe stem rust disease attack causes straw breakage, resulting in a loss of spikes during combine harvesting (Wiese, 1991).



**Plate 4: Susceptible cultivar Duma early infection (left) and killed in later stages (right)**

## 2.3 Control of Stem Rust

The production of wheat in most cereal producing areas in Kenya will be seriously curtailed if the recommended management strategies for combating stem rust are not instituted. The susceptible cultivars suffer yield loss of between 50 and 70% or higher depending on the stage of crop when the initial rust infection occurs (Saari and Prescott, 1975; KARI, 2005a; Wanyera *et al.*, 2006). The most effective, and practical means of controlling wheat stem rust is through the use of resistant cultivars and foliar fungicide application (McIntosh *et al.*, 1995; Agrios, 1997). Foliar fungicide sprays have been successful when applied early, although not economical. Cultural methods such as; early planting, variety mixtures, removal of volunteers through tillage, grazing or spraying herbicides have been used either singly or in combination to ameliorate the effects of the pathogen (Roelfs, 1988; Eversmeyer and Kramer, 2000). When applied independently none of these strategies have proved effective (Nyangito *et al.*, 2002; Wanyera, *et al.*, 2006). The present emphasis is focused on integrated disease management, i.e., sowing resistant cultivars, good crop husbandry and fungicide control.

### 2.3.1 Host-Plant Resistance

Genetic resistance is the most effective, least expensive and most environmentally safe means of controlling stem rust (Jin *et al.*, 2008). When adequate genetic resistance to the disease is achieved, no other control methods are necessary. However, acquiring and maintaining adequate resistance is difficult because of the genetic plasticity of the stem rust pathogen (Roelfs *et al.*, 1992; McIntosh *et al.*, 1995). This notwithstanding, the germplasm that carry resistance genes and other sources with unknown resistance genes are widely available, and are frequently identified to be resistant to new races (Roelfs and Martens, 1988). However, this resource is not inexhaustible and care should be taken not to unnecessarily exhaust it. Therefore, the challenge ahead is continuing to detect new stem rust races that could threaten wheat crop production. Rational planning in terms of breeding for resistance and characterization of variation in the pathogen over long periods of time in order to enhance our defence capability against this dangerous disease should also be pursued (Martens *et al.*, 1983; Griffey *et al.*, 1983; Knott, 2000b).

Resistant cultivars offer the cheapest control alternative, but there is concern about the rate at which race-specific single resistance genes that have provided protection against stem rust for a long time are breaking down (Mengistu and Yeshi, 1991; Pretorius *et al.*, 2000). Known race-specific resistance genes that are effective to race Ug99 are already identified, they include; *Sr*21, 22, 23, 25, 26, 27, 29, 32 , 33, 35, 36, 39, 40, 42, 43, Agi, and Em (McInosh *et al.*,1995; Knott, 2000b; Jin *et al.*, 2007). Additional minor resistance genes known as modifiers may be useful in enhancing the expression of slow rusting adult plant resistance genes. Marker-assisted selection may help in combining some of these currently effective genes in an attempt to enhance their longevity. The best strategy for durable genetic control is to initiate a thorough search for slow rusting, minor resistance genes that have additive effects (Vanderplank, 1984; Expert Panel on the Stem Rust Outbreak in Eastern Africa, 2005) and incorporate them into commercial cultivars.

### 2.3.2 Chemical Control

Several fungicides which are labelled for the control of stem rust are currently being screened for their efficacy. However, large-scale farmers in Kenya are protecting wheat crop against stem rust with foliar fungicides recommended for the control of leaf diseases. The commercial fungicides commonly used include, Tilt 250 EC (propiconazole) and Folicur 250 EC (tebuconazole), which are also effective in reducing rust diseases (Paveley *et al.*, 2006; Milne *et al.*, 2007). However, fungicide application is not widely used by the small-scale farmers because the cost is often high and prohibitive. Moreover, fungicide control is only considered where losses are expected to be very high, and even then in the developing world treatment with fungicides when epidemics occur would not be feasible for many farmers and countries given the constraints to mounting well-coordinated mobilization campaigns (Marasas *et al.*, 2004). Since stem rust epidemics are difficult to predict, it is not economically practical to spray large areas in low yielding/low priced situations common in developing countries, Kenya included. Furthermore, there may be environmental hazards, besides concern of the pathogen developing resistance to the available fungicides.

### 2.3.3 Cultural Control

A greater use of cultural farming techniques would help reduce reliance on fungicides, which are often toxic (sometimes banned) chemicals. This is in line with a growing global awareness of the effect of chemical pollution such as residues in food and soil from pesticides, river and water contamination from crop spraying (Kinyua, 1997). Several cultural methods can however be used to reduce the intensity of an epidemic or provide long-term partial control. The timing of an epidemic is critical to the amount of damage that is sustained (Browning, 1988). The date of disease onset is directly related to the development of an epidemic and is probably the most important single factor in determining the severity of an epidemic (Boshoff *et al.,* 2000). Planting at the onset of rainfall and growing of early-maturing cultivars tend to escape heavy yield losses due to stem rust infection (Agrios, 1997). This approach is no longer feasible in Kenya because there has been no fixed sowing dates and non-adherence to growing recommended resistant cultivars. An attempt has been advocated before in Kenya to discourage double-cropping (two crops a year) to decrease the movement of rust from one crop to the next, but to no avail. This practice facilitates the stem rust infections to 'bridge' two cropping seasons, which renders the management of this disease more difficult.

## 2.4 Screening of Wheat Cultivars for Stem Rust Resistance

Breeding for resistance is a major goal of many wheat improvement programmes (The *et al.*, 1993) and this applies to Kenya as well. However, the identification and utilization of new genes for stem rust resistance is an on-going requirement as existing genes become ineffective or the frequency of resistance genes in wheat cultivars changes as a response to changes in pathogen populations leading to evolution of virulent physiological races (Zwer *et al.,* 1992; The *et al.*, 1993; Burdon and Roelfs, 2000). This ensures continued genetic diversity within cultivar arrays to buffer the extent of loss when relevant pathogenic changes occur.

Inclusion of rust resistance at seedling and adult plant stage are both important in breeding programmes. A variety more susceptible in the seedling stage may become resistant at adult stage and *vice versa*. It is also possible for a variety resistant for 2-3 years to suddenly show susceptibility. While wheat seedlings are often used to screen sources of resistance and to study the genetics of resistance to stem rust (Liu and Harder, 1996b), Adult plant resistance assessment

and the timely detection of stem rust races with new virulence are important to producers aiming at maximum yields (McIntosh *et al.*, 1995; Kurt, 2001b). Similarly, information on pathogenicity enables breeders to utilize the most effective resistance genes in their breeding programmes (Boshoff *et al.*, 2000).

According to McIntosh *et al.* (1995), effective genetic control of rust diseases requires a coordinated effort, including race monitoring, collection and characterization of sources of resistance and resistance breeding. In a recent study of wheat stem rust in South Africa, Boshoff *et al.* (2000) concluded that stem rust disease could be controlled by means of genetic resistance and growing moderately resistant wheat varieties. Since only a few durable resistance genes are available, and have been used in the past for the control of stem rust through incorporation into adapted wheat cultivars, this approach is still a viable option in areas at a greater risk of the disease (Zwer *et al.* 1992; Park, 1997). However, caution should be taken to prevent unnecessary exposure of the few remaining major genes because if depleted the control of stem rust would be very difficult. It would also be advisable to investigate the presence of slow rusting genes in some of the old commercial cultivars and other sources of resistance.

The mode of inheritance of resistance to physiological races of stem rust appears to involve one or two major genes (Singh *et al.*, 2006), most of which are now rapidly breaking down. The new challenge is to transfer the identified resistance genes to the new race(s) into adapted wheat cultivars currently grown in Kenya and reassemble minor genes involved in durable resistance before this dangerous physiological race spreads more out of Eastern Africa highlands and causes major epidemics globally.

## CHAPTER THREE

# MATERIALS AND METHODS

### 3.1 Experimental Sites

The study was conducted during the 2006 and 2007 cropping seasons. Screening in the green house was carried out at the Kenya Agricultural Research Institute (KARI) Njoro, situated in Nakuru district. The field experiments were carried out at two locations, KARI- Njoro and Mau-Narok (Purko Ranch). Kenya Agricultural Research Institute (KARI), Njoro is located $0^0$ 20' S, 35° 56' E and 2185 metres in elevation, with a daily average temperature ranging from 9.7°C (night) and 23.5°C (noon). Variations of daily temperatures are approximately +/– 2°C occurring mostly during the day hours, and receives on average 900 mm of rainfall annually. Dew is formed nearly daily. The soil type is predominantly *Mollic Andosols*. The site falls within agro ecological zone III (Jaetzold and Schmidt, 1983). The Njoro area has been described as a 'hot-spot' for stem rust epidemics because of favourable climatic factors; warm days and cool nights accompanied by formation of dews, necessary for the evolution of new physiologic races (Saari and Prescott, 1985). This microclimate seems to have a strong effect on the frequency of stem rust epidemics in the highlands of Kenya where most of the wheat is produced. Mau-Narok is also situated in Nakuru district, located 0° 39' S, 35° 57' E and lies 2863 metres above sea level (M. A. S. L.). It falls within agro-ecological zone II (UH2) and receives an average annual rainfall of 1200-1400 mm with maximum and minimum temperatures range from 22-26°C and 6-14°C respectively.

### 3.2 Plant Material

Twenty bread wheat cultivars and breeding lines were used for this study; they included nine popular Kenyan wheat cultivars: Njoro Bw1, Njoro Bw2, Kwale, Chozi, Duma, Kenya Popo, Kenya Nyangumi, Kenya Kongoni and Kenya Fahari and eleven advanced breeding lines originating from CIMMYT selected in Kenya after testing in 2005. These were part of an International Stem Rust Screening Nursery in Njoro, established by the Kenya Agricultural Research Institute (KARI) in conjunction with CIMMYT-Mexico, and the Global Rust Initiative. These included: 103141, 103067, 103108, 102091, 10101, 103247, 103249, 97, 6436, 6453 and 102012. Kwale and Njoro Bw2 were released in 1987 and 2001 respectively, are the most

commonly grown bread wheat in Kenyan and are recommended for medium to high altitude areas. Cultivars Njoro Bw1, Duma and Chozi were bred for areas below 1800 m. a. s. l., Chozi the susceptible control was released for cultivation in 1999 has proved highly susceptible to race Ug99 of stem rust. Cultivars recommended for medium altitudes (1800-2100 m) were Kenya Popo, Kenya Nyangumi, Kenya Kongoni and Kenya Fahari. Kenya Nyangumi was popular with farmers because of its high yielding potential (KARI, 2005a). Besides their wide cultivation by farmers, the choice of cultivars was also based on maturity period. They were purposely selected to mature about the same time in order to compare the rate of disease increase.

### 3.3 Urediniospores Isolation, Increase and Race Identification

Stem rust urediniospore samples were isolated by biased sampling from experimental plots of wheat cultivar 'Chozi' in KARI-Njoro by picking single large isolated pustules on stems, cutting them off with a sterile scalpel and placing them into glassine bags for short-term preservation. The isolate Ug99 was used to infect the test cultivars. The inoculum used for evaluation was developed from a single-pustule isolate which was obtained from the diseased stems of wheat cultivar Chozi, reported to carry *Sr*31 in a stem rust nursery at KARI-Njoro.

The sampled pustules were removed using a spatula and the spores suspended in a drop of distilled water and Tween 20 solution on a glass slide. The mixture was stirred vigorously using a spatula, while diluting with distilled water. The suspension was sprayed using a hand operated sprayer on to seven-day-old seedlings of susceptible wheat cultivar Morocco to increase the inoculum (Plate 5). The inoculated seedlings were left for a short time to air-dry before being put into the dark dew chamber. Seedlings were incubated in a dew chamber for 16 hours at 18°C in the dark, and then placed on a greenhouse bench at 22–30°C (Plate 5). The urediniospores that developed after fourteen-days were collected using a vacuum-pump, dried in the laboratory under anhydrous gel and then stored in refrigerator. The stored spores were later used for race identification which was based on 16 differential set for North America (Roelfs, 1988) which were in agreement with the observations made by Jin *et al.* (2007) (Appendices 1 and 2). After race Ug99 confirmation, the collected urediniospores were used for the seedling tests in the greenhouse.




**Plate 5: Universal susceptible cultivar Morocco used for inoculum increase**



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### 3.4 Greenhouse Studies

The twenty wheat cultivars were sown into 13 cm diameter plastic pots filled with forest soil autoclaved at 121°C at 15 pounds pressure for one hour (Roelfs *et al.*, 1992). Each pot contained four cultivars, and four seeds per cultivar were sown in clumps (Plate 6). The pots were placed on trays with gravel filled with water and watered twice per week for 30 minutes. At seven-days the seedlings were inoculated with fresh stem rust urediniospore suspension (2 mg spores/ml), prepared by suspending urediniospores into 500 ml distilled water containing 2 drops of Tween-20 solution, using a small hand sprayer. Each set of treatments was sprayed uniformly from four directions ensuring complete coverage of both the upper and lower leaf surfaces of individual plants. Each set of plants received 100 ml of urediniospore suspension. After inoculation the seedlings were incubated for 16 hours in a dark dew chamber, kept moist by frequent spraying with water to ensure a high relative humidity above 80% and temperature of 18-22°C.

Subsequently, the seedlings were transferred to the greenhouse (Plate 6) and for the first 2 hours they were placed on green house benches with bright sunlight, and every 15 minutes a spray of distilled water was applied on the leaves using a small hand operated sprayer. Thereafter, they were incubated at 22-30°C as per the procedure by Roelfs *et al.* (1992). The experiment was arranged in a completely randomised design (CRD) with three replicates. The scoring of seedlings was done 14 days post-inoculation (Plate 7) based on 0-4 scale as described by Stakman *et al.* (1962), and the measurement proposed by Roelfs *et al.* (1992) by considering the size of the uredinia produced and necrosis or chlorosis of the cells around uredinia formed on the primary leaves. Infection types (IT); 0 , ; , 1 , 2 or combinations thereof were considered low, while 3 to 4 as high (Plate 8). The seedling test was repeated twice.



**Plate 6: Greenhouse with controlled temperature and humidity**



**Plate 7: Potted seedlings of cultivar Chozi (control) 14-days post-inoculation**




**Plate 8: Infection types (ITs) used in scoring seedling reaction to Ug99 (Adopted from Stakman *et al.*, 1962)**

### 3.5 Adult Plant Resistance Test

The twenty bread wheat cultivars were evaluated for their adult plant resistance to stem rust race Ug99 in the field after seedling test in the green house. The cultivars were planted during the 2006/7 cropping seasons. Two yield loss trials were established in two sites one at KARI- Njoro and the second at Mau-Narok (Plate 9). The treatment design consisted of a spit-plot arrangement with fungicide-protected and non-protected (rusted) treatments as main-plots and

wheat cultivars as subplots in a randomized complete block design with three replications per site. Each subplot consisted of 3 m long and 1.5 m wide plots, each containing eight rows spaced 20 cm apart. Sowing was done by hand and a seeding rate of 125 Kg $ha^{-1}$ was used in each planting. The fertilizer was applied by experimental seed-drill planter at the rate of 150 Kg $ha^{-1}$ of Di-ammonium phosphate (N: 18% P: 46% K: 0%). The plots were sprayed for grass weeds control one day after planting with Stomp 500 E (pendimethalin), a pre-emergent herbicide at the rate of 3.0 L $ha^{-1}$. At stem elongation growth stage, (GS) 31 (Zadoks *et al.*, 1974), Buctril MC (bromoxyil iso-octyl esters) post-emergent herbicide at the rate of 1.25 L $ha^{-1}$ (KARI, 2005a) was sprayed to control broad-leaved weeds, and for cereal aphid control, one application of metasystox 250 EC (oxy-demeton-s-methyl) insecticide was applied at the rate of 0.75 L $ha^{-1}$. Non-inoculated subplots were kept disease-free by four applications of foliar fungicide, AmistaXtra 280SC (azoxystrobin 200 g/L + cyproconazole 80 g/L) at the rate of 1.0 L $ha^{-1}$ using a knapsack sprayer at recommended water volume of 200 L/ha at the following wheat growth stages; stem elongation (GS 31), booting (GS 45), heading (GS 55) and flowering (GS 65). The trials were maintained under optimal growing conditions using recommended management practices.

The stem rust epidemic was created by artificially inoculating spreader rows which composed of a mixture of Ug99 susceptible cultivars, Kenya Heroe and Ngamia planted in the pathways at each end of the subplot. The spreader rows were inoculated by dusting them with urediniospores of *Puccinia graminis* f. sp. *tritici* race Ug99 mixed with talc powder at the beginning of stem elongation, (GS 31) as outlined in McIntosh *et al.* (1995). The plots were monitored regularly for the appearance of stem rust symptoms. Disease severity notes and infection response on stems of wheat was taken on the whole plot using modified Cobb scale when 50% of the plants reached the following growth stages; GS 55 (heading); GS 65 (flowering); GS 77 (late milk) and GS 85 (soft dough). The severity was recorded as a visual percentage of the surface covered by pustules (Plate 10). The following intervals were used: 5, 10, 20, 40, 60, 80 and 100 percent infection. The host response readings which refers to the infection type and classified according to the following scale: 0 (No visible infection), R (Resistant; necrotic areas with or without small pustules), MR (Moderately Resistant; small pustules surrounded by necrotic areas), M (Intermediate; pustules of variable size; some necrosis and/or chlorosis), MS (Moderately

Susceptible; medium-sized pustules; no necrosis, but chlorosis possible) and S (Susceptible; large pustules, no necrosis or chlorosis) were also recorded.




**Plate 9: Field experiment at Mau-Narok (Purko Ranch)**



R MR M MS S

**Plate 10: Field responses (types of disease reaction), R = resistant, MR = moderately resistant, M = intermediate, MS = moderately susceptible and S = susceptible used in scoring adult plant resistance in test cultivars**

### 3.6 Data Collection

Disease severity estimations of plots were based on the modified Cobb scale (Peterson *et al.*, 1948), whereas response to infection followed Roelfs *et al.* (1992). Stem rust severity assessments were made on the stems of plants in the whole plot using the modified Cobb scale (Peterson *et al.*, 1948) at 14 days intervals. Mean stem rust severity per plot was determined and the area under the disease progress curves (AUDPC) for each cultivar were calculated using a computer programme developed at CIMMYT following the formula given by Roelfs *et al.*

(1992), which calculates the area of the curve created by the disease scores taken three times during the crop cycle (Lal Ahamed *et al.*, 2004).

$$r = \frac{1}{t_2 - t_1}\left[\ln\frac{x_2}{1-x_2} - \ln\frac{x_1}{1-x_1}\right]$$

Where $t_1$ and $t_2$ are the dates at which disease measurements were made and $x_1$ and $x_2$ are the amounts of disease recorded on these dates. The observed infection rate (r) is the result the interaction of the factors affecting disease development.

The plant response to rust infection at the adult stage was termed 'infection response' according to primarily the size of pustules and associated necrosis or chlorosis on the stems or leaf sheaths of the wheat plants. Infection responses were classified into five discrete categories: R-resistant; MR-moderately resistant; M-intermediate; MS-moderately susceptible and S-susceptible (Roelfs *et al.*, 1992) (Plate 10). Cultivars were evaluated for infection responses and stem rust severity at GS 55 (heading), GS 65 (flowering) and GS 77 (late milk). The final disease severity (FDS) disease notes were taken at GS 85 (soft dough).

The grain yield loss for each cultivar within a site was calculated following the method of Herrera-Foessel *et al.* (2006). Observed yield loss at the two experimental sites was calculated by subtracting the mean diseased yield of plots of the same cultivar treated with a four-spray programme.

$$\% \text{ Grain yield loss} = \frac{(\text{Protected yield} - \text{Non-protected})}{\text{Protected yield}} \times 100$$

The number of spikes per plant was sampled randomly from 10 plants in each plot. Grain yield was harvested from a strip along the centre of each plot, delineated with one square metre quadrant and cut using a sickle and put into paper bags. The cut wheat was threshed using an electric thresher (ALMACO Allan Machine Company), oven-dried to constant weight at 65°C (48 h) and weighed at 13% moisture content. The number kernels per spike were determined by randomly selecting three sub-samples of twenty spikes per plot, which were threshed using a single head thresher (Allan Machine Company Model-ABSC-2) and calculated. The 1000-kernel

weights were determined after counting the grain with an electronic seed counter (Model: PFEUFFER GmbH FlugplatzstraBe 70 D–97318 Kitzingen). The kernel shriveling was determined visually by sampling dried grains of each plot and grading using a scale of 1 to 5 (1 = 20%, 2 =40%, 3 = 60%, 4 = 80%, and 5 = 100% shriveled kernels) (Plate 11). The grain yield was derived from a harvested area of 1 $M^2$ by extrapolation.

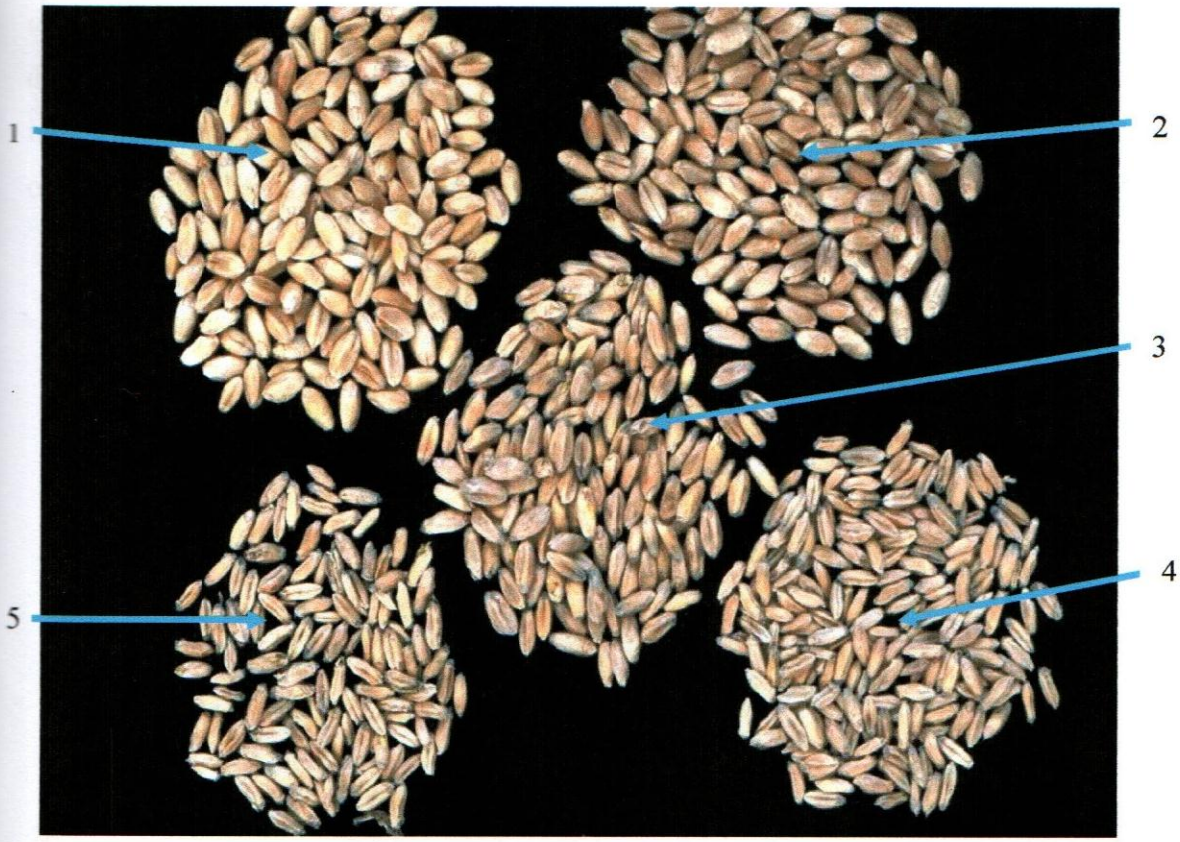



**Plate 11: Healthy wheat kernels (1 & 2) and stem rust-damaged kernels (3, 4 & 5)**

### 3.7 Data Analysis

The grain yields per hectare, 1000-kernel weight, kernels per spike, spikes per plant, kernel shriveling, final disease severity (FDS) and area under the disease progress curve (AUDPC) were subjected to analysis of variance. Data from both sites were analyzed and mean comparisons were made using Tukey's least significant difference (LSD) ($P = 0.5$) test (Steel & Torrie, 1980). Phenotypic correlations among grain yield, yield components, and other traits were determined. Resistance was deduced from the final disease severity (FDS), from the area under the disease

progress curve (AUDPC), infection response determined during adult plant growth stage and from the infection types (ITs) and reaction evaluated at seedling growth stage.

Analyses of variance for the data were analyzed using the Statistical Analysis Systems (SAS, 1999) computer package. Combined split-plot analyses in each site and across the sites were performed for grain yield and yield components using software package ASREML (Gilmour *et al.*, 2002). Cultivars were considered as random whereas treatments as fixed. The area under disease progress curve (AUDPC) values and final disease severity (FDS) were analyzed individually and combined across sites as randomized complete block. The Pearson coefficient of correlation (r) was used to estimate phenotypic correlation between two components.

# CHAPTER FOUR

# RESULTS

## 4.1 Seedling and Adult Plant Resistance

The seedling of susceptible cultivars developed reasonably uniform, large uredia indicating the host susceptibility, while the resistant ones showed little or no hypersensitivity 14 days after infection. Infection types: 0 , ; , 1 , 2 , or combinations thereof were considered as low ITs (Plate 8), indicating that the corresponding resistance gene was effective. The infection types 3 to 4 were considered to be high infection types, indicating that the cultivar or breeding line was susceptible to race Ug99 of stem rust (Jin and Singh, 2006). All the Kenyan cultivars: Njoro Bw1, Njoro Bw2, Chozi, Duma, Kwale, Kenya Kongoni, Kenya Nyangumi, Kenya Fahari and two CIMMYT breeding lines, 103141 and 103108 expressed susceptible, high infection types (ITs '3'-'4') in the greenhouse. Cultivars Chozi, Duma and the breeding line 103108 had the highest IT scores (4), therefore susceptible reaction at seedling stage. Most of the breeding lines 103067 (2), 102091 (0;), 10101 (;1), 103247 (2), 103249 (2), 97 (;2), 6436 (;2), 6053 (0;) and 102012 (2) that tested resistant as seedlings had low infection types '0' ';' '1' or '2' (Table 1). This was based on seedling reaction, and resistance (R) and susceptible (S) cultivar groups.

Stem rust pressure was very high, uniform throughout the experiment and, started early in 2006 crop season at KARI-Njoro compared to the Mau-Narok experimental site, where the infection build-up was slow even after initial created epidemic with artificial inoculation of the spreader rows with urediniospores of race Ug99. The mean rust severity was 12.9% and 31.6% at Mau-Narok and Kari-Njoro respectively (Figures 1 and 2).The control (fungicide-protected) plots were kept free from stem rust and other fungal diseases during the entire crop season, while in the non-protected fungal diseases other than stem rust and stripe rust (*P. striiformis* f. sp. *tritici* Wested. Eriks.) were minimal. Stripe rust was predominant from plant emergence to heading, and stem rust predominated thereafter. All of the Kenyan bread wheat cultivars susceptible at seedling growth stage were also susceptible at adult plant growth stage. But the infection response of Njoro Bw2 and Kenya Nyangumi varied between moderately susceptible (MS) and susceptible (S) as adult plants. In the field at adult plant stage, cultivar Njoro Bw1, Chozi (control), Duma, Kwale, Kenya Popo, Kenya Kongoni and Kenya Fahari showed high

susceptibility responses (S). The remaining cultivars Njoro Bw2 and Kenya Nyangumi were moderately susceptible to susceptible (MS-S) compared to susceptible control (Chozi). Breeding lines displayed variable infection responses to race Ug99 as adult plants in each trial. Line 102091was resistant (R); 103067, 10101, and 6436 were moderately resistant (MR); 97 and 6053 were intermediate (M); 103247, 103249 and 102012 had moderately susceptible to susceptible (MS-S) and the response to infection of lines 103141 and 103108 was susceptible (S).

**Table 1: Seedling reaction and Adult plant severity of wheat cultivars**

| | Seedling Test | | Adult plant Test | | |
|---|---|---|---|---|---|
| | Seedling Reaction | | Stem Rust Severity (%) | | |
| cultivar | IT† | R§ | Mau-Narok | KARI-Njoro | IR□ |
| Njoro Bw1 | 3 | S | 38.0$^{a}$ | 54.3$^{ab}$ | S |
| Njoro Bw2 | 3 | S | 7.3$^{defg}$ | 43.2$^{bcd}$ | MS-S |
| Chozi | 4 | S | 30.0$^{ab}$ | 55.0$^{ab}$ | S |
| Duma | 4 | S | 36.7$^{ab}$ | 58.4$^{a}$ | S |
| Kwale | 3 | S | 7.3d$^{efg}$ | 35.0$^{cde}$ | S |
| Kenya Popo | 3 | S | 11.7$^{defg}$ | 39.1$^{cd}$ | S |
| Kenya Nyangumi | 3 | S | 5.0$^{efg}$ | 21.2$^{fg}$ | MS-S |
| Kenya Kongoni | 3 | S | 11.7$^{def}$ | 45.3$^{abc}$ | S |
| Kenya Fahari | 3 | S | 5.0$^{efg}$ | 46.0$^{abc}$ | S |
| 103141 | 3 | S | 26.7$^{bc}$ | 30.1$^{abc}$ | S |
| 103067 | 2 | R | 1.0$^{g}$ | 14.3$^{g}$ | MR |
| 103108 | 4 | S | 13.3$^{de}$ | 53.0$^{ab}$ | S |
| 102091 | 0; | R | 1.0$^{g}$ | 1.0$^{h}$ | R |
| 10101 | ;1 | R | 5.0$^{efg}$ | 14.0$^{g}$ | MR |
| 103247 | 2 | R | 16.0$^{cd}$ | 30.2$^{efg}$ | MS-S |
| 103249 | 2 | R | 8.3$^{efg}$ | 16.0$^{g}$ | MS-S |
| 97 | ;2 | R | 6.0$^{fg}$ | 11.2$^{g}$ | M |
| 6436 | ;2 | R | 6.0$^{fg}$ | 11.0$^{g}$ | MR |
| 6053 | 0; | R | 6.0$^{fg}$ | 23.3$^{efg}$ | M |
| 102012 | 2 | R | 11.7$^{def}$ | 32.1$^{def}$ | MS-S |
| LSD (0.05) | - | - | 5.3 | 6.6 | - |
| CV % | - | - | 20.8 | 15.7 | - |

† Infection types (IT): Low (0 , ; , 1 to 2), immune and resistant host; High (3 to 4), Susceptible host.
§ Reaction: R = resistant and S = susceptible;
□ Infection response: R = resistant; MR = moderately resistant; M = intermediate; MS = moderately susceptible; S = susceptible.
*a, b, c, d, e, f and g: Means with the same letter in the same column are not significantly different as per LSD means separation procedure (P =0.05)*

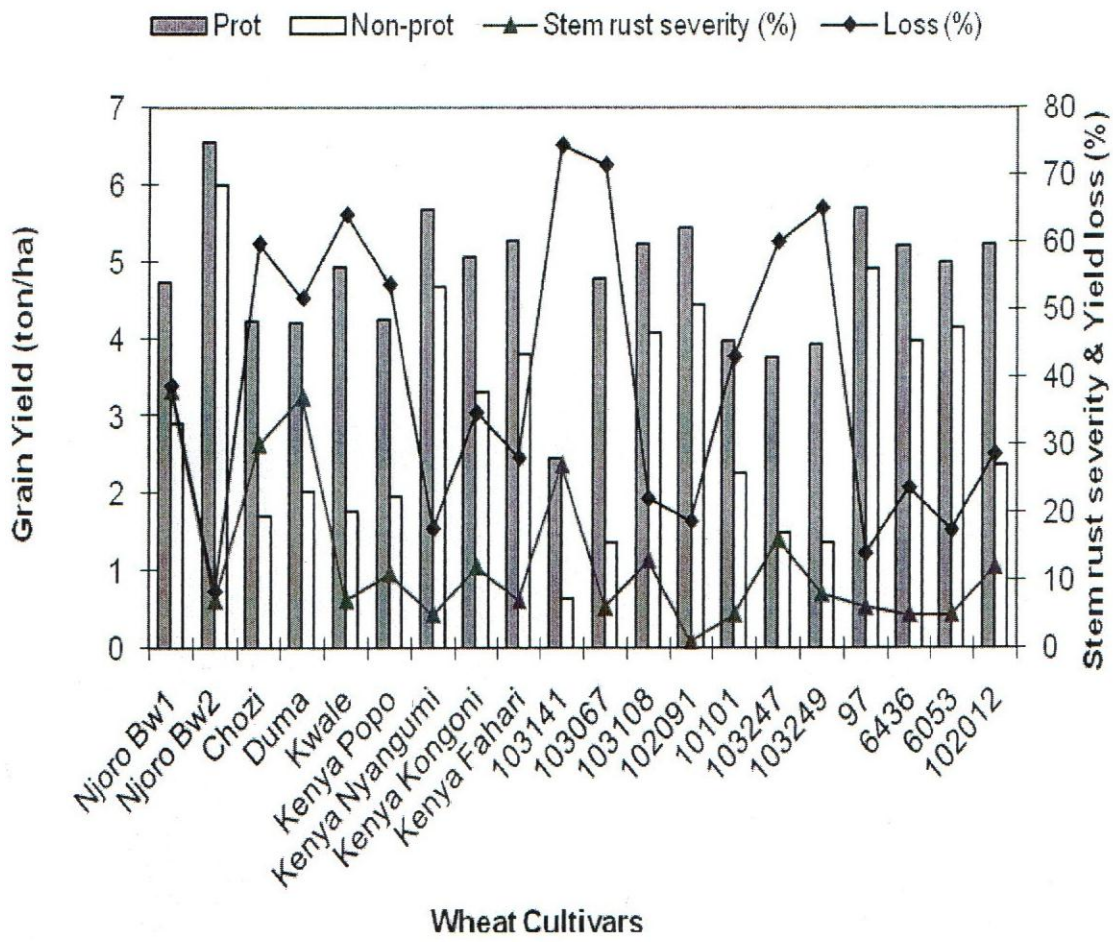



**Figure 1: Stem rust severity and corresponding grain yield of different wheat cultivars without fungicide protection at Mau-Narok**

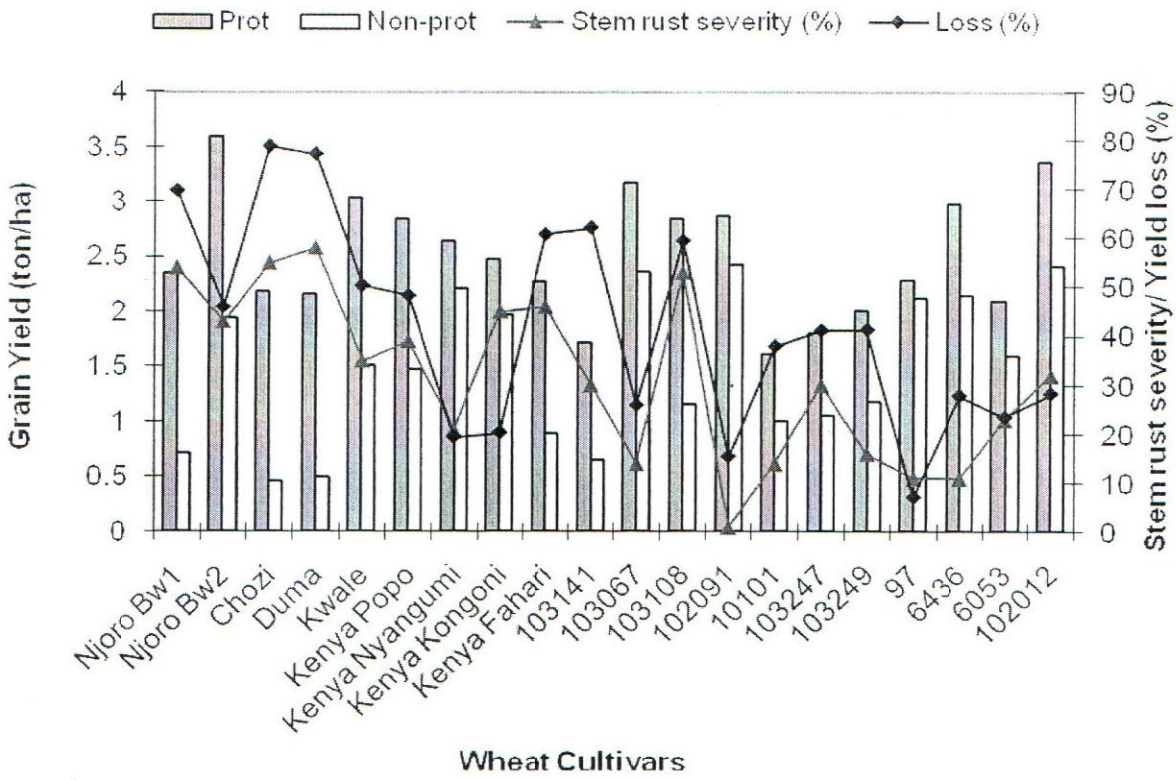



**Figure 2: Stem rust severity and corresponding grain yield of different wheat cultivars without fungicide protection at KARI-Njoro**

## 4.2 Final Disease Severity (FDS) and Area under the Disease Progress Curve (AUDPC)

Average final disease severity of 14.2% and 45.3.6% were recorded at Mau-Narok and KARI-Njoro sites, respectively (Table 2). The mean final disease severity (FDS) and area under the disease progress curve (AUDPC) of susceptible and resistant cultivars differed significantly from each other in both sites and combined analysis across the sites (Appendix 7 and 8). The three most susceptible cultivars, Chozi, Duma and Njoro Bw1, had the highest FDS (Figures 3 and 4) and AUDPC values (Figures 5 and 6). Out of the eleven CIMMYT breeding lines tested, 45.5% were between resistant (R) and moderately resistant (MR), 18.2% moderately resistant to moderately susceptible and 36.3% moderately susceptible (MS) to susceptible (S) infection responses. Stem rust severities in fungicide-protected plots were negligible and non-significant.

The mean final disease severity and the area under the disease progress curve (Table 2) indicated that Njoro Bw1, Chozi and Duma were the most susceptible cultivars across both sites, followed

by Kenya Kongoni and CIMMYT breeding lines 103141, and 103108 (Figures 3 and 4). Their responses to infection (IR) were also susceptible (S). The CIMMYT breeding line 102091 was the most resistant (i.e., showed hypersensitive reaction) in both sites. Three other lines 103067, 10101 and 6436 displayed moderately resistant (MR) host response to infection, manifested as small uredia surrounded by chlorosis at adult plants, and resistant reaction (R) in the seedling greenhouse experiment (Table 1). This showed that these cultivars may possess both major resistance genes and different degrees of adult plant resistance based on slow rusting mechanism as suggested by Herrera- Foessel *et al.* (2006). From the data (Table 2), it is evident that Kwale and Kenya Nyangumi had a much lower FDS and AUDPC values compared to the other susceptible cultivars across the sites (Figures 3 and 4). Although, Njoro Bw2, Kwale and Kenya Nyangumi were generally susceptible at seedling growth stage, their lower AUDPC values seem to indicate the presence of some degree of disease resistance (Table 2). They all displayed pseudo-black chaff (PBC), the phenotypic trait that is associated with slow rusting. The significant interaction between the site x cultivar indicated that stem rust infection progressed differently on the cultivars in the two experimental sites (Appendix 7 and 8).

**Table 2: Final disease severity and area under the disease progress curve of wheat cultivars at Mau-Narok and KARI-Njoro in 2006/7**

| | FDS %[x] | | | AUDPC[y] | | |
|---|---|---|---|---|---|---|
| Cultivar | Mau-Narok | KARI-Njoro | Across sites | Mau-Narok | KARI-Njoro | Across sites |
| Njoro Bw1 | 40.0$^{a}$ | 66.7$^{ab}$ | 53.3$^{abc}$ | 1510.0$^{a}$ | 2566.7$^{ab}$ | 2038.3$^{a}$ |
| Njoro Bw2 | 14.8$^{abc}$ | 63.2$^{abc}$ | 38.7$^{bcdefg}$ | 144.2$^{d}$ | 1936.7$^{abcd}$ | 1040.4$^{cde}$ |
| Chozi | 30.0$^{abc}$ | 80.0$^{a}$ | 55.0$^{a}$ | 1216.7$^{ab}$ | 2520.0$^{ab}$ | 1868.3$^{ab}$ |
| Duma | 36.7$^{ab}$ | 80.0$^{a}$ | 58.3$^{ab}$ | 1453.3$^{a}$ | 2800.0$^{a}$ | 2126.7$^{a}$ |
| Kwale | 8.3$^{bc}$ | 56.7$^{abcd}$ | 32.5$^{cdefgh}$ | 274.2$^{cd}$ | 1563.3$^{abcdef}$ | 918.8$^{cdef}$ |
| Kenya Popo | 11.7$^{abc}$ | 50.0$^{abcde}$ | 30.8$^{defgh}$ | 430.0$^{cd}$ | 1843.3$^{abcde}$ | 1136.7$^{cd}$ |
| Kenya Nyangumi | 5.0$^{c}$ | 33.3$^{cdef}$ | 19.2$^{efghi}$ | 144.2$^{d}$ | 956.7$^{cdefg}$ | 550.4d$^{efg}$ |
| Kenya Kongoni | 11.7$^{abc}$ | 66.7$^{ab}$ | 39.2$^{abcde}$ | 251.7$^{d}$ | 2053.3$^{abc}$ | 1152.5$^{cd}$ |
| Kenya Fahari | 11.7$^{abc}$ | 60.0$^{abc}$ | 35.8$^{bcdef}$ | 251.7$^{d}$ | 2146.7$^{abc}$ | 1199.2$^{bcd}$ |
| 103141 | 26.7$^{abc}$ | 63.3$^{abc}$ | 45.0$^{abcd}$ | 1040.0$^{abc}$ | 2076.7$^{abc}$ | 1558.3$^{abc}$ |
| 103067 | 2.0$^{c}$ | 25.0$^{defg}$ | 13.5$^{ghi}$ | 58.7$^{d}$ | 618.3$^{fg}$ | 338.5$^{fg}$ |
| 103108 | 20.0$^{abc}$ | 66.7$^{ab}$ | 43.3$^{abcd}$ | 474.2$^{bcd}$ | 2473.3$^{ab}$ | 1473.8$^{abc}$ |
| 102091 | 1.0$^{c}$ | 3.0$^{g}$ | 2.0$^{i}$ | 34.2$^{d}$ | 0.0$^{g}$ | 17.1$^{g}$ |
| 10101 | 3.0$^{c}$ | 21.7$^{efg}$ | 12.5$^{hi}$ | 130.0$^{d}$ | 618.3$^{fg}$ | 374.2$^{efg}$ |
| 103247 | 18.3$^{abc}$ | 31.7$^{cdefg}$ | 25.0$^{defgh}$ | 364.2$^{cd}$ | 1061.7$^{cdefg}$ | 712.9$^{defg}$ |
| 103249 | 10.0$^{abc}$ | 21.7$^{efg}$ | 15.8$^{fghi}$ | 264.2$^{cd}$ | 758.3$^{defg}$ | 511.3$^{defg}$ |
| 97 | 6.7$^{bc}$ | 21.7$^{efg}$ | 14.2$^{ghi}$ | 208.3$^{d}$ | 665.0$^{efg}$ | 436.7$^{efg}$ |
| 6436 | 15.1$^{abc}$ | 20.3$^{fg}$ | 17.7$^{fghi}$ | 244.0$^{d}$ | 431.7$^{fg}$ | 337.8$^{fg}$ |
| 6053 | 2.0$^{c}$ | 35.0$^{abcdef}$ | 18.5$^{efghi}$ | 27.2$^{d}$ | 1038.3$^{cdefg}$ | 532.8$^{defg}$ |
| 102012 | 13.3$^{abc}$ | 46.7$^{abcdef}$ | 30.0$^{defgh}$ | 370.0$^{cd}$ | 1446.7$^{abcdef}$ | 908.3$^{cdef}$ |
| LSD ($P = 0.05$) | 7.9 | 8.4 | 21.0 | 779.6 | 1217.6 | 698.6 |
| CV % | 15.7 | 16.1 | 24.1 | 26.5 | 19.9 | 28.4 |

[x]Final disease severity (FDS %) last rating following the modified Cobb scale (Peterson *et al.*, 1948).
[y]Area under the disease progress curve (AUDPC).
*a, b, c, d, e, f, h and i: Means with the same letter in the same column are not significantly different as per LSD means separation procedure (P =0.05)*

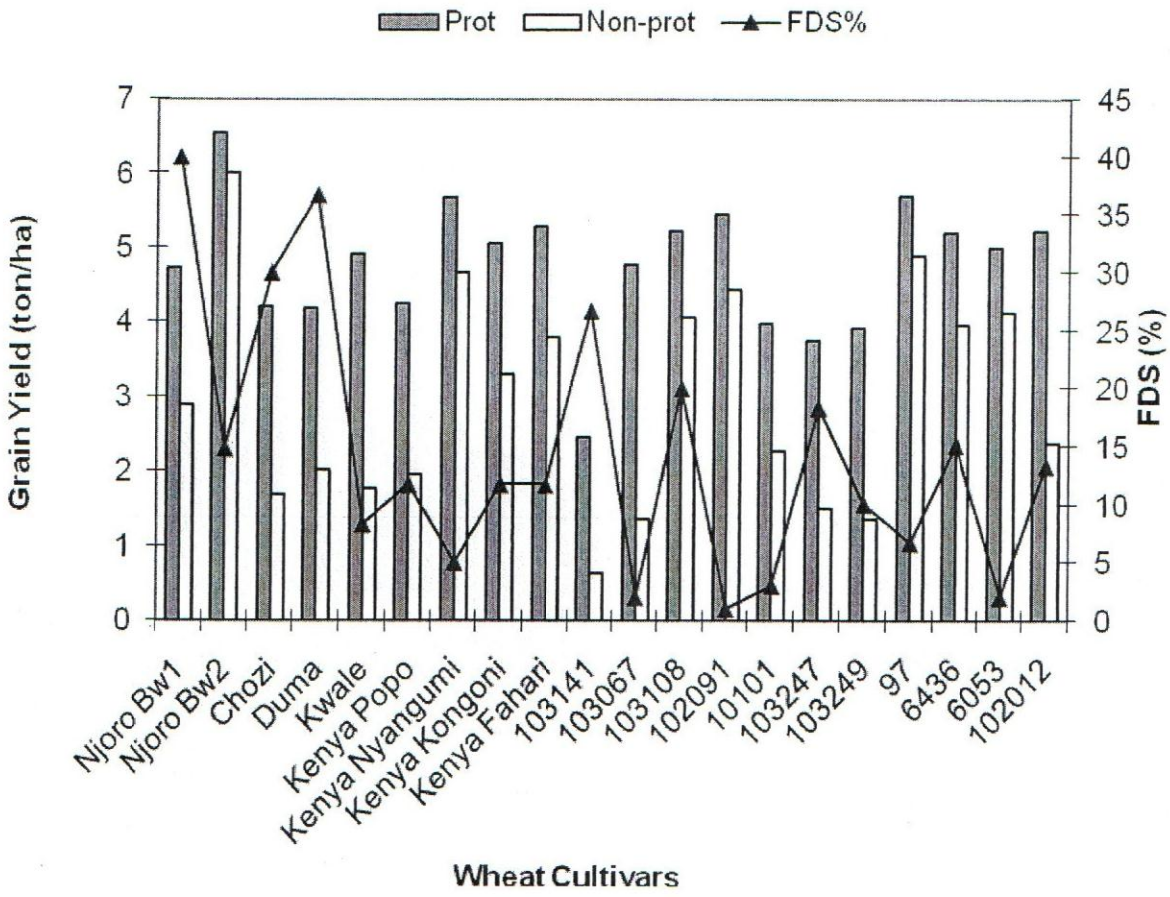



**Figure 3: Relationship between grain yield and final disease severity (FDS %) at Mau-Narok**

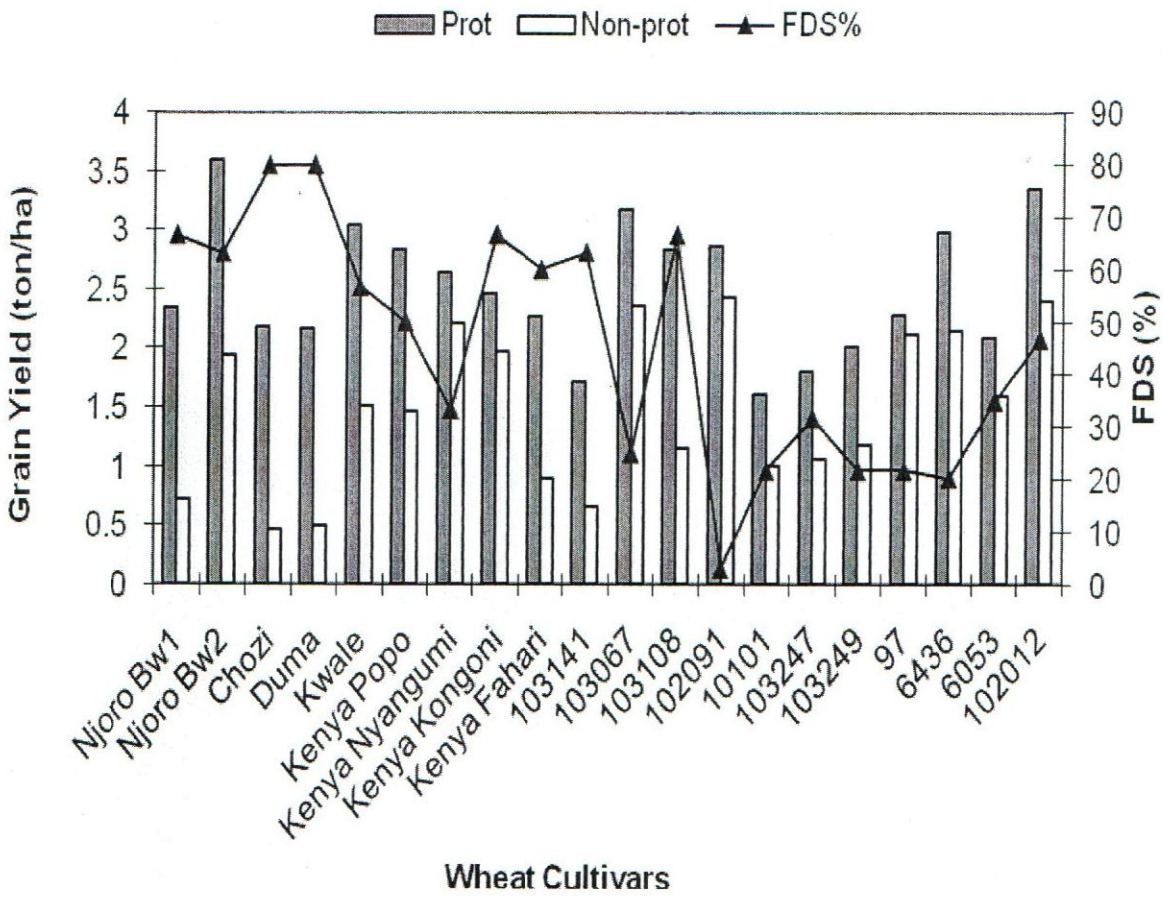



**Figure 4: Relationship between grain yield and final disease severity (FDS %) at KARI-Njoro**

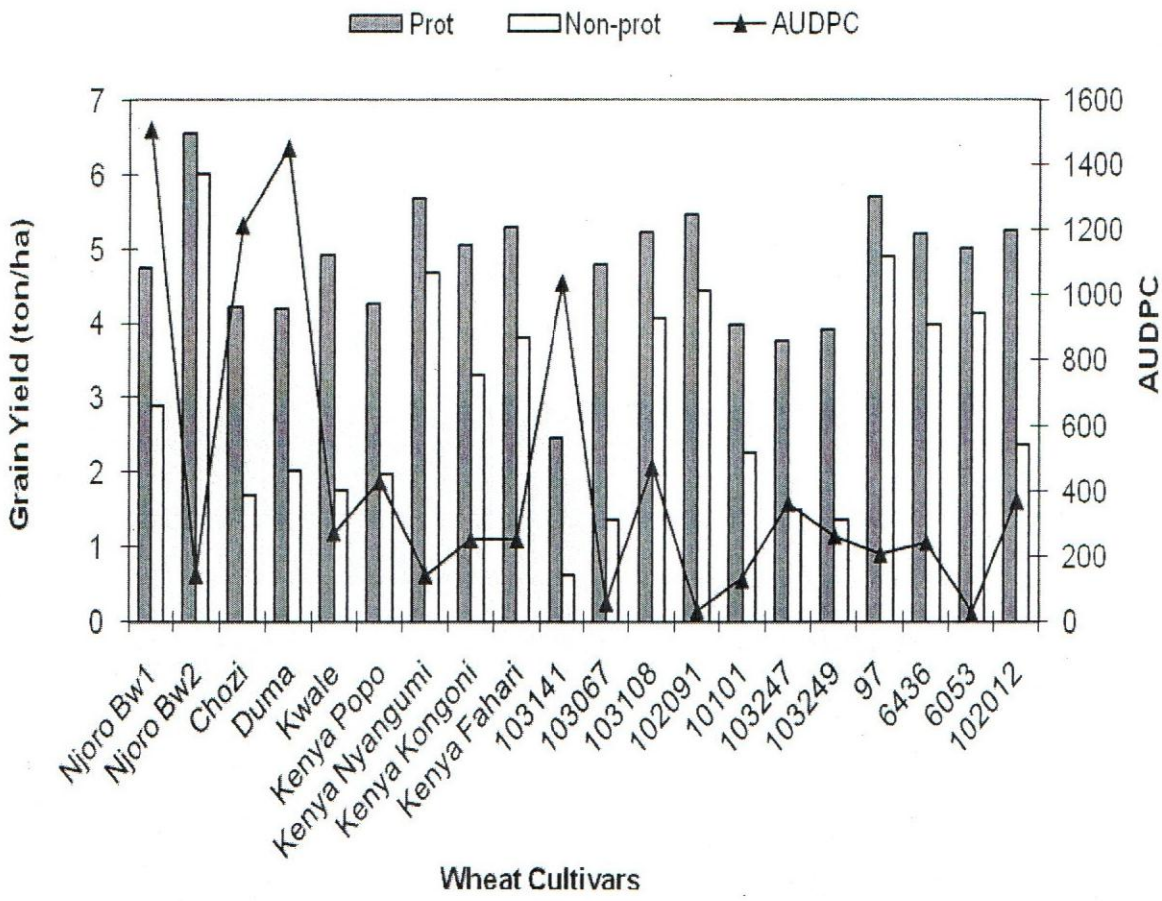



**Figure 5: Effect of area the under disease progress curve (AUDPC) on grain yield of wheat cultivars at Mau-Narok**

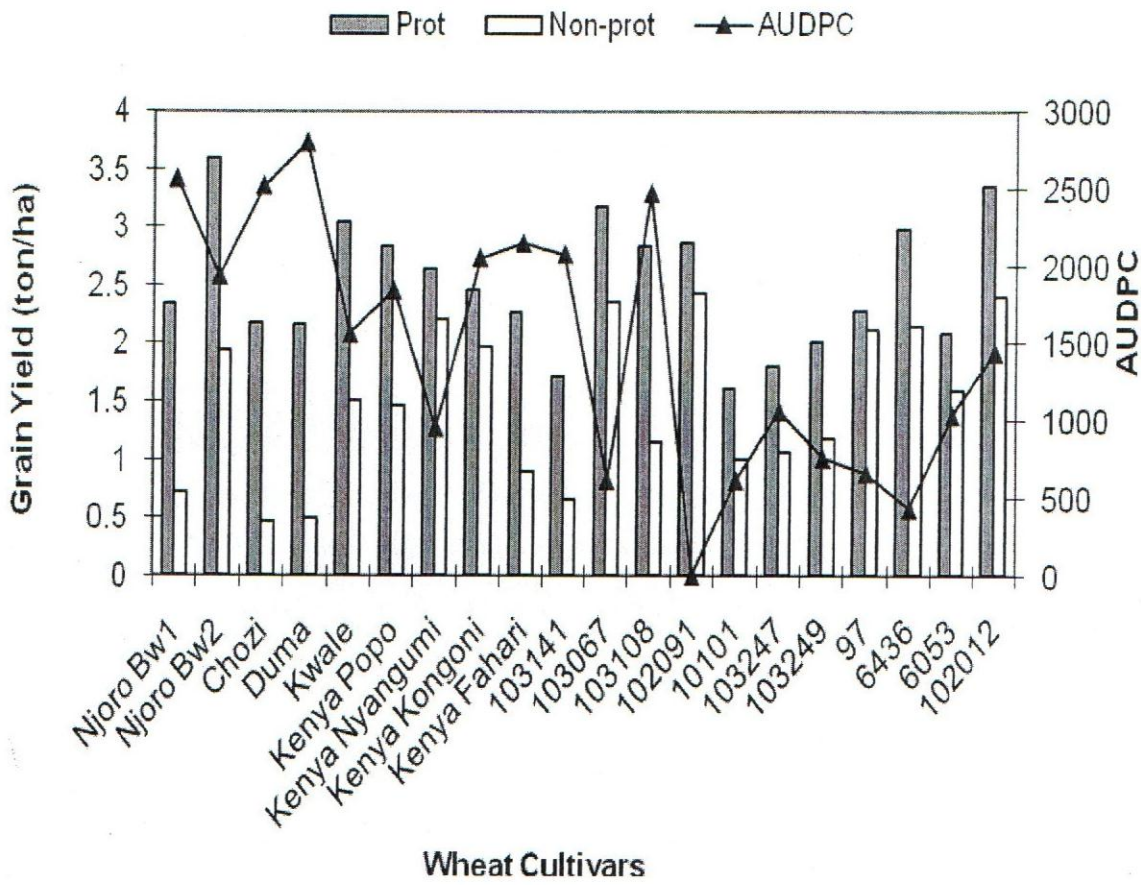



**Figure 6: Effect of area the under disease progress curve (AUDPC) on grain yield of wheat cultivars at KARI-Njoro**

## 4.3 Grain Yield and the Yield Losses

The grain yield was significantly higher for fungicide-protected plots (4.7 and 2.5 t $ha^{-1}$) compared to non-protected plots (3.0 and 1.5 t $ha^{-1}$) at Mau-Narok and KARI-Njoro, respectively (Table 3). Significant a difference in grain yield was observed for each of the main effects: treatment, cultivars and treatment x cultivar interaction. Sites x treatment interaction effect were significant. The three way interaction, site x treatment x cultivar, was significant for grain yield (Appendix 9). The average grain yield over the two sites for each cultivar were significantly higher in the protected plots compared to non-protected plots with the exception of Njoro Bw2, Kwale, Kenya Nyangumi, 102091, 97, 6436 and 6053 (Tables 3), indicating that stem rust caused losses irrespective of the type or level of resistance each cultivar possessed. Grain yield of the protected and non-protected plots varied within sites and the yield losses were more pronounced in susceptible cultivars (Table 3). Grain yield reduction was lowest in one of the resistant CIMMYT breeding line 102091(17.6%), however, not significantly different from the other moderately resistant or tolerant line 97 and local cultivar Kenya Nyangumi (Table 3). Breeding lines 102012, 103067, 103249 and 103247 displayed similar grain yield losses despite the fact that their AUDPC values were significantly different (Table 2). The mean grain yields across the two sites in the fungicide-protected plots of 3.62 t/ha was higher than the National average of 2.5 t/ha (Figures 3 and 4).

**Table 3: Grain yields (t/ha) in non-protected (Non-prot) and fungicide-protected (Prot) and yield loss (%) at Mau-Narok and KARI-Njoro**

| Cultivar | Grain yield | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mau-Narok | | | KARI-Njoro | | | Across sites | | |
| | Prot | Nonprot | Loss | Prot | Nonprot | Loss | Prot | Nonprot | Loss |
| | t/ha | | % | t/ha | | % | t/ha | | % |
| Njoro Bw1 | 4.7 | 2.9$^{ab}$ | 38.7* | 2.4 | 0.7$^{a}$ | 69.8* | 3.5 | 1.8$^{bc}$ | 49.2* |
| Njoro Bw2 | 6.6 | 6.0$^{a}$ | 8.4 | 3.6 | 1.9$^{a}$ | 46.0* | 5.0 | 4.0$^{a}$ | 21.6 |
| Chozi | 4.2 | 1.7$^{bc}$ | 59.7* | 2.2 | 0.5$^{a}$ | 78.9* | 3.2 | 1.1b$^{cd}$ | 66.3* |
| Duma | 4.2 | 2.0$^{bc}$ | 51.7* | 2.2 | 0.5$^{a}$ | 77.3* | 3.1 | 1.3b$^{cd}$ | 64.5* |
| Kwale | 4.9 | 1.8$^{ab}$ | 64.0* | 3.0 | 1.5$^{a}$ | 50.3 | 4.0 | 1.6a$^{bc}$ | 58.8* |
| Kenya Popo | 4.3 | 2.0$^{bc}$ | 53.7* | 2.8 | 1.5$^{a}$ | 48.2 | 3.5 | 1.7b$^{c}$ | 51.4* |
| K. Nyangumi | 5.7 | 4.7$^{ab}$ | 17.6 | 2.6 | 2.2$^{a}$ | 19.3 | 3.7 | 3.4$^{bc}$ | 18.5 |
| K. Kongoni | 5.1 | 3.3$^{ab}$ | 34.7* | 2.5 | 2.0$^{a}$ | 20.2 | 3.8 | 2.6$^{abc}$ | 29.8 |
| K. Fahari | 5.3 | 3.8$^{ab}$ | 28.0 | 2.3 | 0.9$^{a}$ | 60.8 | 3.8 | 2.4$^{abc}$ | 7.8* |
| 103141 | 2.5 | 0.6$^{c}$ | 4.3* | 1.7 | 0.7$^{a}$ | 62.2 | 2.1 | 0.6$^{d}$ | 69.2* |
| 103067 | 4.8 | 1.4$^{ab}$ | 71.3* | 3.2 | 2.4$^{a}$ | 25.8 | 4.0 | 1.9$^{abc}$ | 53.0* |
| 103108 | 5.2 | 4.1$^{ab}$ | 22.0 | 2.8 | 1.2$^{a}$ | 59.5* | 4.0 | 2.6$^{abc}$ | 35.2* |
| 102091 | 5.5 | 4.4$^{ab}$ | 18.7 | 2.9 | 2.4$^{a}$ | 15.3 | 4.2 | 3.4$^{abc}$ | 17.6 |
| 10101 | 4.0 | 2.3$^{bc}$ | 43.0* | 1.6 | 1.0$^{a}$ | 37.9 | 2.8 | 1.3$^{cd}$ | 41.4* |
| 103247 | 3.8 | 1.5$^{bc}$ | 60.0* | 1.8 | 1.1$^{a}$ | 41.1 | 2.8 | 1.3$^{cd}$ | 53.6* |
| 103249 | 3.9 | 1.4$^{bc}$ | 65.0* | 2.0 | 1.2$^{a}$ | 41.3 | 3.0 | 1.3$^{bcd}$ | 56.8* |
| 97 | 5.7 | 4.9$^{ab}$ | 14.0 | 2.3 | 2.1$^{a}$ | 7.0 | 4.0 | 3.5$^{abc}$ | 12.0 |
| 6436 | 5.2 | 4.0$^{ab}$ | 23.7 | 3.0 | 2.2$^{a}$ | 27.9 | 4.1 | 3.1$^{abc}$ | 5.9 |
| 6053 | 5.0 | 4.1$^{ab}$ | 17.4 | 2.1 | 1.6$^{a}$ | 23.5 | 3.5 | 2.9$^{bc}$ | 18.9 |
| 102012 | 5.2 | 2.4$^{ab}$ | 28.6 | 3.4 | 2.4$^{a}$ | 28.3 | 4.3 | 2.4$^{ab}$ | 44.3* |
| LSD (0.05) | 1.7 | | - | 1.6 | | - | 1.4 | | - |
| CV % | 19.8 | | - | 28.3 | | - | 26.4 | | - |

*-Significant at 5% (0.05) level of probability.

*a, b, c and d - Means with the same letter in the same column are not significantly different as per LSD means separation procedure (P =0.05).*

## 4.4 Yield Components affected by Ug99

Significant differences in yield components were observed for each of the main effects, site, treatment, cultivar, and site x cultivar, and treatment x cultivar interaction. Site x treatment interaction effect was significant for only thousand kernel weight. The three way interaction, site x treatment x cultivar, was also significant for thousand kernel weight. Mean thousand kernel

weight loss (based on weight of 1000 grains) across the sites was reduced significantly for all cultivars in non-protected plots compared with the protected plots (Tables 4). Except for the breeding lines 102091, 103067, 102012, 6436 and 6053, the kernel weight of the remaining 15 cultivars was significantly lower in the non-protected plots compared with the fungicide-protected plots (Figures 7 and 8). The kernel weight of Kwale, which is reported to carry resistance gene *Sr*31 was significantly higher than Chozi (control) which possess the same gene, and several other susceptible cultivars. This may suggest that Kwale may also be carrying an unknown minor gene(s) that ameliorates the damage caused by the pathogen. The kernel weight of all the cultivars showed a significant loss in the non-protected plots (Table 4). Breeding lines 10101, 97 and 6436 were moderately resistant compared to 102091, which was the most resistant of all the cultivars under investigation.

**Table 4. Thousand kernel weight (g) and losses (%) of wheat cultivars in non-protected (Non-prot) and fungicide-protected (Prot) plots at Mau-Narok and KARI-Njoro.**

| | Thousand kernel weight (g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mau-Narok | | | KARI-Njoro | | | Across sites | | |
| Cultivar | Prot | Non-prot | Loss (%) | Prot | Non-prot | Loss (%) | Prot (%) | Non-prot | Loss (%) |
| Njoro Bw1 | 50.1 | 35.9$^{bcd}$ | 28.4* | 40.0 | 22.3$^{cdef}$ | 44.2* | 45.1 | 29.1$^{bcd}$ | 35.5* |
| Njoro Bw2 | 44.8 | 41.8$^{abc}$ | 6.7* | 35.5 | 24.9$^{def}$ | 30.0* | 40.2 | 33.3$^{bcd}$ | 17.0* |
| Chozi | 52.1 | 29.0$^{def}$ | 44.4* | 41.5 | 20.1$^{cdef}$ | 50.5* | 46.8 | 24.8$^{cd}$ | 47.1* |
| Duma | 49.5 | 32.7$^{def}$ | 34.1* | 39.9 | 17.7$^{ef}$ | 55.5* | 44.7 | 25.2$^{d}$ | 43.6* |
| Kwale | 49.5 | 34.1$^{cde}$ | 31.0* | 38.9 | 27.7$^{abcd}$ | 28.9* | 44.2 | 30.9$^{abcd}$ | 30.1* |
| Kenya Popo | 46.5 | 34.6$^{def}$ | 25.7* | 39.2 | 26.2$^{abcde}$ | 33.3* | 42.9 | 30.4$^{bcd}$ | 29.2* |
| Kenya Nyangumi | 37.2 | 28.3$^{i}$ | 23.8* | 32.9 | 22.5$^{f}$ | 31.6* | 35.0 | 25.4$^{e}$ | 27.4* |
| Kenya Kongoni | 46.1 | 36.5$^{cdef}$ | 20.7* | 41.6 | 25.9$^{abcd}$ | 37.8* | 43.8 | 31.2$^{abcd}$ | 28.8* |
| Kenya Fahari | 42.9 | 35.4$^{efg}$ | 17.6* | 36.0 | 24.7$^{def}$ | 31.2* | 39.4 | 30.1$^{d}$ | 23.8* |
| 103141 | 44.7 | 28.2$^{gh}$ | 36.9* | 35.4 | 27.5$^{bcdef}$ | 22.2* | 40.0 | 27.9$^{de}$ | 30.4* |
| 103067 | 44.8 | 26.6$^{hi}$ | 40.5* | 37.8 | 30.7$^{abcd}$ | 17.5* | 41.0 | 28.7$^{d}$ | 30.0* |
| 103108 | 50.8 | 40.2$^{ab}$ | 20.9* | 42.8 | 28.7$^{a}$ | 33.0* | 46.8 | 34.4$^{ab}$ | 26.4* |
| 102091 | 48.3 | 38.0$^{bcd}$ | 21.3* | 38.5 | 33.1$^{a}$ | 14.1 | 43.4 | 35.5$^{abc}$ | 18.1* |
| 10101 | 56.7 | 37.2$^{a}$ | 34.5* | 41.6 | 29.6$^{a}$ | 28.8* | 49.2 | 33.4$^{a}$ | 32.1* |
| 103247 | 44.1 | 33.0$^{fgh}$ | 25.1* | 35.0 | 27.1$^{cdef}$ | 22.4* | 39.5 | 30.1$^{d}$ | 23.9* |
| 103249 | 44.1 | 27.0$^{hi}$ | 38.9* | 36.3 | 27.7$^{abcde}$ | 23.6* | 40.2 | 27.4$^{de}$ | 32.0* |
| 97 | 48.8 | 39.6$^{abc}$ | 18.9* | 37.9 | 29.8$^{abcd}$ | 21.4* | 43.4 | 34.7$^{abc}$ | 20.0* |
| 6436 | 42.4 | 36.6$^{ef}$ | 13.9* | 33.4 | 27.3$^{def}$ | 18.4* | 37.9 | 31.9$^{d}$ | 15.8* |
| 6053 | 41.0 | 37.0$^{efg}$ | 9.7* | 37.9 | 30.9$^{abc}$ | 18.5* | 39.4 | 33.9$^{bcd}$ | 13.9* |
| 102012 | 47.8 | 31.1$^{egf}$ | 34.9* | 38.6 | 31.8$^{ab}$ | 17.6* | 43.2 | 31.5$^{abcd}$ | 27.2* |
| LSD(0.05) | 3.0 | | - | 5.6 | | - | 3.1 | | - |
| CV% | 4.5 | | - | 10.6 | | - | 7.5 | | - |

*-Significant at 5% (0.05) level of probability

*a, b, c, d, e, f, g and h - Means with the same letter in the same column are not significantly different as per LSD means separation procedure (P =0.05)*

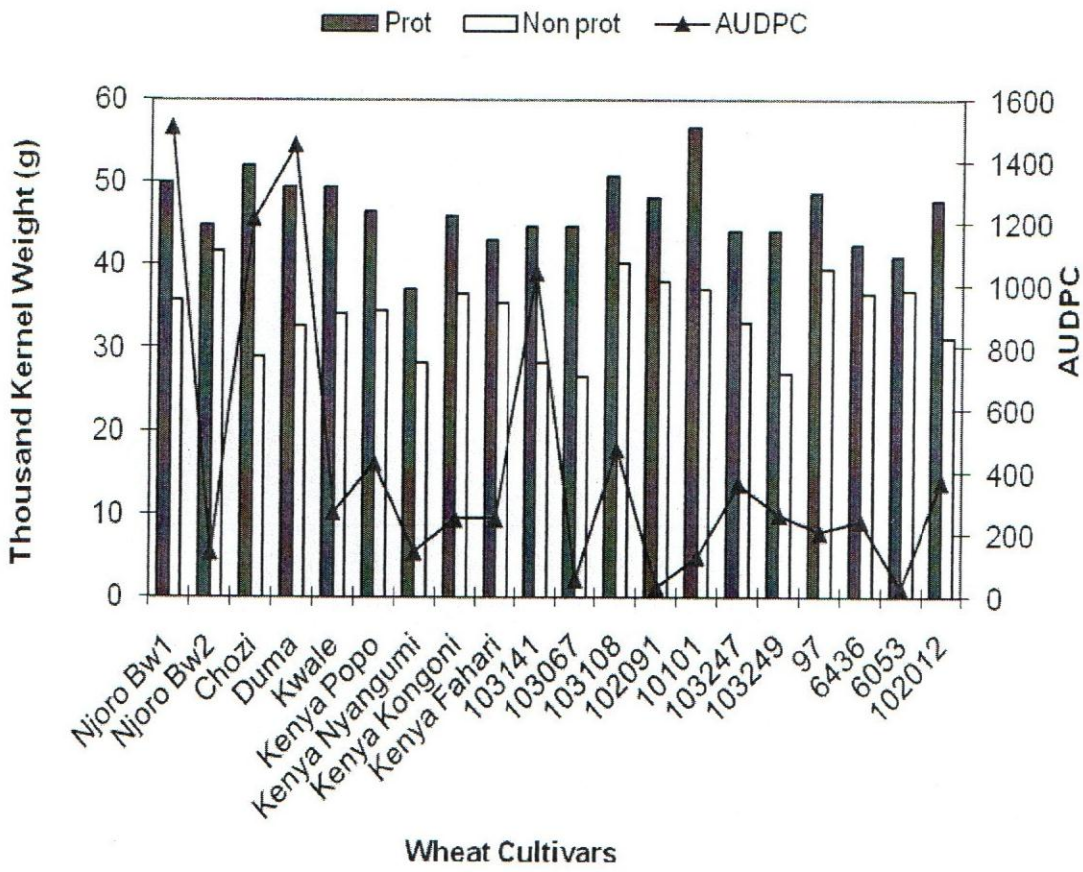



**Figure 7: Effect of area under the disease progress curve (AUDPC) on thousand kernel weight of wheat cultivars at Mau-Narok**

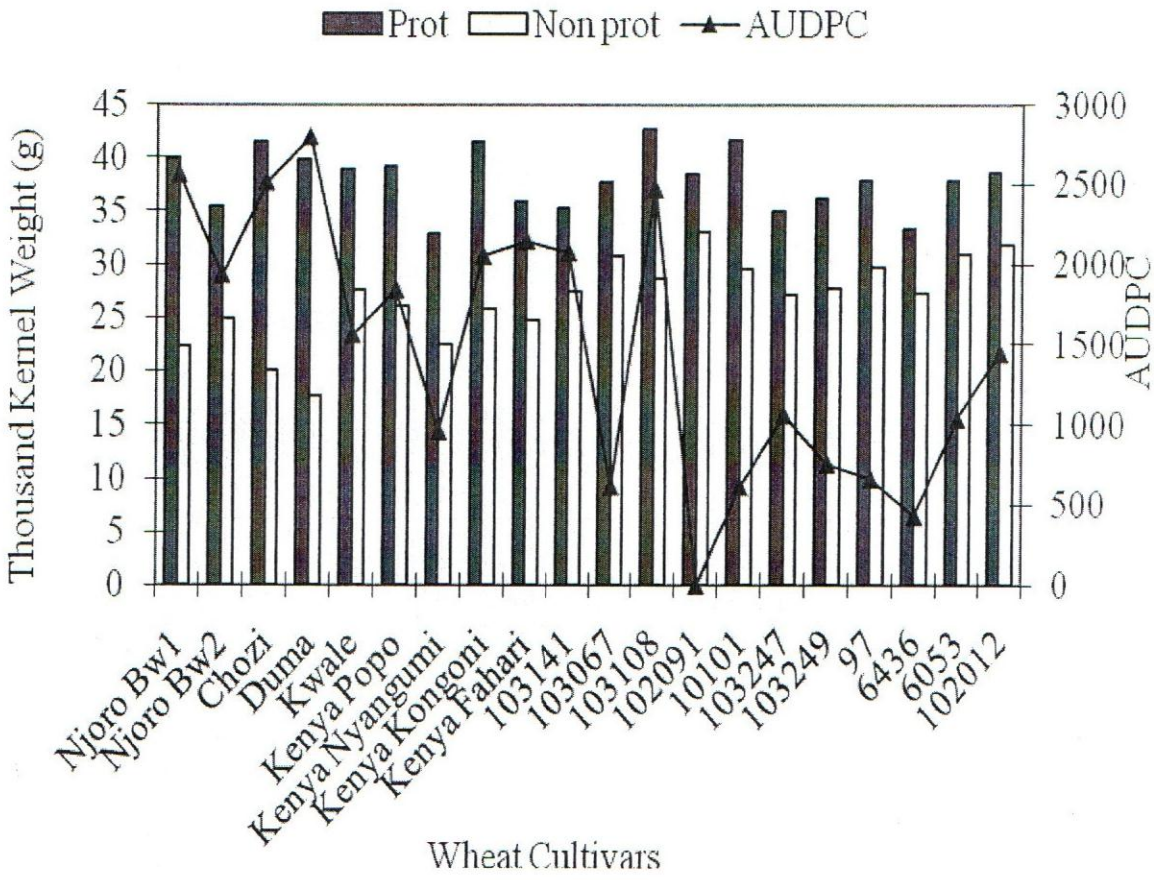



**Figure 8: Effect of area under the disease progress curve (AUDPC) on thousand kernel weight of wheat cultivars at KARI-Njoro**

A significant reduction in spikes per plant was evident for seven cultivars, Njoro Bw2, Kwale, Kenya Fahari, 103141, 103247, 103249 and 6436 (Tables 5). There was no significant loss in other cultivars (Figures 9 and 10). Except for Njoro Bw2, Kenya Nyangumi and breeding lines 102091, 97 and 6053 (Tables 6), the number of kernels per spike of the remaining 15 cultivars were significantly lower in non-protected plots (Figures 11 and 12). The kernel shrivelling or grain integrity based on a 1- 5 scale was significantly reduced for Njoro Bw1, Duma, 103141, 103067, 10101, 103247, 103249 and 102012 cultivars in the non-protected treatments (Tables 7). The scores for kernel shrivelling of the remaining cultivars including, Njoro Bw2, Kwale and Kenya Nyangumi was not significantly reduced in the non-protected treatment (Figures 13 and 14).

**Table 5. Spikes per plant and losses (%) of wheat cultivars in non-protected (Non-prot) and fungicide-protected (Prot) plots at Mau-Narok and KARI-Njoro.**

| | Spikes per plant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mau-Narok | | | KARI-Njoro | | | Across sites | | |
| | Prot | Nonprot | Loss (%) | Prot | Nonprot | Loss (%) | Prot | Nonprot | Loss (%) |
| Cultivar | number | | | number | | | number | | |
| Njoro Bw1 | 14.7 | 10.3$^{bcd}$ | 29.6* | 6.0 | 5.7$^{bcd}$ | 5.5 | 10.2 | 8.2$^{cde}$ | 19.7 |
| Njoro Bw2 | 13.7 | 11.0$^{bcd}$ | 19.5 | 8.3 | 6.0$^{ab}$ | 28.0* | 11.0 | 8.5$^{cde}$ | 22.7* |
| Chozi | 11.7 | 10.7$^{bcd}$ | 8.6 | 7.0 | 5.3$^{cd}$ | 23.9* | 8.8 | 8.5$^{cde}$ | 3.7 |
| Duma | 13.7 | 10.0$^{bcd}$ | 26.9 | 5.7 | 5.3$^{cd}$ | 6.0 | 9.5 | 7.8$^{de}$ | 17.6 |
| Kwale | 18.3 | 11.3$^{ab}$ | 38.2* | 7.3 | 5.7$^{bcd}$ | 22.7* | 12.8 | 8.5$^{cde}$ | 33.7* |
| Kenya Popo | 11.7 | 10.7$^{bcd}$ | 8.6 | 6.7 | 6.0$^{bcd}$ | 10.0 | 8.8 | 8.7$^{cde}$ | 1.8 |
| K. Nyangumi | 16.3 | 13.0$^{abc}$ | 20.4 | 8.7 | 8.3$^{a}$ | 3.9 | 12.3 | 10.8$^{ab}$ | 12.2 |
| K. Kongoni | 17.0 | 13.0$^{ab}$ | 23.5 | 7. 3 | 6.7$^{abcd}$ | 9.0 | 12.2 | 9.8$^{abc}$ | 19.5 |
| K. Fahari | 13.3 | 7.7$^{cd}$ | 42.5* | 6.7 | 5.7$^{bcd}$ | 15.0 | 10.0 | 6.7$^{ef}$ | 33.3* |
| 103141 | 17.0 | 9.0$^{bc}$ | 47.1* | 5.7 | 5.7$^{bcd}$ | 0.0 | 11.3 | 7.3$^{de}$ | 35.3* |
| 103067 | 13.7 | 12.7$^{bc}$ | 7.3 | 8.3 | 6.00$^{ab}$ | 28.0* | 11.0 | 10.0$^{bcde}$ | 9.1 |
| 103108 | 14.0 | 11.0$^{bcd}$ | 21.4 | 6.0 | 5.7$^{bcd}$ | 5.5 | 10.0 | 8.3$^{cde}$ | 16.7 |
| 102091 | 20.3 | 16.3$^{a}$ | 19.7 | 8.3 | 7.3$^{ab}$ | 12.0 | 13.8 | 12.3$^{a}$ | 10.9 |
| 10101 | 8.3 | 8.3$^{d}$ | 0.0 | 4.0 | 4.0$^{e}$ | 0.0 | 6.2 | 6.2$^{f}$ | 0.0 |
| 103247 | 16.7 | 10.7$^{bc}$ | 36.0* | 7.7 | 5.0$^{de}$ | 34.8* | 12.2 | 7.8$^{bcde}$ | 35.7* |
| 103249 | 16.0 | 10.7$^{bc}$ | 33.3* | 7.3 | 5.3$^{cd}$ | 27.3* | 11.7 | 8.0$^{bcde}$ | 31.5* |
| 97 | 13.3 | 11.7$^{bcd}$ | 12.5 | 7.0 | 6.7$^{abcd}$ | 4.7 | 10.2 | 9.2$^{bcde}$ | 9.8 |
| 6436 | 17.3 | 10.7$^{bc}$ | 38.4* | 7.0 | 6.7$^{abcd}$ | 4.7 | 12.0 | 8.8$^{bcde}$ | 26.4* |
| 6053 | 17.3 | 12.0$^{abc}$ | 30.8* | 6.3 | 5.3$^{cd}$ | 15.8 | 11.3 | 9.2$^{bcde}$ | 19.1 |
| 102012 | 12.3 | 11.0$^{bcd}$ | 10.8 | 7.0 | 5.7$^{bcd}$ | 19.0 | 9.7 | 8.3$^{bcde}$ | 13.9 |
| LSD | 4.3 | | - | 1.6 | | - | 2.3 | | - |
| CV % | 29.1 | | - | 21.3 | | - | 29.3 | | - |

*-Significant at 5% (0.05) level of probability.

*a, b, c, d, e and f - Means with the same letter in the same column are not significantly different as per LSD means separation procedure (P =0.05).*

**Table 5. Spikes per plant and losses (%) of wheat cultivars in non-protected (Non-prot) and fungicide-protected (Prot) plots at Mau-Narok and KARI-Njoro.**

| | Spikes per plant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mau-Narok | | | KARI-Njoro | | | Across sites | | |
| | Prot | Nonprot | Loss (%) | Prot | Nonprot | Loss (%) | Prot | Nonprot | Loss (%) |
| Cultivar | number | | | number | | | number | | |
| Njoro Bw1 | 14.7 | 10.3$^{bcd}$ | 29.6* | 6.0 | 5.7$^{bcd}$ | 5.5 | 10.2 | 8.2$^{cde}$ | 19.7 |
| Njoro Bw2 | 13.7 | 11.0$^{bcd}$ | 19.5 | 8.3 | 6.0$^{ab}$ | 28.0* | 11.0 | 8.5$^{cde}$ | 22.7* |
| Chozi | 11.7 | 10.7$^{bcd}$ | 8.6 | 7.0 | 5.3$^{cd}$ | 23.9* | 8.8 | 8.5$^{cde}$ | 3.7 |
| Duma | 13.7 | 10.0$^{bcd}$ | 26.9 | 5.7 | 5.3$^{cd}$ | 6.0 | 9.5 | 7.8$^{de}$ | 17.6 |
| Kwale | 18.3 | 11.3$^{ab}$ | 38.2* | 7.3 | 5.7$^{bcd}$ | 22.7* | 12.8 | 8.5$^{cde}$ | 33.7* |
| Kenya Popo | 11.7 | 10.7$^{bcd}$ | 8.6 | 6.7 | 6.0$^{bcd}$ | 10.0 | 8.8 | 8.7$^{cde}$ | 1.8 |
| K. Nyangumi | 16.3 | 13.0$^{abc}$ | 20.4 | 8.7 | 8.3$^{a}$ | 3.9 | 12.3 | 10.8$^{ab}$ | 12.2 |
| K. Kongoni | 17.0 | 13.0$^{ab}$ | 23.5 | 7. 3 | 6.7$^{abcd}$ | 9.0 | 12.2 | 9.8$^{abc}$ | 19.5 |
| K. Fahari | 13.3 | 7.7$^{cd}$ | 42.5* | 6.7 | 5.7$^{bcd}$ | 15.0 | 10.0 | 6.7$^{ef}$ | 33.3* |
| 103141 | 17.0 | 9.0$^{bc}$ | 47.1* | 5.7 | 5.7$^{bcd}$ | 0.0 | 11.3 | 7.3$^{de}$ | 35.3* |
| 103067 | 13.7 | 12.7$^{bc}$ | 7.3 | 8.3 | 6.00$^{ab}$ | 28.0* | 11.0 | 10.0$^{bcde}$ | 9.1 |
| 103108 | 14.0 | 11.0$^{bcd}$ | 21.4 | 6.0 | 5.7$^{bcd}$ | 5.5 | 10.0 | 8.3$^{cde}$ | 16.7 |
| 102091 | 20.3 | 16.3$^{a}$ | 19.7 | 8.3 | 7.3$^{ab}$ | 12.0 | 13.8 | 12.3$^{a}$ | 10.9 |
| 10101 | 8.3 | 8.3$^{d}$ | 0.0 | 4.0 | 4.0$^{e}$ | 0.0 | 6.2 | 6.2$^{f}$ | 0.0 |
| 103247 | 16.7 | 10.7$^{bc}$ | 36.0* | 7.7 | 5.0$^{de}$ | 34.8* | 12.2 | 7.8$^{bcde}$ | 35.7* |
| 103249 | 16.0 | 10.7$^{bc}$ | 33.3* | 7.3 | 5.3$^{cd}$ | 27.3* | 11.7 | 8.0$^{bcde}$ | 31.5* |
| 97 | 13.3 | 11.7$^{bcd}$ | 12.5 | 7.0 | 6.7$^{abcd}$ | 4.7 | 10.2 | 9.2$^{bcde}$ | 9.8 |
| 6436 | 17.3 | 10.7$^{bc}$ | 38.4* | 7.0 | 6.7$^{abcd}$ | 4.7 | 12.0 | 8.8$^{bcde}$ | 26.4* |
| 6053 | 17.3 | 12.0$^{abc}$ | 30.8* | 6.3 | 5.3$^{cd}$ | 15.8 | 11.3 | 9.2$^{bcde}$ | 19.1 |
| 102012 | 12.3 | 11.0$^{bcd}$ | 10.8 | 7.0 | 5.7$^{bcd}$ | 19.0 | 9.7 | 8.3$^{bcde}$ | 13.9 |
| LSD | 4.3 | | - | 1.6 | | - | 2.3 | | - |
| CV % | 29.1 | | - | 21.3 | | - | 29.3 | | - |

*-Significant at 5% (0.05) level of probability.

*a, b, c, d, e and f - Means with the same letter in the same column are not significantly different as per LSD means separation procedure (P =0.05).*

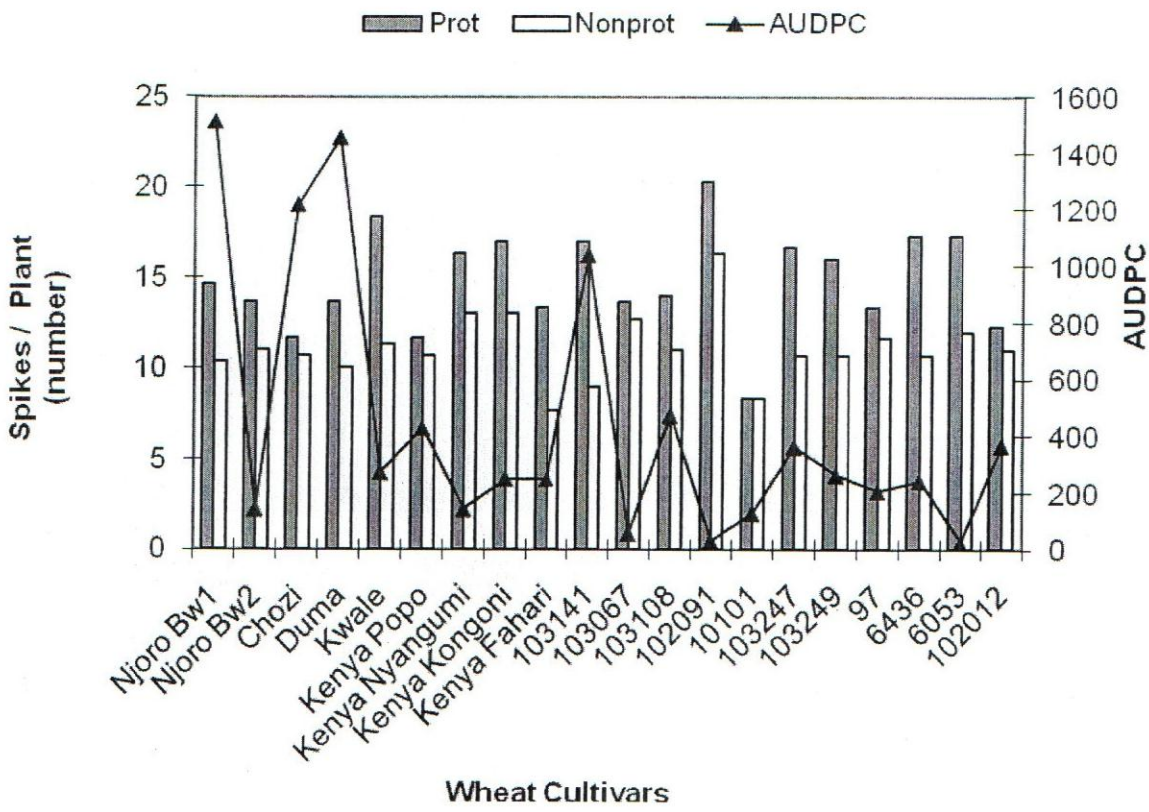



**Figure 9: Effect of area under the disease progress curve (AUDPC) on number of spikes per plant of wheat cultivars at Mau-Narok**

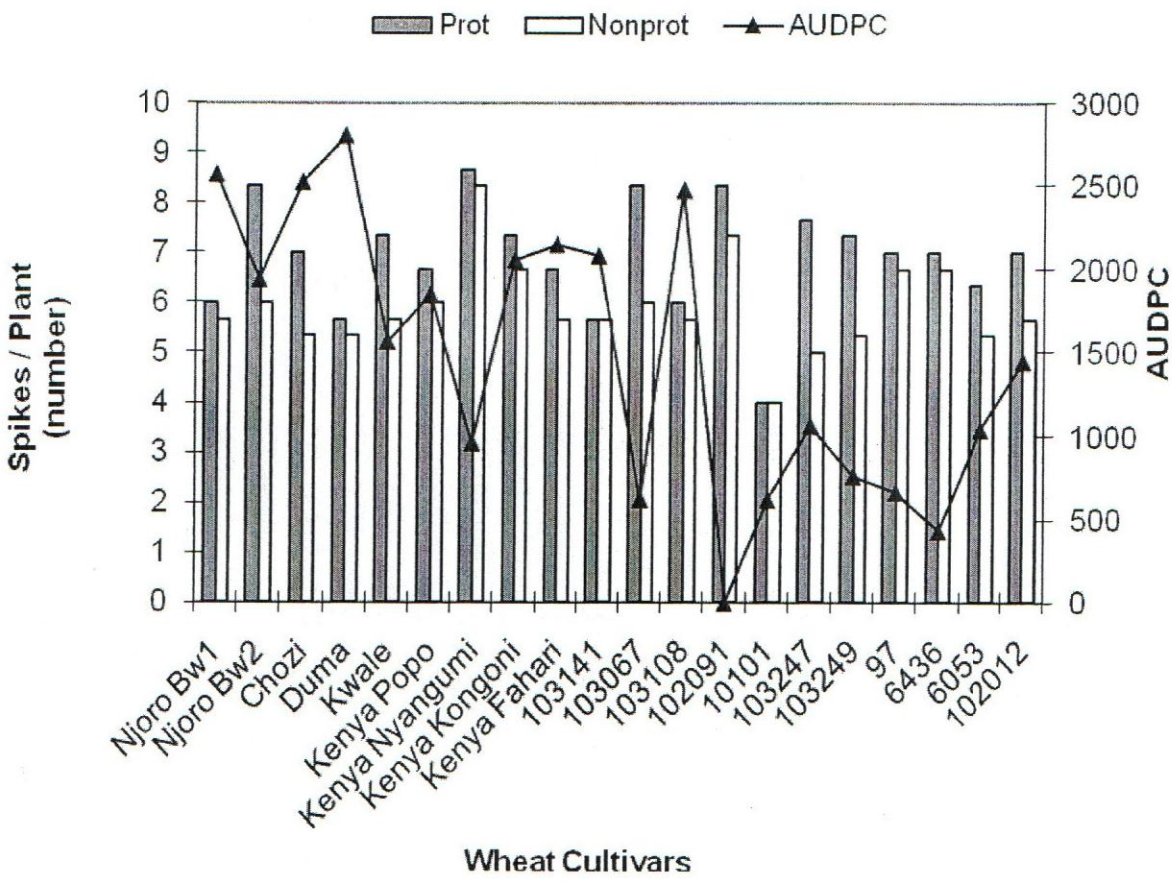



**Figure 10: Effect of area under the disease progress curve (AUDPC) on number of spikes per plant of wheat cultivars at KARI-Njoro**

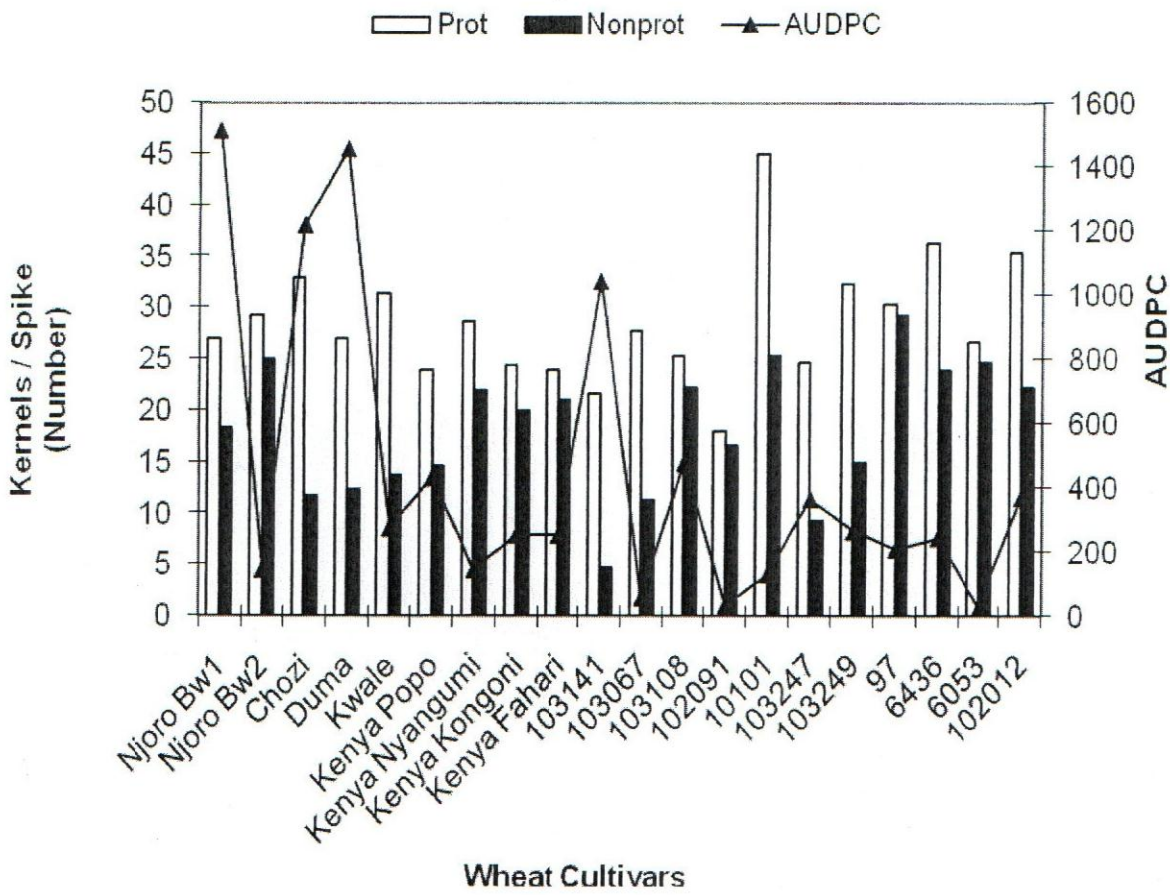



**Figure 11: Effect of stem rust area under the disease progress curve (AUDPC) on the number of kernels per spike of wheat cultivars at Mau-Narok**

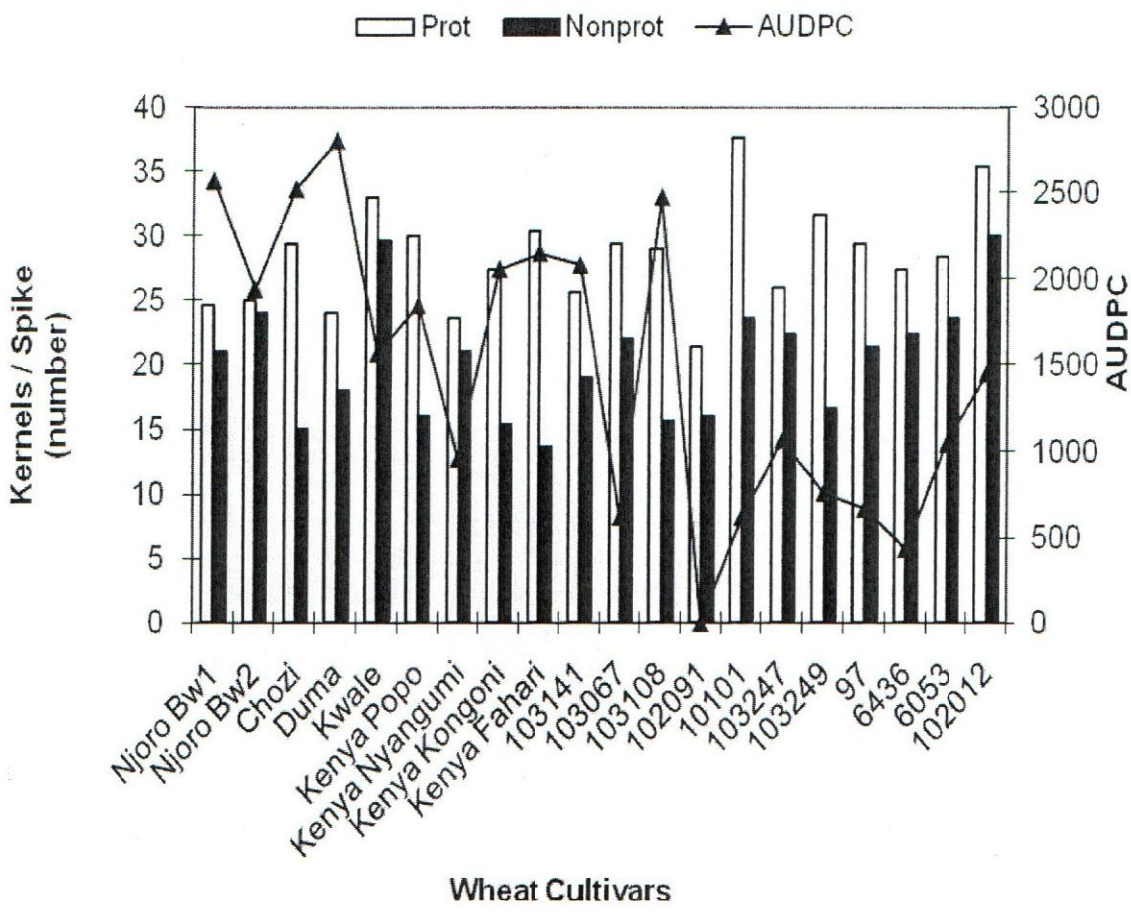



**Figure 12. Effect of stem rust area under the disease progress curve (AUDPC) on the number kernels per spike of wheat cultivars at KARI-Njoro**

**Table 6: Kernels per spike and yield loss of wheat cultivars in non-protected (Non-prot) and fungicide-protected (Prot) plots at Mau-Narok and KARI-Njoro**

| | Kernels per spike | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mau-Narok | | | KARI-Njoro | | | | | |
| | Prot | Nonprot | loss | Prot | Nonprot | loss | Prot | Nonprot | loss |
| Cultivar | number | | (%) | number | | (%) | number | | (%) |
| Njoro Bw1 | 27.0$^{cdef}$ | 18.3 | 32.1* | 24.7$^{cd}$ | 21.0 | 14.9 | 25.8$^{cdefgh}$ | 19.7 | |
| Njoro Bw2 | 29.3$^{bcd}$ | 25.0 | 14.8 | 25.0$^{bcd}$ | 24.0 | 4.0 | 27.2$^{cdef}$ | 24.5 | |
| Chozi | 33.0$^{cdef}$ | 11.7 | 64.6* | 29.3$^{d}$ | 15.0 | 48.9* | 31.2$^{defgh}$ | 13.3 | |
| Duma | 27.0$^{defg}$ | 12.3 | 54.3* | 24.0$^{d}$ | 18.0 | 25.0 | 25.5$^{fgh}$ | 15.2 | |
| Kwale | 31.3$^{cdef}$ | 13.7 | 56.4* | 33.0$^{ab}$ | 29.7 | 10.1 | 32.2$^{bcd}$ | 21.7 | |
| Kenya Popo | 24.0$^{defg}$ | 14.7 | 38.9* | 30.0$^{cd}$ | 16.0 | 46.7* | 27.0$^{efgh}$ | 15.3 | |
| K. Nyangumi | 28.7$^{bcd}$ | 22.0 | 23.3* | 23.7$^{d}$ | 21.0 | 11.3 | 26.2$^{cdef}$ | 21.5 | |
| K. Kongoni | 24.3$^{def}$ | 20.0 | 17.8 | 27.3$^{d}$ | 15.3 | 43.9 | 25.8$^{efgh}$ | 17.7 | |
| Kenya Fahari | 24.0$^{cdef}$ | 21.0 | 12.5 | 30.3$^{d}$ | 13.7 | 54.9* | 27.2$^{defgh}$ | 17.3 | |
| 103141 | 21.7$^{g}$ | 4.7 | 78.4* | 25.7$^{d}$ | 19.0 | 26.0 | 23.7$^{h}$ | 11.8 | |
| 103067 | 27.7$^{defg}$ | 11.3 | 59.1* | 29.3$^{abcd}$ | 22.0 | 25.0 | 28.5$^{cdefgh}$ | 16.7 | |
| 103108 | 25.3$^{bcde}$ | 22.3 | 11.8 | 29.0$^{d}$ | 15.7 | 46.0* | 25.7$^{cdefg}$ | 20.5 | |
| 102091 | 18.0$^{efg}$ | 16.7 | 7.4 | 21.3$^{d}$ | 16.0 | 25.0 | 19.7$^{gh}$ | 16.3 | |
| 10101 | 45.0$^{a}$ | 25.3 | 43.9* | 37.7$^{abc}$ | 23.7 | 37.2 | 41.3$^{a}$ | 24.5 | |
| 103247 | 24.7$^{fg}$ | 9.3 | 62.2* | 26.0$^{bcd}$ | 22.3 | 14.1 | 25.3$^{fgh}$ | 15.8 | |
| 103249 | 32.3$^{bcde}$ | 15.0 | 53.6* | 31.7$^{bcd}$ | 16.7 | 47.4* | 32.0$^{cdef}$ | 15.8 | |
| 97 | 30.3$^{ab}$ | 29.3 | 3.3 | 29.3$^{abcd}$ | 21.3 | 27.3 | 29.8$^{bc}$ | 25.3 | |
| 6436 | 36.3$^{ab}$ | 24.0 | 33.9* | 27.3$^{abcd}$ | 22.3 | 18.3 | 31.8$^{bc}$ | 23.2 | |
| 6053 | 26.7$^{bcd}$ | 24.7 | 7.5 | 28.3$^{abcd}$ | 23.7 | 16.4 | 27.5$^{bc}$ | 24.2 | |
| 102012 | 35.3$^{abc}$ | 22.3 | 36.8* | 35.3$^{a}$ | 30.0 | 15.1 | 35.3$^{bcde}$ | 26.2 | |
| LSD(0.05) | 6.5 | | - | 8.1 | | - | 5.2 | | |
| CV % | 24.3 | | - | 29.0 | | - | 26.1 | | |

*-Significant at 5% (0.05) level of probability

*a, b, c, d, e, f, g and f - Means with the same letter in the same column are not significantly different as per the LSD means separation procedure (P = 0.05).*

**Table 7: Kernel shriveling for wheat cultivars in non-protected (Non-prot) and fungicide-protected (Prot) plots for Mau-Narok and KARI-Njoro**

| | Kernel Shriveling | | | | | |
|---|---|---|---|---|---|---|
| | Mau-Narok | | KARI-Njoro | | Across sites | |
| | Prot | Nonprot | Prot | Nonprot | Prot | Nonprot |
| Cultivar | | | number | | | |
| Njoro Bw1 | 1.0* | 1.8$^{cdef}$ | 1.0* | 2.5$^{abcde}$ | 1.0* | 2.2$^{cde}$ |
| Njoro Bw2 | 1.0 | 1.3$^{f}$ | 2.3* | 3.2$^{abc}$ | 1.7 | 2.3$^{bcde}$ |
| Chozi | 1.0* | 2.8$^{abc}$ | 1.0* | 2.5$^{abcde}$ | 1.0 | 2.7$^{abcd}$ |
| Duma | 1.0* | 2.2$^{abcdef}$ | 2.0* | 3.0$^{abcd}$ | 1.5* | 2.6$^{abcde}$ |
| Kwale | 1.3* | 2.3$^{abcdef}$ | 3.0 | 3.2$^{abc}$ | 2.2 | 2.8$^{abc}$ |
| Kenya Popo | 1.0* | 2.0$^{bcdef}$ | 1.7* | 2.2$^{cdef}$ | 1.3 | 2.1$^{cdef}$ |
| Kenya Nyangumi | 2.0* | 2.5$^{abcde}$ | 3.0* | 3.5$^{ab}$ | 2.5 | 3.0$^{ab}$ |
| Kenya Kongoni | 1.0* | 1.3$^{f}$ | 2.0* | 2.3$^{bcdef}$ | 1.5 | 1.8$^{ef}$ |
| Kenya Fahari | 1.3* | 2.0$^{bcdef}$ | 1.3* | 1.7$^{ef}$ | 1.3 | 1.8$^{ef}$ |
| 103141 | 1.0* | 2.7$^{abcd}$ | 1.3* | 2.2$^{cdef}$ | 1.2* | 2.4$^{bcde}$ |
| 103067 | 1.0* | 2.8$^{abc}$ | 1.7 | 1.8$^{def}$ | 1.3* | 2.3$^{bcde}$ |
| 103108 | 1.0* | 1.5$^{ef}$ | 2.0* | 2.3$^{bcdef}$ | 1.5 | 1.9$^{def}$ |
| 102091 | 1.0* | 1.5$^{ef}$ | 1.0 | 1.2$^{f}$ | 1.0 | 1.3$^{f}$ |
| 10101 | 1.7* | 3.2$^{a}$ | 3.00* | 3.5$^{ab}$ | 2.3* | 3.3$^{a}$ |
| 103247 | 1.0* | 2.2$^{abcdef}$ | 1.7* | 2.5$^{abcde}$ | 1.3* | 2.3$^{bcde}$ |
| 103249 | 1.0* | 3.0$^{ab}$ | 2.3* | 3.0$^{abcd}$ | 1.7* | 3.0$^{ab}$ |
| 97 | 1.0* | 1.3$^{f}$ | 2.0* | 2.3$^{bcdef}$ | 1.5 | 1.8$^{ef}$ |
| 6436 | 1.0* | 1.7$^{def}$ | 3.3* | 3.67$^{a}$ | 2.2 | 2.7$^{abcd}$ |
| 6053 | 1.0* | 1.5$^{ef}$ | 2.3* | 2.7$^{abcde}$ | 1.7 | 2.1$^{cdef}$ |
| 102012 | 1.3* | 2.3$^{abcdef}$ | 2.0* | 2.3$^{bcdef}$ | 1.7* | 2.3$^{bcde}$ |
| LSD(0.05) | 1.2 | | 1.2 | | 0.4 | |
| CV % | 22.4 | | 21.8 | | 22.2 | |

*-Significant at 5% (0.05) level of probability.

*a, b, c, d, e and f - Means with the same letter in the same column are not significantly different as per LSD means*

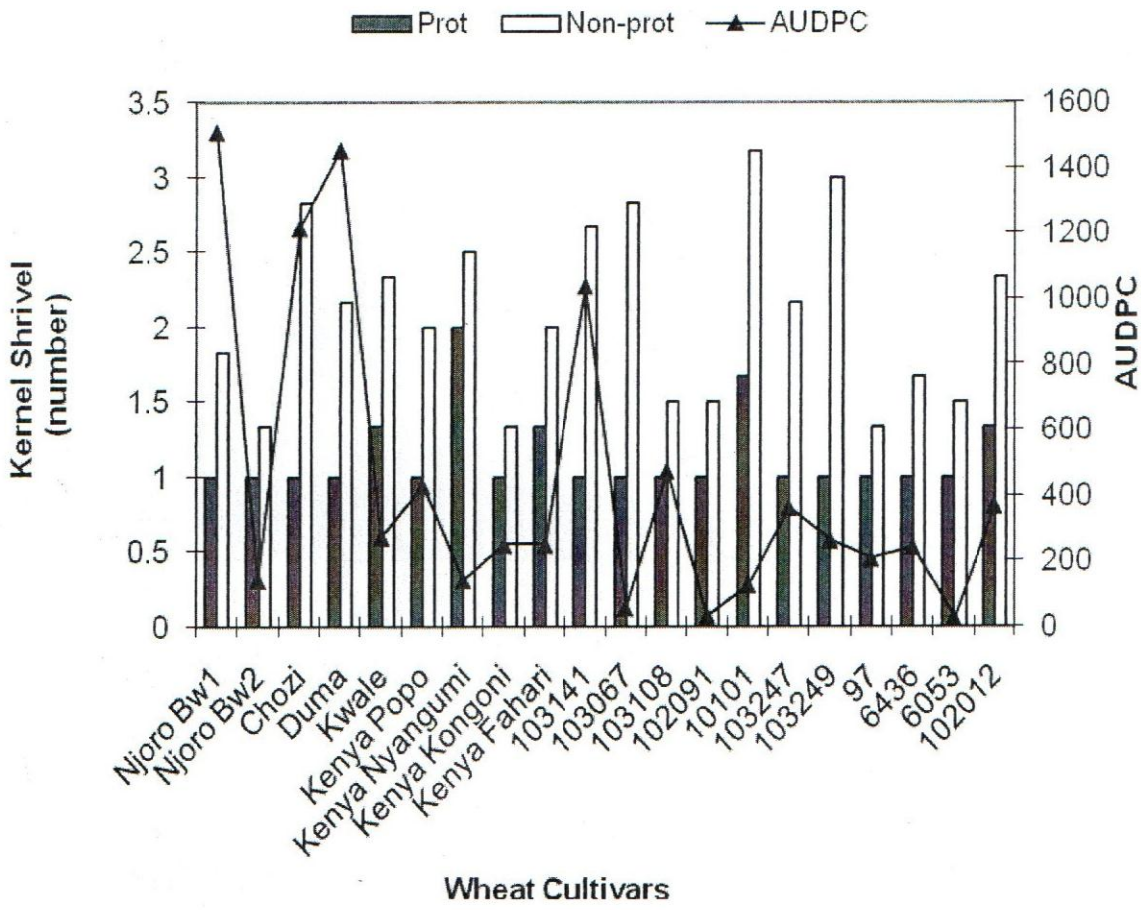



**Figure 13: Effect of area under the disease progress curve (AUDPC) on kernel shriveling of wheat cultivars at Mau-Narok**

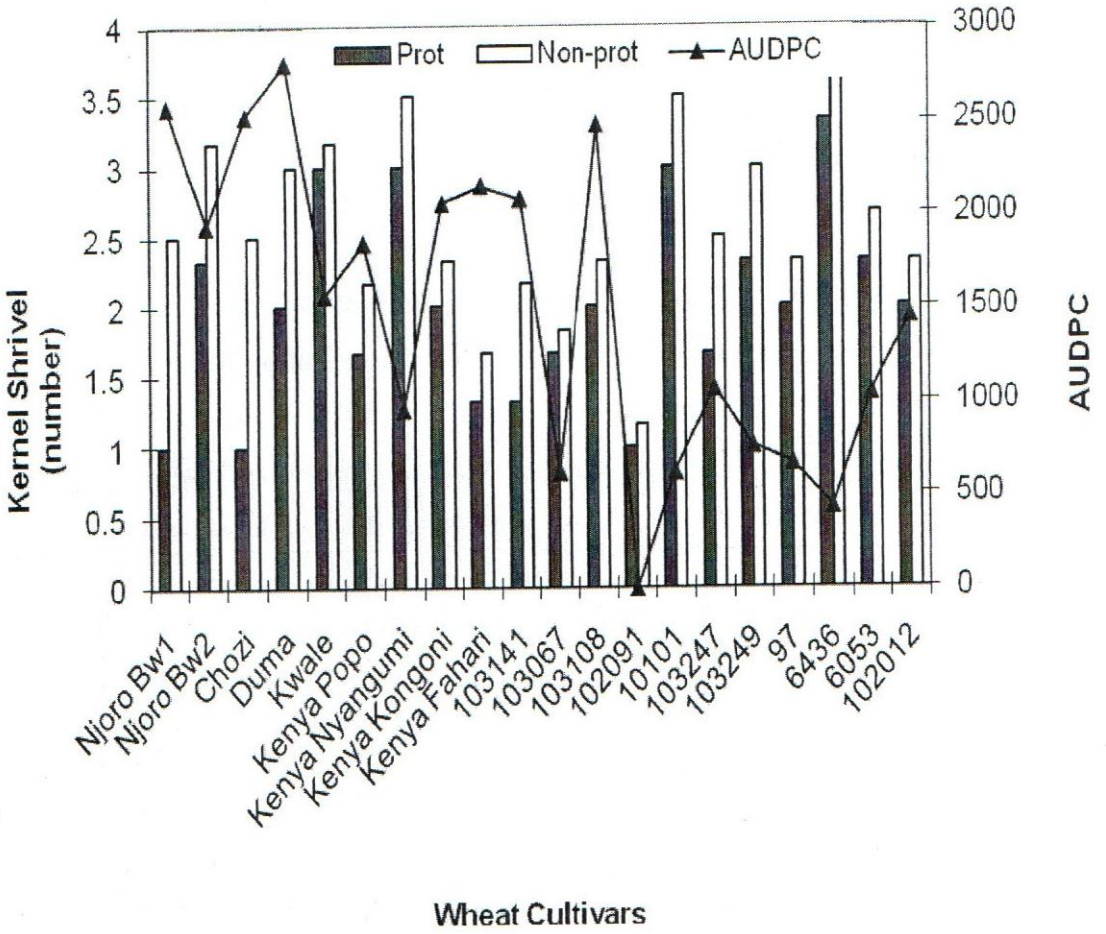



**Figure 14: Effect of area under the disease progress curve (AUDPC) on kernel shriveling of wheat cultivars at KARI-Njoro**

### 4.5 Phenotypic Correlations among Disease, Grain Yield, and Yield Components

The correlation coefficient (r) among disease severity, grain yield, yield loss and yield components depicted both positive and negative values that ranged from 0 to 0.98 (Table 8). The 'r' values of < 0.4, 0.4 - 0.6 and > 0.6 were categorized low, moderate and high, respectively in these results.

It was observed that:

(a) Grain yield had, (i) high, negative and statistically significant relationships with yield loss (r = -0.93*) in non-protected plots, but was not significant in protected plots (r = -0.58), (ii) moderate, positive and significant association with thousand kernel weight (r = 0.56), (iii) high, positive and significant correlation with kernels per spike (r = 0.62), (iv)

moderate, positive and significant correlation with spikes per plant (r = 0.58), (v) moderate, negative and significant correlation with kernel shrivel (r = -0.44) and (vi) moderate, negative and significant correlation with AUDPC (r = -0.44) in non-protected plots.

(b) Besides its negative relationship with grain yield, yield loss recorded significant negative relationships with other yield components in non-protected plots, (i) moderate, negative and significant relationship with thousand kernel weight (r = -0.57), (ii) high, negative and significant association with kernels per spike (r = -0.63), (iii) moderate, negative and significant correlation with spikes per plant (r = -0.54), (iv) moderate, negative and significant correlation with kernel shrivel (r = -0.44) and (v) moderate, negative and significant correlation with AUDPC (r = -0.44).

(c) Apart from being positively correlated with grain yield in protected plots and negative correlation in non-protected plots, thousand kernel weight registered significant relationships with other yield components, (i) moderate, negative and significant correlation with kernels per spike (r = -0.57), (ii) moderate, positive and significant correlation with spikes per plant (r = -0.59), (iv) moderate, negative and significant correlation with FDS (r = -0.48) and (iv) moderate, negative and significant correlation with AUDPC (r = -0.50).

(d) Kernels per spike were moderate, negative and significantly correlated to spike per plant in protected plots (r = -0.58).

(e) FDS was highly, positive and significantly correlated to AUDPC (r = 0.98).

In general, the phenotypic correlations among grain yield, yield loss, and yield-determining components in the non-protected plots were higher compared with no correlations in the fungicide-protected plots (Table 8). The AUDPC and FDS were negatively correlated with grain yield and all yield traits, and highly positively correlated with yield losses. The negative correlation between disease components, AUDPC and FDS was particularly high with thousand kernel weight and kernels per spike. In the non-protected plots, grain yield was positively

correlated with 1000-kernel weight, kernels per spike and spikes per plant. In the protected plots, grain yield was positively correlated with 1000-kernel weight and kernels per spike. Yield loss was negatively correlated with 1000-kernel weight, kernels per spike and spikes per plant of the non-protected plots. Yield loss was negatively correlated to kernel shrivelling in the protected plots, whereas no significant correlation was found between yield loss and kernel shriveling in the non-protected plots. Similarly, both AUDPC and FDS were negatively correlated to the kernel shriveling (Table 8).

**Table 8: Phenotypic correlation coefficient (*r*) among grain yield, yield components, yield loss, AUDPC and FDS of wheat cultivars.**

| Variable | Treatment† | Grain yield | Yield loss | Kernel weight | Kernels per spike | Spikes per plant | Kernel shrivel | FDS |
|---|---|---|---|---|---|---|---|---|
| Yield Loss | Non-prot | -0.93* | 1.00 | | | | | |
| | Prot | -0.58 | | | | | | |
| Kernel weight | Non-prot | 0.56* | -0.57* | 1.00 | | | | |
| | Prot | -0.09 | 0.34 | | | | | |
| Kernels per spike | Non-prot | 0.62* | -0.63* | 0.58* | 1.00 | | | |
| | Prot | -0.04 | 0.14 | 0.32 | | | | |
| Spikes per plant | Non-prot | 0.58* | -0.54* | 0.16 | 0.01 | 1.00 | | |
| | Prot | 0.23 | -0.28 | -0.59* | -0.58* | | | |
| Kernel Shrivel | Non-prot | -0.44* | 0.33 | -0.51* | 0.09 | -0.42 | 1.00 | |
| | Prot | 0.13 | -0.36 | -0.24 | 0.56 | -0.01 | | |
| FDS | Non-prot | -0.38 | 0.53* | -0.48* | -0.34 | -0.42 | 0.01 | 1.00 |
| AUDPC | Non-prot | -0.44* | 0.56* | -0.50* | -0.40 | -0.42 | -0.01 | 0.98* |

*-significant at 5% (0.05) level of probability.
†Treatments: Prot = fungicide-protected plots, and Non-prot = non-protected plots.
FDS - final disease severity.
AUDPC - area under the disease progress curve.

# CHAPTER FIVE

# DISCUSSION

## 5.1 Reaction to Rust in Greenhouse

All the Kenyan wheat cultivars showed susceptibility at seedling stage to race Ug99, while all the breeding lines from CIMMYT, except 103141 and 103108 were either resistant or moderately resistant. These results indicate that the resistant lines contain major gene(s) that is effective against race Ug99 pathogen. Among the Kenyan cultivars, Chozi and Duma were the most susceptible. These cultivars were derived from CIMMYT germplasm that carried the alien resistance gene *Sr*31, which was broken by race Ug99. The deployment of *Sr*31 into major commercial wheat cultivars was initially associated with increased grain yields and resistance to cereal rusts and powdery mildew. This strategy was probably responsible for the reduction of stem rust epidemics to non-significant levels in Kenya for over 50 years.

## 5.2 Reaction to Rust under Field Conditions

Among the Kenyan wheat cultivars screened in the field for adult plant resistance, majority of them produced susceptible response to the infection by the pathogen, with the exception of Njoro Bw2, Kenya Nyangumi and Kenya Kongoni. Both of which displayed the characteristic pseudo black chaff (PBC) appearance at the stem nodes and glumes, which is associated with *Sr*2 resistance gene that confers slow-rusting (Njau *et al.*, 2009). Kenya Nyangumi and Kenya Kongoni are among cultivars released when *Sr*2 complex was the main source of resistance used against stem rust in combination with other unknown adult plant resistance genes in protecting these old cultivars. The reduced disease severity recorded for these cultivars coupled moderately susceptible response suggests the presence of minor genes, which ameliorates the effects of the rust pathogen. The resistance responses scored for lines 102091, 97 and 6436, and the low disease severities indicate the possession of both major and minor genes.

## 5.3 Resistance and Yield Loss

The results showed that severe damage was caused by the new race Ug99 of stem rust on Kenyan commercial wheat cultivars, Njoro Bw1, Njoro Bw2, Chozi, Duma, Kwale, Kenya Kongoni, Kenya Nyangumi and Kenya Fahari. The grain yield losses in the non-protected plots

were significantly high compared to the fungicide-protected treatments in six wheat cultivars and seven CIMMYT breeding lines regardless of their level of stem rust resistance. The grain yield losses of protected compared to non-protected plots of cultivars, Njoro Bw2, Kenya Nyangumi and Kenya Kongoni, and breeding lines, 102091, 97, 6436 and 6053 across the sites were not significantly different. Significant yield losses of 66.3% for Chozi and 69.2% for 103141 the most susceptible cultivar and breeding line, respectively. Some breeding lines however showed resistance to the *Puccinia graminis* f. sp. *tritici* race Ug99 at seedling or adult plant growth stage or at both stages, but all suffered yield loss under unprotected conditions.

Fungicide-protected plots of some cultivars were not entirely disease free, therefore, a reduced yield loss was observed as expected. This was in conformity with observations of other workers. Loughman *et al.* (2005) found that stem rust caused losses irrespective of the level of resistance possessed by the cultivar. Smedegaard-Petersen and Tolstrup (1985) who studied powdery mildew resistance in barley argued that the fact that highly resistant plants do not show any visible disease symptoms after inoculation does not mean that the plants are not affected. The plants respond to infection with energy-demanding physiological processes, probably defence reactions, using host energy that otherwise would go to growth and grain production. In addition, a reduction in the photosynthetic tissue due to hypersensitive flecking on the leaves sheaths can cause yield reductions (Sayre *et al.*, 1998; Herrera-Foessel *et al.*, 2006).

On the other hand, the use of broad-spectrum systemic fungicides often results in yield increases. This yield increase in the presences of low stem rust severity levels may have been due, in part, to a phytotonic effect of fungicides. Such stimulatory effect of fungicide treatments on growth may result in significant yield increases even in the absence of disease (Wegulo *et al.*, 1998) and has been demonstrated in hard winter wheat cultivars (Ransom and McMullen, 2008) and in coffee (Vossen, 1982). This is likely to have adversely affected grain growth because according to McGrath *et al.* (1991), carbon dioxide fixation after flowering supplies 90-95% of the carbohydrate in the grain. Similarly, as reported by Bartlett *et al.* (2002), the green area duration, which is the period from heading to maturity, is closely correlated to wheat grain yield.

Fungicide treatments with strobilurins as an active ingredient, to which group AmistarXtra 280 SC (azoxystrobin 120g/L + cyproconazole 80g/L) belong, have been shown to have a beneficial effect on the plants by delaying senescence, thereby prolonging the duration of the green-leaf area and increasing yield (Burgeno *et al.*, 2000; Bertelsen *et al.*, 2001). At maturity, it was observed that all the protected control plots across the sites delayed drying by between 7 to 12 days. This explains the reason why higher yields were realised in protected plots at both experimental sites, and particularly at Mau-Narok. However, distinguishing between the yield gain because of disease suppression and yield gain because of any direct physiological effects was difficult in field experiments. Quantitative evidence in representative environments is not available (Niks and Lindhout, 2006). However, the yield improvements are economically important and cannot be overlooked.

## 5.4 Tolerance to Stem Rust in Bread Wheat Cultivars

The Njoro Bw2 and Kenya Nyangumi had the lowest yield losses (less than 25%) compared to the susceptible control Chozi had a yield loss of 66% at both sites despite high disease pressure. This suggests that Njoro Bw2 and Kenya Nyangumi have tolerance or unknown slow-rusting genes. Slow rusting describes a reduced rate of epidemic development and is a valuable trait in cereal crops (Welty and Barker, 1992; Singh *et al.*, 2006).The phenotypic expression of pseudo-black chaff (PBC) was strongly evident in Njoro Bw2, Kenya Nyangumi, Kwale and Kenya Fahari. However, in the latter two cultivars, disease scores and yield losses were not consistent across the sites.

The four CIMMYT breeding lines 97, 10101, 6436 and 6053 also showed PBC trait. Thus, they had relatively low disease severity scores and consequently, insignificant yield losses. The PBC has been associated with the stem rust durable resistance gene, *Sr*2- complex present in most cultivars with CIMMYT background (Singh *et al.*, 1991). The presence of adult plant resistance genes in some of the Kenyan commercial cultivars can be exploited in breeding for resistance. Tolerance is defined as the ability of plants to maintain yield or quality performance in the presence of disease symptoms or when the plants appear susceptible to the disease (Caldwell *et al.*, 1958; Schafer, 1971). Individual components of the yield are less likely to describe disease effects on yield than the yield measurement itself, because they were not always consistent

across the sites. Mayfield (1985) found a clear relationship between grain yield and disease severity and showed that prevention of a 1% increase in rust severity avoided a 2% loss in grain yield. By extrapolation, 100% grain yield losses of crops would occur when 50% of stem areas were covered with pustules. According to Gaunt (1981), yield loss rather than yield performance or disease development should more accurately reflect the tolerance of a cultivar. However, it is important to take into account yield performance because a high-yielding cultivar with high stem rust tolerance will be more desirable to breeders and farmers (Caldwell *et al.*, 1958).

Wheat breeding programme both in Kenya and CIMMYT has given emphasis to selecting for the slow-rusting type of resistance (Sayre *et al.*, 1998; Herrera-Foessel *et al.*, 2007). The results of important test cultivars grown in many areas of Kenya, show that this type of resistance was probably present in several cultivars. Mean losses of grain yields in moderately resistant lines ranged between 12.0% (97) to 32 % (6053) as compared to 18% for resistant line 102091. The loss of 66% was observed for the most susceptible cultivar Chozi. The effect of stem rust on the grain yield of resistant line 102091 and moderately resistant line 102068 may reflect loss of photosynthetic stem and leaf sheath area because of hypersensitive flecking or necrosis of the stem and leaf tissues. It may also be due to loss of energy by the plant in defending against other minor diseases or saprophytic fungi (Smedegaard-Petersen and Tolstrup, 1985). For the susceptible cultivars grain yield loss is mainly attributed to the interruptions of movements of photosynthetes, water and mineral nutrients.

Kenyan commercial wheat cultivars: Njoro Bw1 and Chozi released in 2001 and 1999 respectively were highly resistant to stem rust disease at the time of their release probably due to presence of resistance genes, but their race-specific resistances became ineffective just a few years after initial cultivation. Similarly, other commercial cultivars such as Duma, Kwale, Kenya Kongoni and Kenya Fahari that were released more than ten years ago have also become susceptible to the new race Ug99. Data show a definite susceptible seedling reaction and field response and high stem rust severity of the cultivars Njoro Bw2, Kwale and Kenya Nyangumi, in spite of were slower to rusting. Slow rusting describes a reduced rate of epidemic development (Nicks and Lindout, 2006) and is a valuable trait in cereal crops (Herrera- Foessel *et al.*, 2006).

## 5.5 Effect of Rust on Yield Components

Stem rust epidemics in natural field conditions were severe at grain-filling growth stage at KARI-Njoro compared to Mau-Narok. This plant growth stage coincided with stem rust epidemic initiation. Several studies (Bertelsen *et al.*, 2001; Loughman *et al.*, 2005) have also confirmed the same and concluded that stem rust epidemic occurring after soft dough growth stage (GS 85) has little effect on the grain yield. This may explain the lack of significant correlation between final disease severity (FDS) and grain yield at the Mau-Narok experimental site where the stem rust attack started post-flowering.

At KARI-Njoro the stem rust infection started early at stem elongation growth stage (GS 31). This may have adversely affected the number of spikes per plant. There was higher disease damage at KARI-Njoro compared to Mau-Narok experimental site. The reduced yield at KARI-Njoro presumably was the result of the pathogen interfering with the rate of photosynthesis, and consequently reducing the translocation of solutes during grain-filling period as suggested by McGrath and Pennypacker (1991). The yield losses due to stem rust were mostly attributed to reduction in number of kernels per spike. The period 3 to 4 weeks before and after flowering is critical in determining the number of spikes per plant and ultimately the number of kernels in spikes, whereas the grain-filling period is critical in determining the kernel weight (Herrera-Foessel *et al.*, 2006). The kernel weight is the yield component affected most by foliar fungal diseases such as stem rust, which develop primarily during the grain-filling period.

In this study, stem rust epidemic appeared at a much earlier plant developmental stages at stem elongation (GS 31), especially at the experiment in KARI-Njoro; thus both kernel number and size may have been seriously affected. Normally a higher infection around head emergence drastically affects pollination and grain-filling, resulting in few kernels per spike. The reduction in kernels per spike could have been due to rust infection at this critical growth stage of the crop. This was as expected because stem rust infection mainly interferes with physiological processes concerned with grain filling. The experiment at Mau-Narok differed in that the sowing was done two months after the Njoro site. This is because during that time the fields are normally too wet to allow for any farm operation. The inoculation was carried out a month later. Stem rust infection started late and was slow to increase, thus causing the disease severity to be generally

low. The infection coincided with heading and grain-filling growth stages, which had drastic effect on the yield components, especially kernels per spike and spikes per plant. As observed, the grain yield losses of test cultivars between the protected and non-protected plots at Mau-Narok were much lower compared to KARI-Njoro, and the yield losses were attributed mainly to changes in kernel weight. In a similar study by Sayre *et al.* (1998), grain yield losses due to leaf rust in bread wheat was associated with reductions in kernel weight, kernels per square metre, spikes per square metre, and grain-filling rate. This relationship between reduction in kernel weight and yield losses caused by rust has been found by many authors (Singh *et al.*, 1991; Herrera-Foessel *et al.*, 2006).

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# CHAPTER 6

# CONCLUSIONS AND RECOMMENDATIONS

This study showed that a grain yield loss in excess of 80% was caused by race Ug99 of stem rust on susceptible wheat cultivars, such as Chozi, Duma, Njoro Bw1 and breeding line 103067. However, the levels of resistance in test cultivars were not equal and varied greatly under high disease pressure. Results indicated that race Ug99 of stem rust is highly virulent on popular commercial wheat cultivars grown in Kenyan with few exceptions, hence, posing serious threat to wheat production. Out of the twenty screened cultivars seven recorded less yield losses. Similar yield potential in non-protected and fungicide-protected control were observed for Njoro Bw2, Kenya Nyangumi and Kenya Kongoni and CIMMYT lines 102091, 97, 6436 and 6053.

The other commercial cultivars Chozi, Duma, Njoro Bw1, Kwale, Kenya Popo, Kenya Fahari and CIMMYT lines 103141, 103108, 103247, 6053 and 102012 were found to be susceptible. However, Njoro Bw2, Kenya Nyangumi and Kenya Kongoni appeared to be less damaged by the rust pathogen compared to the other susceptible cultivars. Even though the three cultivars were susceptible at seedling growth stage, their adult plant infection responses showed low to moderate levels of disease severity in the field, suggesting the existence of adult plant resistance. This trait could be used for breeding new cultivars. The rest of Kenyan cultivars were susceptible to Ug99 at both seedling and adult plant stages and therefore, they cannot be grown without heavy yield losses.

The following recommendations can be made:

1. Genetic studies are needed to characterise the adult plant resistance in cultivars and breeding lines that displayed high levels of tolerance or resistance to race Ug99.
2. Resistant breeding line 102091 and identified slow-rusting cultivars should be exploited as sources of resistance.
3. A stem rust survey should be conducted annually to monitor and characterize pathogenic variability of *Puccinia graminis* f. sp. *tritici* in the major wheat producing areas of Kenya.
4. Investigations are required to re-establish suitable cultivars for each wheat-growing area, periods of sowing, rainfall, and maximum occurrence of air-borne urediniospores of stem

rust in Kenya in order to forecast the on-set of an epidemic. Hence, the application of fungicide protection to the crop.

Fungicides should be screened for use in combination with slow-rusting or tolerant commercial cultivars to reduce the yield losses.

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# APPENDICES

**Appendix 1: Identification of race Ug99 (TTKS) of wheat stem rust**

| USDA-Differential line | *Sr* gene | Infection Type (IT)[a] | Virulence/avirulence |
|---|---|---|---|
| ISr5-Ra | 5 | 4 | H |
| CnS-T-mono | 21 | 3 | H |
| Vernstine | 9e | 4 | H |
| ISr7b-Ra | 7b | 4 | H |
| ISr11-Ra | 11 | 4 | H |
| ISr6-Ra | 6 | 4 | H |
| ISr8a-Ra | 8a | 4 | H |
| CnSr9g | 9g | 4 | H |
| W2691SrTt-1 | 36 | 0; | L |
| W2691Sr9b | 9b | 4 | H |
| BtSr30Wst | 30 | 3 | H |
| Combination VII | 17 | 4 | H |
| ISr9a-Ra | 9a | 4 | H |
| ISr9d-Ra | 9d | 3 | H |
| W2691Sr10 | 10 | 3 | H |
| Triumph 64 | Tmp | 2 | L |

[a]ITs at the seedling stage following the descriptions of Stakman *et al.* (1962), where IT - 0, ;, 1, 2, or combinations thereof were considered low (L), infection types, and 3 & 4 were considered high (H), infection types.

**Appendix 2: A key for defining the *Puccinia graminis tritici* - code races of *Puccinia graminis* f. sp. *tritici***

| | Subset[a] | Infection type produced on host lines with *Sr* | | | |
|---|---|---|---|---|---|
| | 1 | 5 | 21 | 9e | 7b |
| | 2 | 11 | 6 | 8a | 9g |
| | 3 | 36 | 9b | 30 | 17 |
| Pgt - code | 4 | 9a | 9d | 10 | Tmp |
| B | | Low | Low | Low | Low |
| C | | Low | Low | Low | High |
| D | | Low | Low | High | Low |
| F | | Low | Low | High | High |
| G | | Low | High | Low | Low |
| H | | Low | High | Low | High |
| J | | Low | High | High | Low |
| K | | Low | High | High | High |
| L | | High | Low | Low | Low |
| M | | High | Low | Low | High |
| N | | High | Low | High | Low |
| P | | High | Low | High | High |
| Q | | High | High | Low | Low |
| R | | High | High | Low | High |
| S | | High | High | High | Low |
| T | | High | High | High | High |

[a]Pgt - code consists of the designation for subset 1 followed by that for subset 2, 3 and 4.

**Appendix 5: Description and Pedigree of twenty wheat cultivars used in the study**

| Cultivar/Line accession # | Pedigree | Source | Remarks/ Year of release |
|---|---|---|---|
| 1. Njoro BW1 | KM14 (PASA MUTANT) | Kenya | (2001) |
| 2. Njoro BW2 | TNMU | CIMMYT | (2001) |
| 3. Chozi | F12.71/COC//GEN | CIMMYT | (1999) |
| 4. Duma | AU/UP301//GLL/SX/3/PEW"S"-4-MAIN "S" -/MAYA "S"//PEW "S" | CIMMYT | (1993) |
| 5. Kwale | Kinglet | Canada | (1987) |
| 6. Kenya Popo | KL. At1/Tob66//cfn/3/Bb/4/6648-2 | Kenya | (1982) |
| 7. Kenya Nyangumi | Tzpp//SKE$^{6}$/LR64 HDM/3/AFM/4/KSW/K4500-6 | Kenya | (1979) |
| 8. Kenya Kongoni | C18154/2*/3*Rom/3/wis.245-ll-50-17/C18154/2/2*Fr | Kenya | (1981) |
| 9. Kenya Fahari | Tob66/SRPC527//Cl8l54/3/2*FROCOR | Kenya | (1977) |
| 10. 103141 | CHEN/ARE.SQ//2*WEAVER// OASIS/5*BORL95 | CIMMYT | Advanced breeding line |
| 11. 103067 | OASIS/5*BORL95//Tukuru | CIMMYT | " |
| 12. 103108 | KAUZ//ALTAR84//..../3/KAUZ/4/SW94.1 5464 | CIMMYT | " |
| 13. 102091 | GAN/AE.SQ(236)/3/KAUZ*2/ BOW//KAUZ/4/HP1761 | CIMMYT | " |
| 14. 10101 | CMH79A.1380/GAN FRENCH/6/CMH79A.955/4/AGAR/3/.... | CIMMYT | " |
| 15. 103247 | KRICHAUFF/PASTOR | CIMMYT | " |
| 16. 103249 | BUC/PRL/WEAVER/3/Tukuru | CIMMYT | " |
| 17. 97 | BABAX/LR42//BABAX/3/BABAX/LR42// BABAX | CIMMYT | " |
| 18. 6436 | INIA CABURE/INIA TIJERETA | CIMMYT | " |
| 19. 6453 | PF8944/BBGL//BR23/EMB27 | CIMMYT | " |
| 20. 102012 | TAM200/TUI//SW89-5124*2/FASAN | CIMMYT | " |

## Appendix 6: Growth stages of small grains

| CODE | Description |
|---|---|
| **0** | **GERMINATION** |
| 00 | Dry seed |
| 01 | Start of imbibition |
| 03 | Imbibition complete |
| 05 | Radicle emerged from seed |
| 07 | Coleoptile emerged from seed |
| 09 | Leaf just at coleoptiles tip |
| **1** | **SEEDLING GROWTH** |
| 10 | 1st leaf through coleoptile |
| 11 | 1st leaf unfolded |
| 12 | 2 leaves unfolded |
| 13 | 3 leaves unfolded |
| 14 | 4 leaves unfolded |
| 15 | 5 leaves unfolded |
| 16 | 6 leaves unfolded |
| 17 | 7 leaves unfolded |
| 18 | 8 leaves unfolded |
| 19 | 9 leaves unfolded |
| **2** | **TILLERING** |
| 20 | Main shoot only |
| 21 | Main shoot and 1 tiller |
| 22 | Main shoot and 2 tillers |
| 23 | Main shoot and 3 tillers |
| 24 | Main shoot and 4 tillers |
| 25 | Main shoot and 5 tillers |
| 26 | Main shoot and 6 tillers |
| 27 | Main shoot and 7 tillers |
| 28 | Main shoot and 8 tillers |
| 29 | Main shoot and 9 tillers |
| **3** | **STEM ELONGATION** |
| 37 | Flag leaf just visible |
| 38 | Flag leaf ligule just visible |
| **4** | **BOOTING** |
| 41 | Flag leaf sheath extending |
| 43 | Boots just visible swollen |
| 45 | Boots swollen |
| 47 | Flag leaf sheath opening |
| 49 | First awns visible |
| **5** | **EAR EMERGENCE** |
| 51 | 1st spikelet of ear emerged |
| 53 | One-fourth of ear emerged |
| 55 | One-half of ear emerged |
| 57 | Three-fourths of ear emerged |
| 59 | Emergence of ear completed |
| **6** | **FLOWERING** |
| 61 | Beginning of flowering |
| 65 | Flowering half-way complete |
| 69 | Flowering complete |
| **7** | **MILK DEVELOPMENT** |
| 71 | Seed water ripe |
| 73 | Early milk |
| 75 | Medium milk (An increase in the solids of the liquid of the endosperm is notable when crushing the seed between fingers) |
| 77 | Late milk |
| **8** | **DOUGH DEVELOPMENT** |
| 83 | Early dough |
| 85 | Soft dough (Fingernail impression not held) |
| 87 | Hard dough (Fingernail impression not held; head losing chlorophyll) |
| **9** | **RIPENING** |
| 91 | Seed hard (difficult to divide by thumbnail) |
| 92 | Seed hard (can no longer be dented by thumbnail) |

| | | | |
|---|---|---|---|
| 30 | Pseudo stem erection | 93 | Seed loosening in daytime |
| 31 | 1st node detectable | 94 | Over-ripe; straw dead/ collapsing |
| 32 | 2nd node detectable | 95 | Seed dormant |
| 33 | 3rd node detectable | 96 | Visible seed giving 50% germination |
| 34 | 4th node detectable | 97 | Seed not dormant |
| 35 | 5th node detectable | 98 | Secondary dormancy induced |
| 36 | 6th node detectable | 99 | Secondary dormancy lost |

(**Adopted from Zadok *et al.*, 1974**)

**Appendix 7: ANOVA of stem rust FDS % for cultivars across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 132.5707 | 256.46 | 0.0001* |
| Treat | 1 | 950.3132 | 1838.38 | 0.0001* |
| Site x Treat | 1 | 132.5707 | 256.46 | 0.0001* |
| Cultivar | 19 | 7.7109 | 14.92 | 0.0001* |
| Site x Cultivar | 19 | 1.7699 | 3.42 | 0.0001* |
| Treat x Cultivar | 19 | 7.7109 | 14.92 | 0.0001* |
| Site x Treat x Cultivar | 19 | 1.7699 | 3.42 | 0.0001* |
| Rep | 4 | 1.0442 | 2.02 | 0.0943 |
| Error | 156 | 0.5169 | - | - |

CV = 24.1%
*-Significant at 5% (0.05) level of probability.

**Appendix 8: ANOVA of stem rust AUDPC for cultivars across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 4949.920 | 312.73 | 0.0001* |
| Treat | 1 | 40460.307 | 2556.26 | 0.0001* |
| Site x Treat | 1 | 4949.920 | 312.73 | 0.0001* |
| Cultivar | 19 | 350.662 | 22.15 | 0.0001* |
| Site x Cultivar | 19 | 62.851 | 3.97 | 0.0001* |
| Treat x Cultivar | 19 | 350.662 | 22.15 | 0.0001* |
| Site x Treat x Cultivar | 19 | 62.851 | 3.97 | 0.0001* |
| Rep | 4 | 40.387 | 2.55 | 0.0413 |
| Error | 156 | 15.828 | - | - |

CV = 28.4%
*-Significant at 5% (0.05) level of probability.

**Appendix 9: ANOVA of stem rust on grain yield across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 296.46 | 323.37 | 0.0001* |
| Rep | 4 | 5.12 | 5.58 | 0.0003* |
| Treat | 1 | 493.70 | 538.51 | 0.0001* |
| Rep x Treat | 4 | 4.30 | 4.69 | 0.0013 |
| Cultivar | 4 | 5.25 | 5.73 | 0.0001* |
| Site x Cultivar | 19 | 1.14 | 1.24 | 0.2334 |
| Treat x Cultivar | 19 | 3.31 | 3.61 | 0.0001* |
| Site x Treat x Cultivar | 19 | 2.24 | 2.44 | 0.0014 |
| Error | 152 | 0.92 | - | - |

CV = 26.5%
*-Significant at 5% (0.05) level of probability

**Appendix 10: ANOVA of stem rust on thousand kernel weight across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 3788.81 | 505.73 | 0.0001* |
| Rep | 4 | 50.65 | 6.76 | 0.0001* |
| Treat | 1 | 8391.97 | 1120.16 | 0.0001* |
| Rep x Treat | 4 | 12.86 | 1.72 | 0.1491 |
| Cultivar | 19 | 79.46 | 10.61 | 0.0001* |
| Site x Cultivar | 19 | 29.62 | 3.95 | 0.0001* |
| Treat x Cultivar | 19 | 54.05 | 7.22 | 0.0001* |
| Site x Treat x Cultivar | 19 | 25.03 | 3.34 | 0.0001* |
| Error | 152 | 7.49 | - | - |

CV = 7.5%
*-Significant at 5% (0.05) level of probability.

**Appendix 11: ANOVA for stem rust on spikes per plant across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 2489.70 | 310.36 | 0.0001* |
| Treat | 1 | 108.00 | 13.46 | 0.0003* |
| Site x Treat | 1 | 75.94 | 9.47 | 0.0025 |
| Cultivar | 19 | 24.15 | 3.01 | 0.0001* |
| Site x Cultivar | 19 | 8.70 | 1.09 | 0.3711 |
| Treat x Cultivar | 19 | 12.83 | 1.60 | 0.0624 |
| Site x treat x Cultivar | 19 | 9.38 | 1.17 | 0.2908 |
| Rep | 4 | 37.15 | 4.63 | 0.0015 |
| Error | 156 | 8.02 | - | - |

CV = 29.3%
*-Significant at 5% (0.05) level of probability

**Appendix 12: ANOVA of stem rust on kernels per spike across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 57.04 | 1.47 | 0.2277 |
| Rep | 4 | 199.62 | 5.13 | 0.0007 |
| Treat | 1 | 4977.70 | 128.00 | 0.0001* |
| Rep x Treat | 4 | 42.18 | 1.08 | 0.3662 |
| Cultivar | 19 | 182.49 | 4.69 | 0.0001* |
| Site x Cultivar | 19 | 55.70 | 1.43 | 0.1194 |
| Treat x Cultivar | 19 | 60.88 | 1.57 | 0.0718 |
| Site x Treat x Cult | 19 | 54.49 | 1.40 | 0.1337 |
| Error | 152 | 38.88 | - | - |

CV = 26.1%
*-Significant at 5% (0.05) level of probability.

**Appendix 13: ANOVA of stem rust on kernel shriveling across the sites**

| Source of variations | df | MS | F | Pr □ F |
|---|---|---|---|---|
| Site | 1 | 13.54 | 50.15 | 0.0001* |
| Rep | 4 | 2.26 | 8.38 | 0.0001* |
| Treat | 1 | 142.60 | 528.25 | 0.0001* |
| Rep x Treat | 4 | 1.48 | 5.58 | 0.0004* |
| Cultivar | 19 | 2.80 | 10.38 | 0.0001* |
| Site x Cultivar | 19 | 1.83 | 6.77 | 0.0001* |
| Treat x Cultivar | 19 | 34.65 | 6.75 | 0.0001* |
| Site x Treat x Cultivar | 19 | 17.71 | 3.45 | 0.0001* |
| Error | 152 | 0.27 | - | - |

CV = 22.2%
*-Significant at 5% (0.05) level of probability.

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