INHERITANCE OF RESISTANCE GENES -Sr26, Sr36- AND EFFECTIVENESS OF OTHER MAJOR AND SLOW RUSTING GENES AGAINST RACE 'PgtUg99' OF STEM RUST (*Puccinia graminis* Pers.f.sp *tritici*) IN BREADWHEAT

MACHARIA KAMAU GODWIN

Reg. No: KM12/1498/05

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EGERTON UNIVERSITY

AUGUST, 2009

DECLARATION AND RECOMMENDATION

DECLARATION

This proposal is my original work and has not been presented in any other form for a degree in this or any other university

Signed.....Date.....30th July '2009.....

Macharia Kamau Godwin

KM12.

RECOMMENDATION

This thesis has been submitted to the graduate school with our approval as supervisors:

Signature.....Date.....30th July 2009.....

Prof., R.S Pathak
Department of Crops, Horticulture and Soils
Egerton University, NJORO

Signature.....Date.....1st August '09.....

Prof., Miriam G. Kinyua
Department of Biotechnology
Moi University, ELDORET

DEDICATION

To my (late) dad Johnson Macharia

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Many a time, my spirit dithered: it is in such circumstances that my family's hand came handy. My mother Sifira, wife Patricia, daughter Alicia, brothers and sisters all granted me a shoulder. Dorcus my classmate inspired the philosophy of not letting up. Last but most important, I reckon that all was possible because of the almighty God; He blesses, He is able and His promises are eternally true.

ABSTRACT

The emergence of the highly virulent race-PgtUg99- of stem rust threatens wheat production not only in Kenya but regionally and globally too. With most of the Kenyan commercial cultivars being susceptible, the need to explore new sources of resistance and to elucidate the mode of inheritance of confirmed resistance is paramount. A few major genes are reportedly resistant to PgtUg99. Study of the inheritance of two of such genes, Sr26 and Sr36 from Australian cultivars Eagle and Cook respectively was carried out. Kenyan cultivars Kwale and Njoro Bw II, extensively grown in the higher potential areas alongside Duma and Chozi popular in the drier zones were selected in generating study populations. Furthermore, the usefulness of other selected major genes, vis-à-vis the slow rusting additive genes of resistance, in mitigating wheat yield loss under high PgtUg99 pressure in the field was investigated. Crosses were made between the two resistance sources (Eagle, Cook) and the four Kenyan cultivars (Kwale, Njoro Bw II, Duma, Chozi) to give F_1, F2 and BC_1F_1 Populations. These populations were then inoculated with PgtUg99 inoculum and infection types classified. The observed ratio of the resistant versus susceptible seedlings for each population was tested against expected ratio for one gene through chi-square. In the field experiment, a replicated yield trial of 30 cultivars was planted at Njoro, Kenya in paired spilt plot design with fungicide protected and unprotected plots through two seasons. The parameters: infection rate (*r*), Final Disease Score (FDS), the Area Under Disease Progress Curve (AUDPC) were recorded as measures of epidemiology of PgtUg99 on the different hosts. These were then hierarchically delimited into dendrograms depicting the varying levels of resistance. A record of grain yield/acre, 1000 kernel weight, and biomass and test weight with the attendant losses was established. Both resistance genes Sr26 and Sr36 were found to be dominant and simply inherited. Results from the field experiment revealed that the FDS on susceptible cultivars was highly positively correlated to the rate of disease progress (Pearsons correlation =0.99). The FDS and AUDPC were significantly positively correlated to yield loss (Pearsons correlation=0.72). Rust severity on average was 60%,50% and 25% for the susceptible, slow rusting and race-specific groups of cultivars respectively. Chozi, lost as much as 80% grain yield while Kwale and Njoro Bw II recorded about 50% grain yield reduction. Losses in 1000-kernel weight ranged between 4-30% across all cultivars. The results of experiments confirmed the destructive nature of race PgtUg99 on susceptible cultivars. The dominance of genes Sr26 and Sr36 suggest that they are amenable to incorporation into the PgtUg99 susceptible high yielding Kenyan wheat cultivars through simple crossing and backcrosses. Besides, it was evident that wheat grain yield losses can be significantly reduced by utilizing genes Sr25, Sr Tmp, Sr Sha7 in combination. Similarly, the study supports that 'slow rusting' cultivars such as 6356, with resistance as effective as that of major genes should be considered for commercial production especially where this resistance provides in high yielding backgrounds. It may be inferred that low rate of stem rust disease progress predicts low final disease severity and as such, slow rusting cultivars can ideally be identified from among those with low final disease severities pointedly, 20-30 MS/S on the modified cobb scale.

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LIST OF ABBREVIATIONS

AUDPC	Area Under Disease Progress Curve
CAN	Calcium Ammonium Nitrate
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
DAP	Di-Ammonium Phosphate
FAO	Food and Agricultural Organization
FDS	Final Disease Score
IFPRI	International Food Policy Research Institute
KARI	Kenya Agricultural Research Institute
R	Resistance is the ability of a host cultivar to restrict or even prevent the production of disease symptoms by a pathogen due to a gene for resistance
S	Susceptibility is the inability of the host to prevent a pathogen from attacking it
Sr	Stem rust
UN	United Nations
T	Tolerance is the ability of a host to avoid or minimize loss in productivity although it has been infected by a pathogen

CHAPTER I

GENERAL INTRODUCTION

1.1 Background

Globally, wheat is the major crop in basic food commodities, followed by coarse grains and rice. According to the Food and Agriculture Organization of the United Nations the worldwide production of wheat was more than 500 million tons in 2004 (FAO, 2005; Appendix A). The average per-capita global consumption of wheat in 2005 was 68kg- 61kg and 75kg in developing and developed countries, respectively. Moreover, it is also used as animal feed, with more than 110 million tons used for this purpose in 2004 (Bhalla, 2006).

Owing to the current world population growth estimated at around 100 million per year and expected to exceed 10 billion by 2050 (Rosegrant *et al.*, 1997), agricultural economists at the International Food Policy Research Institute (IFPRI) have projected a 40% increase in food demand within the next 20 years (Rajaram, 2001). Braun *et al.* (1998) argue that at least one billion tons of wheat should be produced by year 2020 with production levels doubling by mid 21st century (Bhalla, 2006).This dictates the need to intensify production from the same amount of arable land. An upward trend estimated at 2 to 4 fold increase in per-capita income, with resulting changes in dietary requirements has been reported was also in forecast (Tilman, 2002).

Wheat is the second most important cereal crop after maize in Kenya (KARI 1989) and is gradually becoming an important source of food for humans and feed for livestock. Demand for wheat and wheat products is growing at 7% per annum and even though production is increasing, only about 50% of domestic consumption requirements are being met (Gamba *et al.*, 2002; Hassan *et al.*, 1993). Increasing population, rapid urbanization, rising income levels, and changing tastes and preferences are major factors contributing towards this demand.

Wheat was introduced into East Africa by missionaries towards the end of the 19th century (Payne *et al.*, 2000). In 1907, Lord Defamer made the first attempts at extensive cultivation of wheat in Kenya. Faced with the challenge of stem rust, Delamere recruited and financed the first wheat breeding program in the country (Dixon, 1960). The first wheat variety grown in Kenya was of Australian origin (Thorpe, 1958; Payne *et al.*, 2000). In addition there were Italian and Egyptian varieties which formed the founding parental genepool. It was not until 1927 that formal wheat breeding research program was initiated at the National Plant Breeding Station (NPBS), Njoro then under the ministry of Agriculture. Following the interaction between

production and stem rust (Dixon, 1960) in the first 50 years of wheat in Kenya, remarkable success was achieved through the internationalization of the breeding program. Screening of international wheat nurseries and other collections mainly from the international centre for wheat and maize improvement (CIMMYT), Mexico led to deployment of resistant parental materials in the crossing block and ultimately release of stem rust resistant varieties (Dixon, 1960).

In the dawn of the 1980's, there was a wide adoption of progenies of the high yielding 'Veery group' of wheat all over the world Kenya included (Rajaram, 2001; Singh *et al.*, 2006). These wheats were a derivative of a cross between spring and winter wheats and were found to yield better than other cultivars both in high yielding environments and under irrigation. The difference arising from this group of wheat owed to the fact that they carried the 1B/1R translocation from rye. Many of the semi-dwarf wheats that were sown on 63% of the dry land wheat area in developing countries by 1990 (Byerlee and Moya, 1993) possessed the 1B/1R translocation. This alien translocation is also the carrier of the stem rust resistance gene Sr31 (Singh *et al.*, 2006), to which the new race of wheat stem rust, TTKS (Ug99) -the basis of this study- is virulent (Pretorius, 2000). This is the only race globally that shows virulence on Sr31 (Singh *et al.*, 2006).

1.2 The Statement of the problem

Wheat stem rust has been one of the most serious problems threatening the wheat industry in Kenya right from the introduction of the crop at the turn of the 20th century. In the decades between 1970 and 1990, the disease was largely restrained due to the release of numerous stem rust resistant varieties containing resistance gene Sr31 derived from the wheat- rye translocation 1BL-1RS and a combination of other vertical resistance genes. The detection of the new race of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) PgtUg99 in 1999 posses renewed threat to wheat production in Kenya, because Sr31 has been shown to be susceptible (Pretorius, 2000). The global rust initiative (GRI) has warned that only 0.3% of the more than 44 million hectares planted to known varieties of wheat globally is moderately resistant to PgtUg99 (B.G.R.I, 2005). This very low statistic is further highlighted in CIMMYT's own projection: that in Africa alone, grain yield losses of this important staple approaching $1 billion in value within the next 15 years are eminent (CIMMYT, 2005). With Kenya being a net wheat importer, producing only a third of her estimated 0.9 million metric tons annual consumption needs (KARI, 1998), the

problem of PgtUg99 is certainly challenging. If immediate steps are not taken to identify and deploy new resistant varieties or incorporate possible sources of resistance into the adapted varieties, the PgtUg99 provides yet another reason for denied incomes to Kenyans making a living from the wheat industry, distorted food import priorities and most worrying reduced availability of wheat-derived food and feed products. This therefore prompted this study 'inheritance or resistance genes Sr26, Sr36 and evaluation of the effectiveness of other major and slow rusting genes against race PgtUg99 of stem rust in bread wheat' with these as its objectives:

1.3 Thesis Objectives

The broad objective of this study was to identify, and deploy high yielding stem rust resistant wheat germplasm for Kenya.

The specific objectives were:

i. To determine the inheritance of resistance genes - Sr26 and Sr36
ii. To compare seedling and adult plant resistances to PgtUg99 in major gene and slow rusting cultivars
iii. To determine the effects of PgtUg99 on yield and yield components of selected wheat cultivars
iv. To select PgtUg99 resistant cultivars from the study material and recommend them for further yield tests

1.4 Null Hypotheses

i. Breeding populations do not show segregation for resistance to the race PgtUg99
ii. There are no differences in the expression of seedling and adult plant resistances among wheat cultivars
iii. The effects of PgtUg99 epidemics on yield are not significant
iv. There are no selectable PgtUg99 resistant cultivars with desirable yield potential from the study material

1.5 Justification of the study

Disease virulence continues to be the chief cause of production loss in wheat (Klatt, 1988). Stem rust, the most devastating of all wheat diseases can reduce an apparently healthy wheat crop three weeks before harvest to a black tangle of broken stems and shriveled grain by harvest

time (Roelfs, 1992), leading to reduced grain quantity and quality as well as poorer straws for use as hay for livestock.

Earlier efforts by breeders, through utilization of slow rusting Sr2 complex, major gene Sr31 and an array of other 'vertical-resistance' genes circumvented the threat by stem rust (Singh and Rajaram, 1995), until the resurgence of PgtUg99. The first sign of the reappearance of stem rust in Kenya was in 1996 (KARI, 1998).

By 2001, virtually all commercial Kenya bread wheat varieties were susceptible (Njau *et al.*, 2001) and in by 2005, all wheat farms in Kenya, Uganda and Ethiopia were affected by the new strain.

Fungicides are expensive to use (Fraser, 1985). CIMMYT(2005) notes that although large scale farmers extensively use chemicals to spray, the cost can run to more than US $ 100 per acre and a total of US $10million annually (KARI,2005). Besides, chemicals pose serious health problems to the users and to the fauna and flora.

Calamity looms for tens of millions of farmers and hundreds of millions of consumers in the high risk zone, including Kenya and predicted dispersal route of this pathogen (Singh *et al.*, 2006). Landless laborers and small scale farmers who have few alternative sources of livelihoods are at the highest risk if expeditious interventions are not put in place. Moreover, large production losses would have significant implications for rural and national economic growth rates in the country.

Growing resistant cultivars is the most cost effective and environmentally friendly means of managing pests and diseases (Russell, 1978). Singh *et al.* (2006) propose that the fastest way to reduce yield losses in susceptible but potentially high yielding wheat cultivars and elite lines would be to incorporate diverse sources of resistance into them through backcrossing.

A study of the inheritance of resistance genes Sr26 and Sr36 is important in efficiently using these genes for breeding. Information on the effectiveness of the different types of vertical resistant and slow rusting genes in checking the epidemiology of PgtUg99 and by large protecting grain yield losses in bread wheat is limited. Evaluating the effectiveness of the various genes proposed in this study would be a milestone in mitigating PgtUg99 in Kenya and the region.

1.6 Scope and limitation of the study

The inheritance of only two major genes of resistance-Sr26 and Sr36- was addressed in this study (Chapter 2). These are not the only genes conferring resistance to PgtUg99 and the scope may be expanded in future research. The green house facilities utilized in outlining the genetics of the two genes, lacked adequate lighting and temperature control systems and an attempt was made to delay the study until the ambient temperatures were considered favorable. Lack of temperature control hindered the chance to encourage seed production in the Eagle-derived crosses limiting them to 'grass dwarfs' and information of the segregation of Sr26 from Eagle in the F_2 and in backcrosses (BC_1F_1) was inevitably impossible. Chapter 4 describes results obtained on the effectiveness of various major and slow rusting genes against potential yield losses. This research was done only at one site, Njoro and though it would have been replicated in a second and perhaps third site, this was not feasible within the available time frame and resources. During the two seasons for the study there was less than normal rainfall which necessitated irrigation. The choice of the test materials was based on the fact that they had shown good adaptability at Njoro and beside their resistances; they appeared of acceptable agronomic type. The isolate used throughout these studies was that of PgtUg99. As far as possible the field trial was cushioned from exogenous inoculums by surrounding it with the non-host species-oats. The spreader rows were inoculated with PgtUg99 to initiate epidemics. However, since stem rust spores are wind borne, there was a chance of unintended spores landing on the experimental materials, but these were assumed not to significantly impact on the results of the field studies.

CHAPTER II

EXPANDED LITERATURE REVIEW

2.1 The host

The term wheat is normally used to refer to the cultivated species of the genus *Triticum.* The genus *Triticum* is complex and includes diploids, tetraploids, and hexaploids. A number of species have been cultivated over the years. Currently cultivation is restricted almost entirely to the tetraploid durum wheat (*Triticum turgidum* L.) and the hexaploid or bread wheat (*Triticum aestivum* L.).

2.1.1 The origin and evolution of wheat

Wheat falls into three groups with chromosome numbers of 14, 28, and 42 (Sakamura, 1918). The basic chromosome number and the number of chromosomes in a genome are 7. Durum wheat carries one genome (A) in common with einkorn wheat and has one additional genome (B). A cross between durum and bread wheat often has 14II+ 7I at meiosis. Thus, bread wheat carries the A and B genomes plus a third genome (D).

The genus *Triticum* evolved through amphiploidy. Cultivated *Triticums* evolved following the scheme shown in Figure 1.

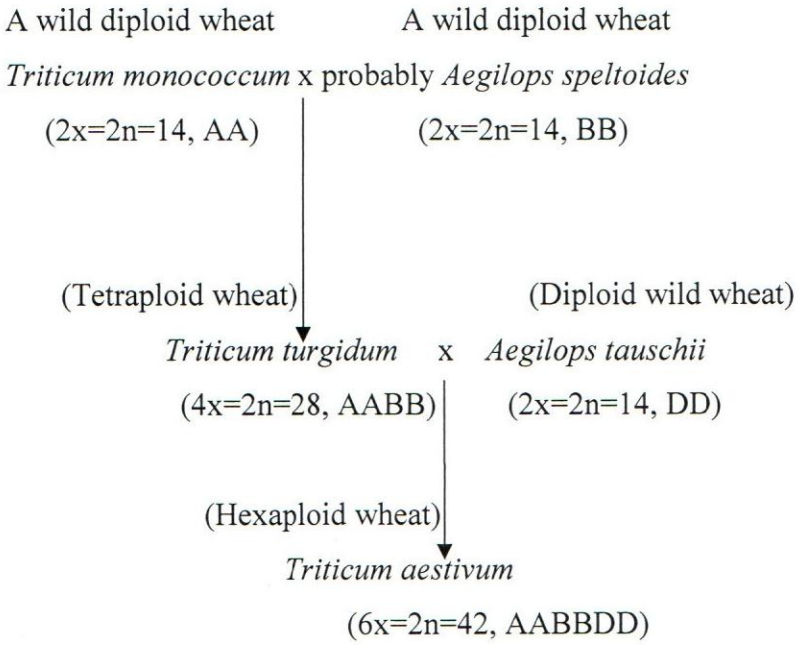


Figure 1. The evolution of *Triticum turgidum* (durum wheat) and *Triticum aestivum* (bread wheat) (Knott, 1989).

2.1.2 Variability in durum and bread wheat

The cultivated bread and durum wheats are grown over wide areas in many different environments. Both species are very diverse both at the cellular and morphological levels and many thousands of cultivars of each are known. Tremendous variability exists for resistance to stem rust (*Puccinia graminis* Pers. f. sp *tritici* Eriks. and Henn) and other rusts.

2.2 The stem rust pathogen

Stem rust alongside leaf and yellow rusts belong to the genus *Puccinia*. Specifically, stem rust which is also called black rust is caused by *Puccinia graminis* Pers. The name black rust is associated with the black teliospores that are produced towards the end of the growing season of an infected wheat plant. *Puccinia graminis* is characterized by marked specialization on the host genera to produce formae specialis. Thus;

P. graminis f. sp. *avenae*: specific to oats and some related grasses

P. graminis f. sp. *secalis*: specific to rye and some related grasses

P. graminis f. sp. *tritici*: specific to wheat, barley, and many of the relatives of wheat

A formae specialis does not produce pustules on a species outside its host range (Knott, 1989).

The genus *Puccinia* is a member of the family pucciniaceae of the order uridinales of the class Basidiomycetes (Littlefield, 1981). In the basidiomycetes, meiosis occurs in a basidium and results in the production of four haploid, single-celled basidiospores.

2.2.1 Life cycle

Stem rust has five spore types and both sexual and asexual stages (Figure 2). The asexual stage occurs on wheat and related grasses and the sexual stages on an alternative host, barberry (*Berberis* spp) or *Mahonia* spp. This rust is referred to as being macrocyclic-since it has the five spore stages as well as heteroecious-since it has an alternate host. Berberis and Mahonia, the alternate hosts are of significance mainly in areas with cold winters where the asexual stage cannot survive but the teliospores do.

2.2.1.1 The five spore stages

The pustules of stem rust that are seen on wheat during most of its growing cycle are called uredia and produce urediospores. Urediospores are oblong in shape and reddish brown in color. They contain two genetically different nuclei i.e. are dikaryotic. A large number of spores are produced by each uredium over several weeks.

In the absence of strong wind current, most spores remain within the crop canopy and cause reinfection. On the contrary, wind carries spores sometimes to long distances. Luig (1985) observed that spores have been carried from southern Africa to Australia in at least three occasions. The more common phenomena involve movement of spores from field to field over short distances.

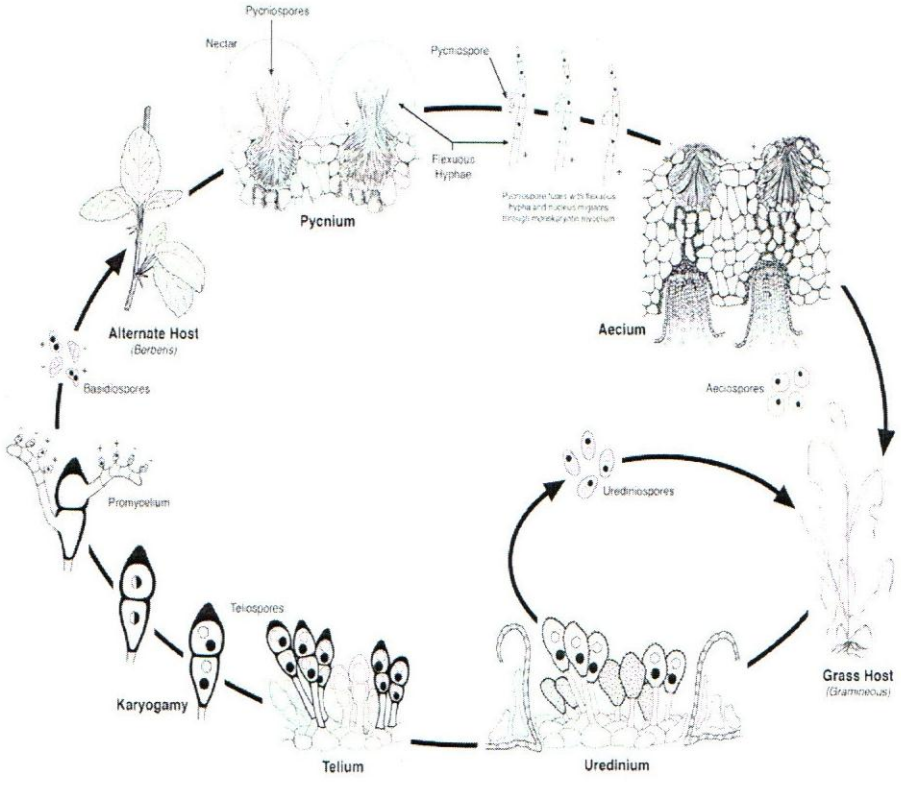


Figure 2. Reproduction cycle of *Puccinia graminis* f. sp *trtici* (After Kurt and Szabo, 2005)

Urediospores germinate when free water is present on the wheat plants. Germ tubes grow until they reach a stoma. This is followed by the development of an appressorium over the stomatal opening, through which an infection peg pushes and a vesicle develops in the substomatal cavity (Staples and Macko, 1984). Infection hyphae grow from the vesicle and produce haustoria mother cells. Penetration pegs then push into the host cells and give rise to haustoria (Harder and Chong 1984).

Infections usually become visible in 5-8 days and sporulation begins 7-14 days depending largely on temperature; 30^0C is the optimum temperature for these events. Where the alternate host is not known to exist, wheat stem rust can survive entirely in the asexual stage.

As the growing season ends, the uredia begin to turn black as urediospore production stops and production of black teliospores follows. The teliospores are resistant to extremes of weather and germinate only after alternate periods of freezing and thawing, or wetting and drying. Teliospores give rise to basidiospores which are small and slightly oval shaped. Basidiospores are forcibly discharged in the air and carried to alternate host. They then germinate rapidly, produce an infection peg and penetrate directly into epidermal cells (Roelfs, 1985).

Barberry genotypes may be either resistant or susceptible to particular stem rust races. Once infected, there is development of sub epidermal, flask-shaped pycnia on the upper leaf surface of barberry. In 7-14 days after infection the pycnia open and a viscous liquid (honey dew) containing pycniospores appears. The pycniospores function as male gametes. The pycnia are of two mating types, + and -. Successful mating can only occur between opposite types. Pycniospores are transferred from one pycnium to another by insects attracted to the honey dew or by splashing raindrops.

Ultimately, aeciospores are produced from the pycniospores which are then carried by wind to nearby wheat fields where they mate and initiate heavy, early infections. The infections on wheat develop into uredia, thus completing the life cycle.

2.2.2 Epidemiology

The epidemic development of diseases within the growth cycle of the host is determined by the initial amount of disease, X_o, and the rate at which the disease increases, described as the apparent infection rate r (Van der plank 1963; 1968). To reduce the severity of disease, the reproductive rate of the pathogen must be decreased. The major components of resistance which affect the reproduction of the pathogen, are a reduction in the infection frequency (IF) or lesion number, a lengthening of the latent period (LP), and decrease in spore production (SP).

A rust epidemic occurring in the field (polycycle) can be thought of as resulting from a series of monocycles, each of which in turn comprises distinctive processes and the whole of which is integrated into disease progress curve to describe the epidemic (Figure 3a) (Teng *et al.*, 1977). In a stem rust epidemic component processes of the rust monocycle recognized are (Figure 3b):

1. Spore production during an infectious process
2. Spore liberation (a passive detachment by wind and plant movement)
3. Spore survival

4. Spore deposition
5. Spore germination
6. Penetration of host tissue by germ tube of the fungus
7. A latent period between penetration and pustule eruption

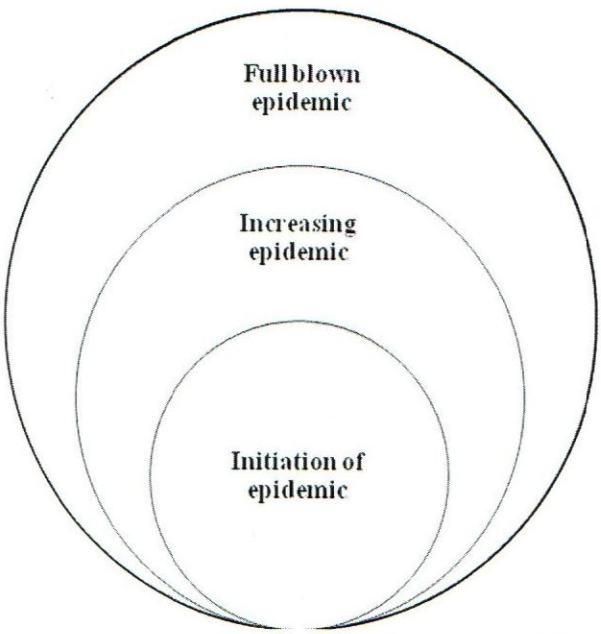


Figure 3a. Polycyclic events characterizing an epidemic in the field (After Teng *et al.*, 1977)

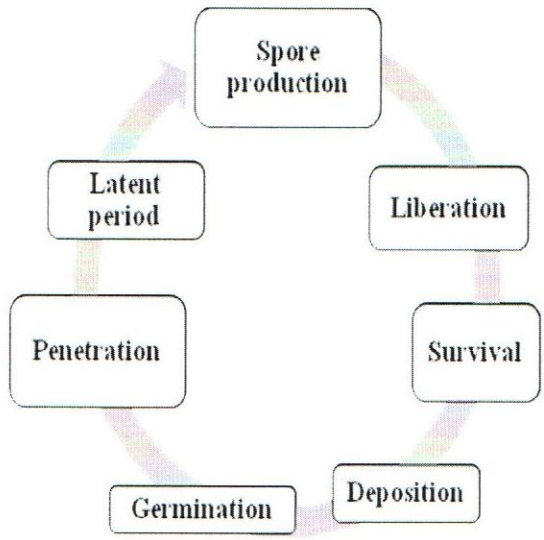


Figure 3b. Steps through distinct rust monocycles (After Teng *et al.*, 1977)

2.2.3 Genetic variation in the pathogen population

The distribution and frequency of pathogen genes and genotypes in a particular area is a function of several factors.

Mutation. Mutations are the most likely and most predictable events that contribute to short-term rust pathogen variability (McIntosh, 1988). Genes for virulence in the rust pathogens are generally recessive. Recessive mutations occur much more frequently than dominant ones, although their frequency is low, perhaps of the order of $1x10^{-5}$ or 10^{-6}. Since the rusts are dikaryotic, a double mutant will be needed.

Recombination. The sexual cycles of rust pathogens have obvious implications both for the evolution of new pathotypes and for seasonal carry-over of inoculum (McIntosh, 1988). For stem rust and leaf rust, the sexual cycle can be an important source of new combinations of genes of virulence wherever the alternate host occurs.

Somatic hybridization. In many fungi, variation can result from the fusion of hyphae from different pathogen genotypes, followed by nuclear exchange and the reassortment of different nuclei in binucleate or multinucleate cells. Nelson *et al.* (1955) observed the fusion of hyphae from different stem rust biotypes on a thin agar film.

Selection pressure. The genotypes of the predominant commercial wheat cultivars as well as those of the barley, and other grasses on which rust survives between seasons will influence the pathogen genotypes that survive.

2.3 Race identification/naming

The first key to what are now designated physiologic races of *Puccinia graminis* was published by Stakman and Levine in 1922. This work followed the discovery of races 1 and 2 by Stakman and Pierneisel in 1916. In 1918, race 3 was described by Levine and Stakman and race 4 by Melchers and Parker. The physiologic race of 1922 was based on the 12 differential host cultivars Little club, Marquis, Kanred (later replaced by Reliance), Kota, Arnautka, Mindum, Marz (known now as Spelmer), Kubaka, Acme, Einkorn, White spring emmer (now called Vernal), and Khalpi. Infection types were divided into groups: 0, 1, and 2 indicating host resistance and 3 and 4 indicating susceptibility. The X infection type was considered separately as a heterogeneous host response.

Physiologic races were determined by a dichotomous key of resistant and susceptible host responses with the heterogenous responses in a separate section of the key. The differential hosts selected by Stakman and co-workers in 1916 were used worldwide until the earlier years of the 80s (Roelfs and Matens, 1989). The changes in the classification of *Puccinia graminis* f. sp. *tritici* resulted from two major scientific events; the development of the gene-for-gene concept, principally by Flor (Flor, 1971) and the development of host lines differing primarily by a single effective gene of resistance.

The earliest lines that contained single genes for resistance to wheat stem rust were developed by Knott, Anderson, Watson and Luig, and Loegering and Harmon (Roelfs and Matens, 1989).

The acceptance of single-gene differentials for race identification came rapidly, involving different systems of nomenclature, i.e., Australia in 1963 (Watson and Luig, 1963), Canada in 1965 (Knott and Anderson, 1956) and the United States in 1972 (Roelfs and McVey, 1979).
A method that has gained wide application is that developed and improved at the Cereal Rust Laboratory, St. Paul, MN.

In this case, differential lines that carry single genes Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9d, Sr9e, and Sr15 in Chinese spring background and Sr9b, Sr10, Sr13, Sr15, and Sr36 in the background of W2691, a highly susceptible line developed at the University of Sydney, Vernstein (Sr9e), combination VII (Sr13 and Sr17) and Triumph (Sr Tmp) are employed as testers.

To designate a race, a coding system is employed. Twelve of the testers are divided into three sets of four lines (Table 1). The first set includes the genes Sr5, Sr7b, Sr9d, and Sr9e. The other two sets include some of the more recently identified genes of race identification. When four lines are classified for resistance or susceptibility there are 16 possible combinations. These are coded from B to T, omitting the vowels (Table 1). The pathogenecity of a race is coded using three letters, each indicating its pathogenicity on one set of four lines.
Code BBB would indicate that all the tester lines are resistant while TTT would indicate that all 12 are susceptible.

Table 1. The U.S coding system used to designate stem rust races and indicate their pathogenicity (Roelfs *et al.*, 1982)

		Wheat lines carrying		
Set1	Sr5	Sr9d	Sr9e	Sr7b
Set2	Sr11	Sr6	Sr8a	Sr9a
Set 3	Sr36	Sr9b	Sr13	Sr10
Code[a]		Host reaction		
B	R†	R	R	R
C	R	R	R	S
D	R	R	S	R
F	R	R	S	S
G	R	S	R	R
H	R	S	R	S
J	R	S	S	R
K	R	S	S	S
L	S	R	R	R
M	S	R	R	S
N	S	R	S	R
P	S	R	S	S
Q	S	S	R	R
R	S	S	R	S
S	S	S	S	R
T	S	S	S	S

[a] Three letters designate race. The first letter indicates the reaction of the lines in set 1; the second letter set 2 etc.
† R=Resistant S=Susceptible

2.4 Races, Biotypes, Cultures and Isolates

Races are types differing in pathogenecity. A race is a specific combination of virulence and avirulence on a defined set of differentials (Knott, 1989). A race is not necessarily one genotype and can indeed be heterogeneous for other characters such as spore color.

Roelfs (1984) defined a biotype as "a population of individuals of the same genotype". The term biotype is used when additional host genotypes are employed to subdivide a race.

The terms culture and isolate have often been used interchangeably. However, an isolate is more likely to be derived from a single urediospore, and therefore to be a pureline. A culture often originates from a field collection and its purity is less certain.

2.5 Infection of the host tissue

Infection of cereal or grass plants by germinating urediospores follows an intricate series of stages and structures, which apparently are essential for establishment of a successful parasitic relationship of the stem rust fungus with its host (Staples and Macko, 1984).

Urediospores germination occurs if the spore is in contact with a film of water on the stem or leaf surface (Kurt and Szabo, 2005). The germinating urediospore produces a germ tube that typically orientates its growth on the host leaf or stem surface perpendicular to the long axis of the epidermal cells. Proper orientation requires that the germ tube is appressed to the waxy cuticle on the epidermis to maximize the chances that the germ tube will encounter a stoma through which penetration of the host may occur. At the stoma the germ tube stops elongating and forms an appressorium over the stomatal opening. Germination and appressorium formation occur at night when dew is present on the plant surface.

Development in *Puccinia graminis* typically stops after formation of the appressorium until dawn when fungal growth resumes. The resumption in development probably depends only indirectly on light through its stimulation of photosynthesis and reduction of CO_2 concentration at stomata in the host plant (Kurt and Szabo, 2005). Yirgou and Cardwell (1968) showed that penetration of the host by *P. graminis* is inhibited by CO_2 but will occur nearly equally well in light or dark in CO_2 -free air.

Under favorable conditions, a narrow penetration peg grows out from the lower surface of the appressorium, through the stoma, and into the sub stomatal cavity below the epidermis.
The appressorium then forms the haustorial mother cell which in turn produces a narrow peg that penetrates the host cell wall by enzymatic dissolution (Harder and Chong, 1984) as well as pressure. Upon penetrating the plant cell wall, a specialized fungal hypha expands to form an enlarged haustorium in the periplasmic space of the host cell.

The success of formation of more haustoria from the primary haustorium depends on the ability of the initial haustorium in extracting nutrients from the host without inducing a resistant response (Staples and Macko, 1984; Wietholter *et al.*, 2003).

Low receptivity to infection by *Puccinia graminis* is a major feature of the slow rusting character of some wheats (Rowell, 1982). Once a compatible relationship has been established in a rust infected host, the normal direction of phloem transport is altered to divert nutrients to the infected tissue at the expense of actively growing plant tissue (Mendgen, 1981). This change is

characterized by massive increases of respiration and accumulation of cytokinins in the infected area. Sugars accumulate in the lesion area, and an invertase provides hexoses used by the fungus for growth and sporulation. The infection process by which *Puccinia graminis* meets these needs is a highly regulated response system that involves signaling and response in the host and pathogen (Kurt and Szabo, 2005).

2.6 Symptomology

Infections in cereals or grasses occur mainly on stems and leaf sheaths, but occasionally they may be found on leaf blades and glumes as well. The first macroscopic symptom is usually a small chlorotic fleck, which appears a few days after infection. About 8-10 days after infection, a pustule several millimeters long and a few millimeters wide is formed by rupture of the host epidermis from a mass of brick-red urediospores produced in the infection. These pustules are generally linear or diamond shaped and may enlarge up to 10mm long. The Powderly masses of urediospores appear similar to rust spots on weathered iron surface (Kurt and Szabo, 2005). With age the infection ceases production of brick- red urediospores and produces a layer of black teliospores in their place, causing the stems of heavily infected plants to appear blackened late in the season.

2.7 Rating system

Infection types for uredinia on seedling leaves are commonly described based on a scale developed by E.C. Stakman and co-workers starting 1910. This gives infection types as 0, 1, 2, 3 or 4 with an extra class designated as X, for heterogenous or mesothetic infections (Stakman et al., 1962) (Figure 4, Table 2). In practice, infection types form a continuum and there is no clear-cut demarcation between types. For convenience, infection types 0, 0; ,1 , 2 and X are rated resistant while 3 and 4 are rated susceptible.

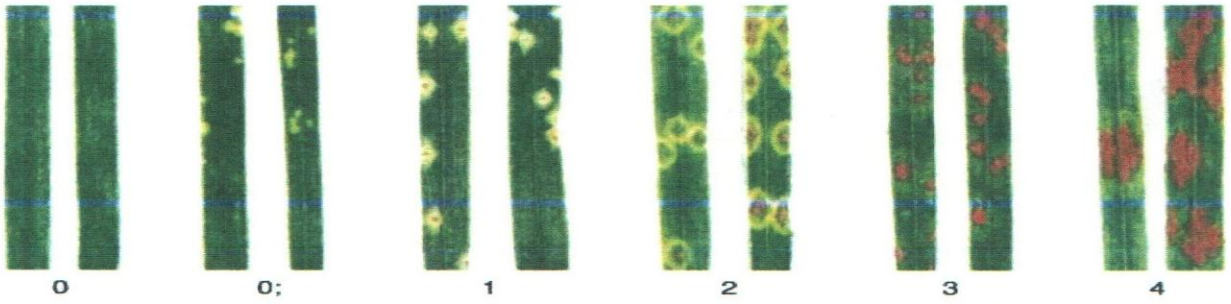


Figure 4. Infection types (ITs) of *Puccinia graminis* f. sp. *tritici* : 0 - 4 scale. IT=0 (no uredia); IT=0; (fleck); IT=1 (small uredia); IT=2 (small to medium uredia); IT=3 (medium uredia without chlorosis or necrosis) IT=4 (large uredia without chlorosis or necrosis) (Leonard and Szabo 2005)

Table 2. Infection codes used in classifying the reaction to stem rust on seedling wheat leaves. (Adapted from Stakman *et al.*, 1962)

Host response	Infection type	Description of symptoms
Immune	0	No sign of infection to the naked eye but minute flecks may be visible under low magnification
Very resistant	0;	No uredia, but distinct flecks of varying sizes, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic or necrotic areas
Moderately resistant	2	Small to medium sized uredia, typically in a dark green island surrounded by a chlorotic area
Mesothetic /heterogenous	X	A range of infection types from resistant to susceptible scattered randomly on a single leaf; caused by a single race not a mixture
Moderately susceptible	3	Medium sized uredia, usually surrounded by a light green chlorosis
Susceptible	4	Large uredia with limited amount of chlorosis; may be diamond- shaped

2.8 Recorded stem rust

Stem rust was once the most notorious disease in most wheat –growing regions of the world. Several references in the Bible relate to epidemics of cereal rusts and smut which were inflicted on Israelites as punishment for their sins (Chester, 1946). Fragments of stem rust wheat from the Bronze age have been discovered in Israel (Kislev, 1982).

Numa pompilius (715-672 BC) described the Roman festival of Robigalia that was established to protect cereal crops through prayer and sacrifice to the rust gods (Leonard and Szabo 2005). Aristotle and Theophrastus associated cereal rust epidemics with warm, wet weather (Chester, 1946). In recent history, several authors have documented losses associated with stem rust (Table 3).

Table 3. Examples of recorded global losses to stem rust

Year	Region	Estimated yield losses (%)	Source
1932	Eastern and central Europe	5-20	Zadoks,1963
1951	Scandinavia	9-33	Zadoks,1963
1974	Southern Australia	-	Watson,1981
1948,1951,1952,1956	North China and inner Mongolia	-	Roelfs,1978
1935	North Dakota and Minnesota	50	Leonard,2001
1950-55	North America and Canada	40	Roelfs,1978

2.9 Economic importance of stem rust

Stem rust is primarily a warm weather disease, but it can cause great damage to susceptible wheat crops over broad geographic regions. A crop that appears healthy three weeks to harvest can be devastated by explosive build up of stem rust if sufficient inoculum arrives from heavily infected wheat crop in some distant region. Severe infection of stem rust interrupts nutrient flow to the developing heads, resulting in shriveled grain (Kurt and Szabo, 2005). Furthermore, stems weakened by rust infection are prone to lodging and further loss of grain (Roelfs *et al.*, 1992).

2.10 Wheat and stem rust in Kenya

Progress in the breeding of wheat in Kenya has been on the background of past achievements vis-à-vis set-backs from changing rust races (Dixon, 1960).

Two stem rust physiologic forms were differentiated in 1927 (Green *et al.*, 1968). Later another form appeared on wheat variety Reliance at Njoro in 1930 and a forth one was recognized in 1931(Anonymous, 1933). By 1947, eight stem rust races (on the basis of Kenya differentials) had been identified (Green *et al.*, 1968, Payne *et al.*, 2000). Four more races appeared three years later with little or no stable resistance available in the Kenyan genepool (Table 4). Based on international race differentiation system, the races K1, K5 and K12 were identified as American 'race 21'. K8, K10 and K11 as American race 24 (Thorpe, 1958).

Table 4. Races of stem rust recorded in Kenya from 1928 to 2003

Race Kenya No.	International No.	Year Identified
K1	21	1928
K2	17	1928
K3	34	1930
K4	116	1931
K5	21	1936
K6	107	1940
K7	?	1943
K8	24	1948
K9,10,11	122,24,24	1950
K12	21	1951
K13,14	?	1953
K15	?	1954
K16	?	1955
K17,18,19	?	1956/57
K20	?	1958
PgtUg99	TTKS	2003

The absence of winter coupled with an abundance of wild and cultivated host material for survival have been thought to be the key factors in the broad virulence spectrum of wheat stem rust in Kenya (Payne et al., 2000). Leppik (1970) draws attention to *Berberis holstii* which is endemic to Kenya and at times prone to heavy rust infection, as the probable alternate host for wheat stem rust in East Africa. However earlier observations revealed that aecidial cultures taken from this species of barberry failed to infect wheat leading to the conclusion that *Berberis holstii* is non-functional in the stem rust cycle and that there is no known alternate host of the rust in Kenya (Thorpe, 1958).

Studies with 164 collections of wheat stem rust on local wild and exotic grasses produced no positive hosts either (Harder *et al.*, 1972). Localized surveys to date indicate that grass species are not important in the epidemiology of wheat stem rust in East Africa. Certainly however, since large areas of grassland are yet to be explored, it is possible that grass species may play some role.

The close genetic relationship of the earlier wheat varieties caused them to be destroyed as a group by new rust races (Thorpe, 1958). The year 1953 marked the beginning of a new era in the Kenya wheat industry (Dixon, 1960). There was a large scale introduction and scrutiny of

diverse germplasm through the 'International spring wheat nursery'-funded by the Rockfeller Foundation programme in Mexico and 'Near East nurseries' -organized by F.A.O (Payne et al.,2000). The opportunity derived from these nurseries was twofold: (a) the evaluation of the material on the broadest possible basis and (b) the chance to select new sources of resistance based not only on Kenya observations but on world-wide basis year by year (Thorpe, 1958, Dixon, 1960).

The influx of new wheat germplasm in the 50's opened a chance for large number of crosses, on average three hundred thirty five per year were made and cultivar releases were numerous averaging nearly four per year from 1960-1968 (Pinto & Hurd 1970), (Appendix D). Despite the progress made through the introduced sources of resistance, in 1968 losses due to stem rust and leaf rust were substantial, reaching over £500,000 (Hurd *et al.*, 1969). There was an attempt to spread the risk of huge losses by growing a large number of varieties with different but unidentified sources of resistance (Evans *et al.*, 1969). In the mid 1970's the government of Kenya provided facilities at the National Plant Breeding Research Centre, Njoro with logistical support from CIMMYT to allow screening and off-season generation advancement of breeder's material from West Asia and North Africa (WANA) and other countries.

Wheat is planted in Kenya at times chosen to make maximum use of rainfall while the crop is growing, yet have it ready for harvest during a dry period. In most of Kenya's wheat growing areas ranging from 1800-2800m meters above sea level (Payne *et al.*, 2000), the long rains usually occur between March and August, the short rains during October and November. Dry conditions usually prevail from December to March (Green *et al.*, 1968). Most wheat is planted from March to June and harvested from August to November (Figure 5). Markedly, only a small proportion of the crop is grown above 2400m and on a small acreage. On this land rust inoculum can be produced, and remains standing in January and February as a reservoir for rust in the main wheat growing season. Urediospores produced on this wheat could be in the air after the new crop at lower altitudes has emerged.

The wheat growing season has temperature characteristically ranging from 18-30^{0}C with days uniformly about 12.5 hr long. Dews are heavy and precipitation occurs frequently as showers during the main growing season.

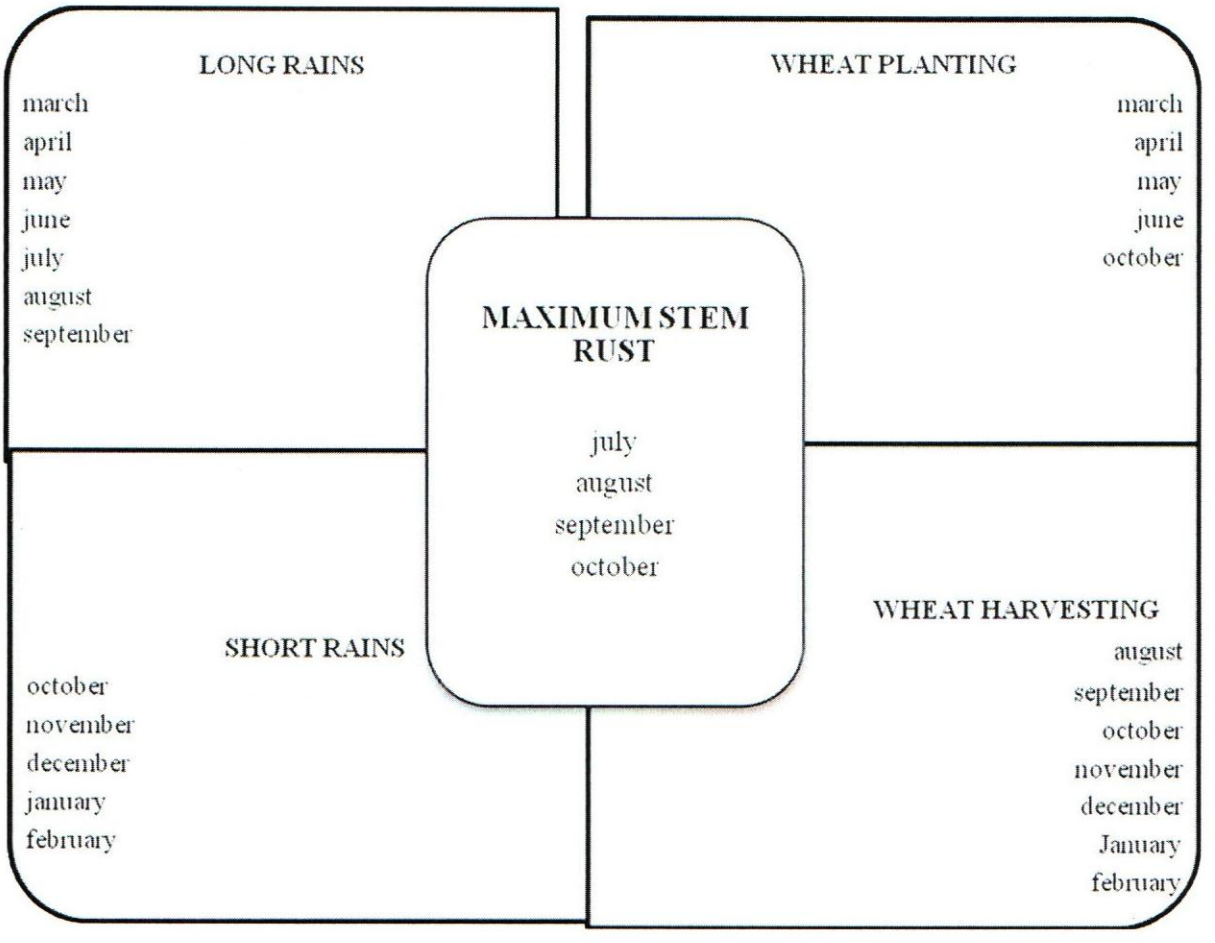


Figure 5. Periods of planting and harvesting of wheat: rainfall, and maximum occurrence of airborne urediospores of stem rust in Kenya. (After Green *et al.*, 1968)

As at First World War, only 10,000 acres of wheat were grown in Kenya (Thorpe, 1958). By the year 1940 production had risen steadily to some 200,000 acres. Production subsequently rose to 340,000 acres in 1955 following the great stimulus provided by World War II. The Kenya development plan of 1970-1974 estimated that 500,000 ha of land were suitable for wheat production (Payne *et al.*, 2000). Only about a third of this land was on wheat by 1994. Presently, wheat is grown in many agro-ecological zones that have different planting dates (Ralph and Schimtz, 1983; Kamidi, 1995).This provides the ever present reservoir of rust inocolum often termed the 'green- bridge' (Roelfs *et al.*, 1992).

2.11 Stem rust control

Cultural methods

Cultural practices are primarily aimed at breaking the life cycle of the rust. Early infection sometimes arises from volunteer plants and delayed planting may help. Cultivation to remove volunteer plants from an earlier planted crop may also reduce sources of inoculums. Barberry bushes, if they occur should be eradicated since they act as an intermediate host.

Chemical control

Several effective fungicides for example Dichlone, Propaconizole, Tebuconazole, Sulfur, and Tridemefon are available (Knott, 1989). One spraying may be sufficient, but depending on the chemical, the weather, and the length of the growing season, two or more application may be necessary. Chemicals are expensive and there is an added cost in their application.

Furthermore, throughout the world, people are growing increasingly concerned about the use of chemicals and the potential danger of residuals in food, of contamination of water supplies and injury to the people applying them.

Genetic control

Development and use of resistant cultivars is the most effective method of control of rusts (Johnson, 1992). Use of resistant cultivars adds no extra cost to farmers. Breeding for resistance in many wheat growing countries is a never-ending task that goes through a repeating cycle. Wheat breeders are increasingly interested in types of resistance and methods of using resistance that will result in long-lasting control of the rusts, also termed durable resistance (Johnson, 1984; Bruce *et al.*, 2002).

2.12 Host- parasite specificity

The hypersensitive response is doubly effective against biotrophs such as rust fungi because of the accompanying up-regulation of a multitude of defense genes (Bohland *et al.*, 1997; Heath, 1997; Munch-Garthoff *et al.*, 1997; Lin *et al.*, 1998) as well as the loss of an essential nutritional base when host cells bearing fungal haustoria collapse and die. Thus the hypersensitive response to pathogen invasion is one of the most important natural defenses that *P. graminis* must overcome to establish a successful parasitic relationship Kurt and Szabo (2005). Germ tube walls of *P. graminis* f. sp. *tritici* contain a glycoprotein that induces a hypersensitive lignifications response when applied to healthy wheat leaves of both resistant and susceptible genotypes.

2.13 Host resistance

The 20th century started with the demonstration of simple Mendelian genetic control of rust resistance in wheat (Jeffrey *et al.*, 2007). During this century large yield losses in wheat were avoided by the identification, cataloguing and deployment in international and local breeding programs of rust resistance genes. Resistance is classified into two classes: the vertical, unstable, monogenic and qualitative type, and the horizontal, stable, polygenic, and quantitative type (Parleviet, 1979). Vertical resistance delay the onset of an epidemic (X_0) while horizontal resistance slows it down once it has started (reduce *r*) (Van der plank, 1963).

Currently fifty seven different host genes (McIntosh, 1979) for stem rust resistance have been isolated (see appendix B). Stem rust resistance genes in wheat are officially designated when the chromosomal arm on which they are located has been determined and when it has been established that the new gene is distinct from other known stem rust resistance genes on that arm Kurt and Szabo (2005).

Among the 45 officially designated stem rust resistance genes (McIntosh *et al.*,1995), only twenty were found originally in hexaploid bread wheat (*T. aestivum*) ; nine were transferred to bread wheat from tetraploid *T. turgidum* (either wild or cultivated durum wheat), three were from diploid *T. monococcum*, two were from cultivated rye, and eleven other stem rust resistance genes were transferred to bread wheat from various wild relatives (*Triticum comosum, T. speltoides, T. tauschii, T. timopheevi, T. ventricosum and Thinopyrum ponticum*).
Furthermore, twenty four other presumably distinct resistance genes have received provisional designations pending further characterization; eleven of these were found in bread wheat, one is from durum and two are from *Thinopyrum* species.

All the officially and provisionally designated stem rust genes are known to be race specific (Kurt and Szabo, 2005). Sr2, is however an exception; it has long provided stable, partial resistance in the adult plant in many cultivars grown throughout the world (McIntosh et al., 1995).

When two or more genes for race specific resistance are effective against a race of *P. graminis* f. sp. *tritici* the gene conditioning the highest level of resistance is epistatic to the gene(s) conditioning lower levels of resistance (Roelfs, 1988). Both the resistance in wheat and the avirulence in *P. graminis* f. sp. *tritici* are often incompletely dominant (Roelfs, 1988; Roelfs and Groth, 1988). Wheat is a self pollinated crop, so commercially grown cultivars are highly

homozygous implying that the incomplete dominance of resistance has little or no practical significance.

2.14 Inheritance of resistance

Resistance to stem rust has been reported to be monogenic, digenic, or more complicated in inheritance (Ausemus *et al.*, 1946).

Knott and Anderson (1956) used diallel crosses to determine whether resistant parents carried genes at the same or different loci. They also employed backcrosses to simplify genetic analysis.

Usually, the first observation on pattern of monogenic inheritance is recorded on the response of F_1 hybrids. A resistant response similar to the resistant parent indicates dominance of resistance. The ratio of resistant versus susceptible F_2 plants indicates the number of resistance genes segregating in the cross. Following Mendel's law of segregation and in the absence of linkage, the expected F_2 segregation and interpretations are (Roelfs *et al.*, 1992):

3 resistant: 1susceptible=1 dominant gene;

1 resistant: 3 susceptible=1 recessive gene;

13 resistant: 3 susceptible= 1 dominant+ 1 recessive gene;

7 resistant: 9 susceptible=2 recessive genes;

15 resistant; 1 susceptible=2 dominant genes;

9 resistant; 7 susceptible=2 complementary genes.

When a susceptible parent is used in backcrossing resistant F_1s, the expected backcross segregation ratios are:

1resistant: 1 susceptible=1 dominant gene;

3resistant:1 susceptible=2 dominant genes;

7 resistant: 1 susceptible=3 dominant genes.

CHAPTER III

INHERITANCE OF RESISTANCE GENES SR26 AND SR36 TO RACE PGTUG99 OF STEM RUST IN BREAD WHEAT

3.1 Introduction

The increase of race PgtUg99 of stem rust since it was first reported (Pretorius *et al.*, 2000) is reminiscent of race 15B which caused tremendous damage to wheat crops in the United States and Canada (Mohammed and Ausemus, 1957).

Biffen demonstrated that host resistance to stem rust was inherited in a Mendelian manner (Biffen, 1905). The host- pathogen interaction produces a visible response which is actually the result of the interaction of the host and pathogen genotypes and the environment existing immediately before and following infection (Rowell, 1984). The genetic interactions in *P. graminis* f. sp. *tritici* with the primary host apparently follow the gene-for-gene system (McIntosh and Watson, 1982). Each interaction involving host reaction-pathogen pathogenicity gene pair probably results in a different low infection type (Roelfs and McVey, 1979). The genetic background of the host can affect the expression of specific genes for resistance in that host (Roelfs, 1988). Low infection types produced on two host lines with the same designed resistance gene often differed to a wide range of cultures (Roelfs and McVey, 1979; Roelfs and Groth, 1988). When genes for specific resistance are transferred to cultivars that are susceptible to a wide range of diseases, the low infection types are often greater whereas resistance genes transferred into cultivars resistant to a wide range of diseases, often have a reduced low infection type (Roelfs, 1988).

The study of the mode of inheritance to stem rust, other characters and the linkage between them, if any, is an essential consideration in any planned plant breeding program.

3.2 Literature Review

Much of the early genetic work on the inheritance of rust resistance in wheat was done in conjunction with wheat breeding (Knott, 1989). A susceptible cultivar would be crossed with a resistant parent. In the usual pedigree breeding system (Poehlman, 1987), F_2 populations and F_3 families would be studied, often in the field with natural rust epidemics. The families were then classified into homozygous resistant, segregating, and homozygous susceptible classes. In some cases seedling tests in the greenhouse with specific rust races were carried out. As a result of these studies a considerable body of data on resistance to leaf and stem rust was developed, which was summarized by Ausemus *et al.*, (1946). Resistance was variously reported to be monogenic, digenic, or more complicated in inheritance.

Genetic studies may involve crossing resistant and susceptible cultivars, or crossing various parents with one or more known gene(s) for resistance-'allellism test' (Roelfs *et al.*, 1992).When resistance is dominant, clearly identifiable, and simply inherited, its inheritance may be determined directly from the segregation of F_1 plants from a backcross.

To determine the number of genes for resistance it is necessary to decide whether each family is segregating or susceptible in the F_3 generation. If there is one gene for resistance, the ratio will be 1 segregating: 1 susceptible family, for two genes 3:1, for three genes 7:1, etc (Roelfs *et al.*, 1992). The segregations within the segregating families often provide additional information on the types of gene action involved.

When a single gene is involved, the infection type on the F_1 and backcross F_1 plants will indicate whether the resistance is dominant or recessive. When the gene conditions a moderately resistant infection type, it is not uncommon to find that the F_1 plants are intermediate in reaction (Knott, 1989).

3.3 Materials and Methods

3.3.1 Parental genotypes

The parental cultivars used in this study are:

i. Susceptible: Njoro Bw II, Kwale, Chozi, and Duma

ii. Resistant: Eagle, Cook

The varieties Njoro Bw II and Kwale are commercially popular in the high altitude environments ordinarily associated with high rainfall while Duma and Chozi were bred for the lower drier regions and have been proposed for the non-traditional wheat growing zones of Kenya (Kimurto *et al.*, 2004).

Cultivar Eagle is the source of resistance gene Sr26 (McIntosh, 1995) and shows resistance to PgtUg99 (Pretorius 2000; Jin and Singh, 2006). This gene is a translocation from *Thinopyrum elongatum* (McIntosh, 1995; Singh *et al.*, 2006). The resistance gene Sr36 initially derived from *Triticum timopheevi* (McIntosh, 1995), exhibits an immunity (no symptoms) to race PgtUg99 at both seedling and adult plant stages (Singh *et al.*, 2006) and is present in cultivar Cook. The two resistant varieties were selected as resistance donors because they had also shown good agronomic potential and could be grown easily in preliminary screening nurseries. A brief pedigree information for the parental stocks is summarized in Table 5.

Table 5. Pedigree of 6 parental cultivars involved in the inheritance study

Parent	Origin	Pedigree¶	Resistance gene
Eagle	Australia	Timgalen / condor/2/condor	Sr 26
Cook	Australia	Scout pureline selection	Sr 36
Njoro Bw II	Kenya	Ias-58/4/Kalyansona/Bluebird//Cajeme-F-71/3/Alondra/5/Bobwhite	Susceptible
Kwale	Kenya	Kavkaz/TanoriI F- 71/3/Maya 4//Bluebird/Inia	Susceptible
Chozi	Kenya	F-12-7/Cocoraque 75//Genaro 81	Highly Susceptible
Duma	Kenya	Aurora/Up301//Gallo/Super X/3/Pewee/4/Maipo/Maya 74//Pewee	Highly Susceptible

¶ Crosses represented following Purdy nomenclature (Purdy *et al.*, 1968; Skovmand *et al.*, 1997)

3.3.1.2 The F_1s

16 F_1 populations were generated by crossing resistance x susceptible for all parents including reciprocals. An additional 2 F_1 populations were developed by crossing resistance x resistance, reciprocals inclusive.

In developing these populations the parental materials were planted in three crossing blocks on 3rd, 10th and 17th march 2007 respectively at KARI-Njoro. The staggered plantings were necessary in order to synchronize availability of pollen and also to manage the volume of crossing work. The plot sizes were 1 m, 2 rows for the susceptible cultivars and 6 m, and 8 rows for Eagle and Cook with 20cm row spacing. Purposely, emasculation and pollination (Figure 6) was effected on 20 healthy well formed spikes of the intended female parent before the anthers matured; while they were still green in the florets as suggested by Kinyua (1997). Care was taken not to damage the stigma.

The emasculated ears were then covered with glassine bags for two to three days. Spikes of the male parents were selected for pollination, before they shed pollen. Pollination followed the go-go method (Kinyua, 1997), in which the glassine bags were trimmed at the top and the spike shaken back and forth to release the pollen grains from the protruded anthers. Optimal growth conditions including weed control, watering and disease control were provided to the pollinated plants to ensure for good seed development.

Figure 6. Emasculation in the crossing block

3.3.1.3 The BC_1F_1 populations

Seeds from the 18 F_1 populations were planted in plots of 4 rows x 2 m (alongside the parental germplasm) to give rise to 18 F_1 populations. Out of these, the 8 F_1 progeny derived from the Sr26 donor parent -Eagle didn't flower and hence were unavailable for development of backcrosses. These F_1s presented as 'grass clumps' a condition also reported by Hermsen (1967); Moore, (1968); Canvin and Mcvetty (1975). These are described in detail in sections 3.3.4, 3.4.2. The 8 Cook derived F_1s flowered normally. Emasculation on these F_1s was on average done on 20 spikes per population. These spikes were pollinated with pollen from each of the recurrent parents to give rise 8 BC_1F_1 seeds.

3.3.1.4 The F_2 populations

Eight F_2 populations were generated by allowing natural selfing of each of the Cook derived F_1s. Up to 30 spikes were selected and threshed giving rise to between 400 -650 seeds.

3.3.2 Seedling tests

Soil: sand mixture, 1:1 (v/v) was filled into 30-by 50-cm flats. This potting media had initially been autoclaved to minimize possible damage to seedlings from soil borne pathogens especially nematodes. Two rows of each parent and F_1, ten rows of F_2, five rows of BC_1F_1 seeds were sown in each flat on 14th February 2008. Each row contained 10 to 20 seed, planted 2cm deep, with 4-cm spacing between rows. The planted trays were placed in an isolated chamber within the greenhouse and watered daily for two weeks to obtain healthy, clean seedlings for inoculation. Temperature ranged between 15-26^{0}c through the two weeks growing period. Between twenty and thirty plants were obtained for the parents. All the other crosses gave rise to a varied number of plants (Table 6) which were inoculated and subjected to the inheritance studies.

Table 6. Normal and 'grass clump' phenotypes observed in F_1, F_2 and BC_1F_1

Population[a]	Seeds Planted	Plants germinated/inoculated as seedlings	[b]Phenotype of adult plants
Parents			Normal
Njoro Bw II	20	20	Normal
Kwale	20	19	Normal
Duma	20	18	Normal
Chozi	20	20	Normal
Cook	20	20	Normal
Eagle	20	18	Normal
F_1s			
Nj Bw II/Eagle	50	46	Grass clump
Eagle/Nj Bw II	50	43	Grass clump
Nj Bw II/Cook	50	48	Normal
Cook/ Nj Bw II	50	37	Normal
Kwale/Eagle	50	49	Grass clump
Eagle/Kwale	50	48	Grass clump
Kwale/Cook	50	45	Normal, sterile
Cook/ Kwale	50	42	Normal, sterile
Duma/Eagle	50	35	Normal, sterile
Eagle/Duma	50	36	Normal, sterile
Duma/Cook	50	48	Normal
Cook/ Duma	50	44	Normal
Chozi/Eagle	50	47	Grass clump
Eagle/Chozi	50	44	Grass clump
Chozi/Cook	50	37	Normal
Cook/ Chozi	50	42	Normal
Eagle/Cook	40	18	Grass clump
Cook/Eagle	40	31	Grass clump
F_2s			
Nj Bw II/Cook	120	96	Normal
Cook/ Nj Bw II	80	78	Normal
Kwale/Cook	100	90	Normal
Cook/ Kwale	110	106	Normal
Duma/Cook	90	69	Normal
Cook/ Duma	90	60	Normal
Chozi/Cook	85	82	Normal
Cook/ Chozi	50	76	Normal
Backcrosses (BC_1F_1)			
Nj Bw II/2*/Cook	100	67	Normal
Cook/ *2/Nj Bw II	70	44	Normal
Kwale/2*/Cook	64	52	Normal
Cook/ *2/Kwale	75	63	Normal
Duma/2*/Cook	42	40	Normal
Cook/ *2/Duma	60	48	Normal
Chozi/2*/Cook	50	43	Normal
Cook/ *2/Chozi	35	27	Normal

[a]Crosses represented following Purdy nomenclature (Purdy *et al.*, 1968;Skovmand *et al.*, 1997)
[b]Grass clump phenotype elucidated in text (sections 3.3.4,3.4.2)

3.3.2.1 Inoculation

The inoculum used in this study was that of PgtUg99 provided by the pathology department of KARI-Njoro. This inoculum had been collected earlier from an Sr 31 bearing cultivar-Chozi using a cyclone collector and was in storage in dessicators at 5-8^{0}C. To confirm that the isolate was pure, the inoculum was first applied on a selected set of differentials (Appendix C) previously planted in trays and identification determined through the methods of Roelfs and Martens (1989). Through this procedure a code is obtained corresponding to patterns of reactions depicted by the differential materials. In this test, the code TTKS popularly recognized to represent race PgtUg99 of *Puccinia graminis* (Singh *et al.*, 2006) was observed.

Prior to inoculation of the seedlings (as well as the differentials), the inoculum was left overnight on the laboratory benches in order to rehydrate. 3grams of the spores were weighed and then suspended in 500ml distilled water in which 5-7 drops of the surfactant tween 20 were added, followed by thorough agitation to produce the desired inoculum mixture. This mixture was applied on the seedlings through an atomizer with a fine nozzle with the trays placed on a revolving table to ensure uniform spread of the spores. Inoculated seedlings were left overnight in the growth chamber at a temperature of 16-20^0 C in the dark.

To enhance spore germination, germ tube growth and appressorium formation, conditions of free water were created within the chambers by pouring water in the troughs in which the trays were suspended and by spraying the polythene- masking its walls with a fine mist of distilled water. Twenty hours post inoculation, the seedlings were removed from the growth chambers and allowed to slowly dry in the adjacent well light cubicles at a temperature of 25^0 C for about 4 h. The plants were then moved to the green house at a temperature of 15-28^0 C where they were watered for 14 days.

On the 15th day, scoring for seedling reactions was done on the basis of the 0-4 scale of Stakman *et al.*, (1962), (Figure 4).

3.3.3 Chi square (X^2) test of expected segregation ratios

The number of resistant and susceptible F_2 families was tested against the 3:1 ratio for segregation of one gene using chi-square goodness- of- fit test at α=0.05% as suggested by Knott, (2000). Similarly by applying Proc Chi of SAS, chi-square was calculated based on the formula below (Steel and Torrie,1980) and used in testing the 1:1 ratio expected for a

BC_1F_1population at α=0.05%. To simplify the analyses plants that showed intermediate reactions were classified as susceptible.

$$\text{Chi square, } X^2 = \sum \left[\frac{\text{Observed-Expected}]-0.5 \ ]^2}{\text{Expected}} \right.$$

df =2-1=1

At α=0.05%,

where 0.5 is Yates correction factor

3.3.4 Validation of the 'grass clump'phenotype

The grass clump observation was corroborated in this study by planting seeds harvested from both straight and reciprocal Eagle F_1 hybrids. Seeds of all the parents Njoro Bw II, Kwale, Duma, Chozi, Cook and Eagle were similarly sown in 15cm diameter pots at depths of 1cm and placed in the green house where they were watered regularly till maturity. Growth was scored by measuring stretched height (cm) from the soil surface to the longest leaf tip of the seedlings starting two weeks post sowing and on weekly intervals for eight weeks thereafter. The relative height (*r*Height) was calculated as height at 10weeks/height at 2 weeks for each of the populations. Digital pictures were taken as records of the 'grass clump phenotype'

3.4 Results

3.4.1 Inheritance of resistance to PgtUg99

Typical reddish brown pustules were observed on the leaf blades of some of the inoculated plants in the greenhouse at the end of the incubation period. These reactions were classified accordingly and used in characterizing the inheritance of Sr26 and Sr36.

3.4.1.1 Seedling infection of the parents and Eagle-derived F_1 progeny

The results obtained with the Sr26 donor parent Eagle and subsequent F_1s when inoculated with PgtUg99 are presented in Table 7a.

Table 7a. Seedling reaction of F_1 plants for eight crosses involving variety Eagle (Sr26) and four Kenya bread wheat cultivars

Test Population	Total plants inoculated	Number of plants with infection type 0, 0; , 1- ,1 ,1+ , 2	3, 3-, 3+, 4
Parent		Resistant	Susceptible
Eagle	20	20	0
Njoro Bw II	20	0	20
Kwale	19	0	19
Chozi	20	0	20
Duma	18	0	18
F_1s			
Nj Bw II/Eagle	38	38	0
Eagle/Nj Bw II	35	35	0
Kwale/Eagle	41	41	0
Eagle/Kwale	39	39	0
Chozi/Eagle	35	35	0
Eagle/Chozi	40	40	0
Duma/Eagle	28	28	0
Eagle/Duma	31	31	0
Eagle/Cook	17	17	0
Cook/Eagle	27	27	0

Eagle had low infection types of '0' and '0;' of complete resistance of Sr26 to race PgtUg99. The proportion of plants with low infection types amongst the Eagle derived F_1s pointed to dominance of Sr26 and confirmed its major gene nature. The seedling reaction of the parental genotypes was consistent with their adult plant responses (discussed in Chapter 4) in which case; Eagle had a low infection type 6RMR while Njoro Bw II, Kwale, Chozi and Duma scored 44SMS, 43SMS, 85S and 70S respectively on the basis of the modified Cobb scale (Peterson *et al.*, 1948).

3.4.1.2 Seedling infection of the parental and Cook-derived F_1, F_2, BC_1F_1 progenies

Results obtained with Cook, the Sr36 donor parent and its progeny are given in Table 7b.

Table 7b. Seedling reaction of Parents, F_1, F_2 and BC_1F_1 plants for eight crosses involving resistant parent Cook (Sr36) and the four susceptible Kenya bread wheat cultivars

Test population		Number of plants 0;1-11+	2 2N2+	3 3-3+ 4	Ratio Observed	Expected		
	Plants inoculated	Resist.	Segreg.	Suscep.	R:S	R:S	X^2	*P-value*
Parents								
Cook	20	20	-	0	-	-	-	-
Njoro BW II	20	0	-	20	-	-	-	-
Kwale	19	0	-	19	-	-	-	-
Chozi	20	0	-	20	-	-	-	-
Duma	18	0	-	18	-	-	-	-
F_1s								
Nj Bw II/Cook	43	43	-	-	-	-	-	-
Cook/Nj Bw II	30	30	-	-	-	-	-	-
Kwale/Cook	36	36	-	-	-	-	-	-
Cook/Kwale	33	33	-	-	-	-	-	-
Chozi/Cook	27	27	-	-	-	-	-	-
Cook/Chozi	38	38	-	-	-	-	-	-
Duma/Cook	37	37	-	-	-	-	-	-
Cook/Duma	36	36	-	-	-	-	-	-
F_2s								
Nj Bw II/Cook	96	67	6	23	67: 29	3:1	1.3889	0.2386
Cook/Nj Bw II	78	61	7	10	61:17	3:1	0.4274	0.5133
Kwale/Cook	90	74	6	10	74:16	3:1	2.5037	0.1136
Cook/Kwale	106	78	9	19	78:28	3:1	0.1132	0.7365
Chozi/Cook	83	62	4	17	62:21	3:1	0.004	0.9495
Cook/Chozi	76	53	3	20	53:23	3:1	1.1228	0.2893
Duma/Cook	69	51	3	15	51:18	3:1	0.0435	0.8348
Cook/Duma	60	39	5	16	39:21	3:1	3.2	0.0736
BC_1F_1								
(Nj Bw II/Cook)/Nj Bw II)	71	39	5	27	39:32	1:1	0.6901	0.4061
(Cook/Nj Bw II)/Nj Bw II)	44	26	7	11	26:18	1:1	1.4545	0.2278
(Kwale/Cook)/Kwale)	52	24	10	18	24:28	1:1	0.3077	0.5791
(Cook/Kwale)/Kwale)	65	28	9	26	28:35	1:1	0.7778	0.3778
(Chozi/Cook)/Chozi	43	26	2	15	26:17	1:1	1.8837	0.1699
(Cook/Chozi)/Chozi	27	16	2	9	16:11	1:1	0.9259	0.3359
(Duma/Cook)/Duma	40	21	5	14	21:19	1:1	0.1	0.7518
(Cook/Duma)/Duma	48	28	7	13	28:20	1:1	1.3333	0.2482

Low infection type in variety Cook confirmed that Sr36 was resistant to race PgtUg99. Most plants in the Cook derived F_1s gave resistant reactions suggesting that resistance was dominant to susceptibility. All reciprocal hybrids (Table 7b) were not different from straight crosses implying that there was no maternal effect in the transmission of resistance by Sr36. The F_2 populations segregated in a 3:1 (resistant: susceptible) ratio, confirming that resistance in Cook to race Pgt Ug99 was conditioned by a single gene, Sr36. All the BC_1F_1 progeny segregated 1:1 (resistant: susceptible) (Table 7b), further supporting that resistance in Cook is monogenic and dominant.

3.4.2 Inheritance of the 'grass clump' character

The height in cm of the resistant parent Eagle and its F_1 progeny in different crosses, recorded weekly over a 10 week growth period, are given in Table 8,Figure 7. The parameter, relative height (*r*Height) for each population gives an approximate rate of growth.

Table 8. Height (cm) of five parental wheat cultivars and their F_1 progenies through 10 weeks of growth in the greenhouse at Njoro

	Height (cm)										
Population	Weeks after planting										
Parents	2	3	4	5	6	7	8	9	10	SE$^{\beta}$	*r*Height
Eagle	11.2	12.1	17.2	20.8	31.7	40.2	48.6	51.4	62.9	0.37	5.62
Nj Bw II	10.6	12.8	17	22.4	35.3	39.8	47.1	50.5	59.4	0.31	5.60
Kwale	10.8	11.7	16.4	23.1	29.6	37.7	42.8	52.1	58.9	0.25	5.45
Duma	12	12.9	15.9	21.6	29.3	38.2	40.9	48.6	56.2	0.24	4.68
Chozi	11	12.4	17.4	23.6	34.8	44.3	51.6	57.4	61.7	0.52	5.61
F_1s											
NJ Bw II/ Eagle	10.4	10.9	11.1	11.6	11.4	11.8	12.1	11.9	11.6	0.22	1.12
Eagle/ NJ Bw II	10.8	11.3	11.3	11.8	12.2	11.6	11.7	11.9	12.1	0.18	1.12
Kwale/ Eagle	12.1	12.4	13.7	15.2	22.4	28.5	37.4	41.3	44.5	0.45	3.68
Eagle/Kwale	11.3	12.7	14.4	16.7	23.1	27.7	35.9	40.8	45.2	0.24	4.0
Duma/Eagle	10.5	11.9	16.1	20.6	29.9	41.1	45.2	52.6	61.7	0.30	5.88
Eagle/Duma	10.9	11.5	16.9	22.4	30.2	42.3	45.6	53.9	60.5	0.23	5.55
Chozi/Eagle	9.6	16.3	10.6	11.4	11.5	11.3	12.3	12.9	13.1	0.20	1.36
Eagle/Chozi	11.3	12.5	12.5	13.1	12.8	13.4	14.2	14.4	14.2	0.11	1.26

$^{\beta}$=Standard error

Low growth rates were recorded in Njoro Bw II/Eagle and Chozi/Eagle crosses (plate 1A and 1B). Similar results were obtained in the reciprocal crosses and were suggestive of no maternal effects for the grass clump trait in these crosses. The growth rates in Duma/ Eagle and Kwale/ Eagle hybrids were however comparable to those of their parents though they produced sterile spikes (Plate 1D and 1E).

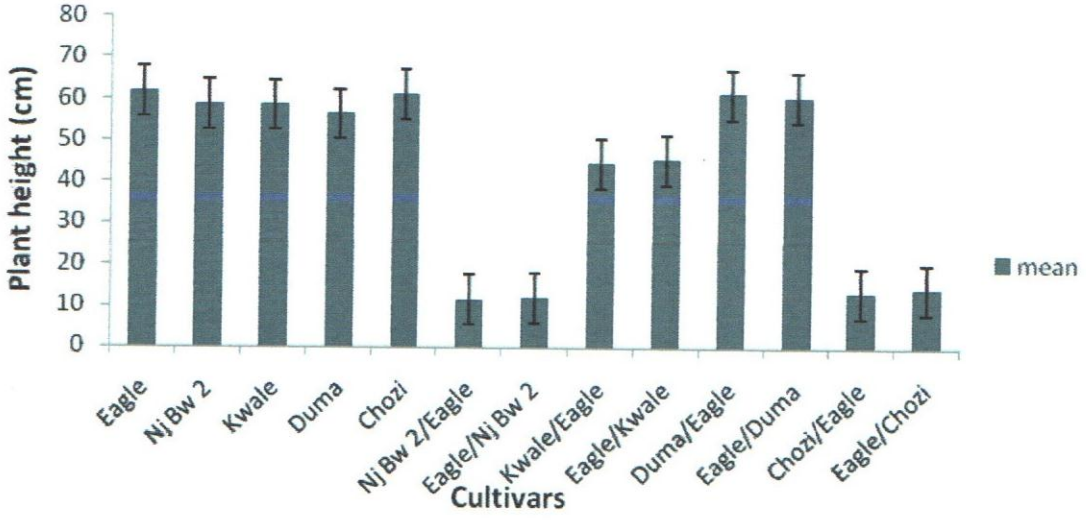
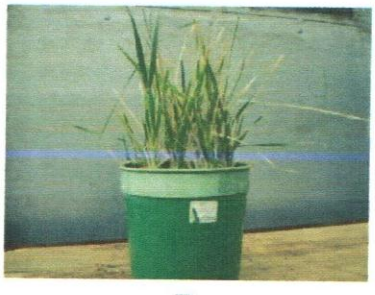


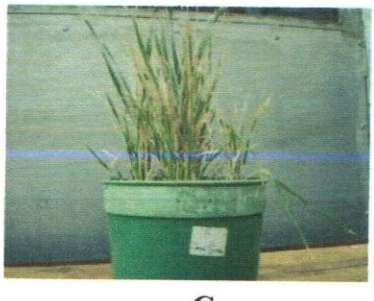
Figure 7: Adult height (cm) after 10 weeks of growth for five wheat parental cultivars and their progeny.

The following photographs (Plate 1: A, B, C, D, E and F) depict the grass clump phenotype in some of the Eagle-derived hybrids.

Plate 1: **A**
Njoro Bw II/ Eagle F_1hybrid



B
Chozi/ Eagle F_1hybrid



C
Cook/ Eagle F_1 hybrid

D

Sterile spike of a Duma/Eagle hybrid

E

Normal but sterile Kwale/Eagle hybrid

F

Eagle (left), Cook (right), Cook/Eagle 'grass dwarf' hybrid (centre).

Plate F above illustrates normal growth depicted in stem rust resistance donor Eagle (left) and Cook (right). The Cook / Eagle hybrid is characterized by reduced height and profuse tillering.

3.5 Discussion

Breeding for disease and pest resistance is one method of protecting crops from damage by biotic factors. Heritable resistance is a valuable attribute because it is easy for the grower to use and reduces the need for other methods of control, including the application of chemicals.

The potential value in using resistance genes in control of cereal rusts lie in either one or both of two approaches: (1) Manipulation of host plants to remain one step ahead of the prevailing pathogenic potential of the parasite or (2) Breeders to deploy combinations of genes for resistance in the host plants, which will decrease the probability of a given parasite overcoming that resistance. Both approaches are possible with increased knowledge of host: parasite specificity. The combination of two or more resistant genes would be useful in protecting commercial cultivars for longer periods than with one gene.

3.5.1 Inheritance of resistance to PgtUg99

The inheritance of the resistance genes Sr26 and Sr36 investigated in this study indicated that both are dominant (Tables 7a and 7b) implying that both genes could be pyramided in one variety. By pyramiding these genes it would be anticipated that the pathogen will not be able to undergo a sequence of mutations corresponding to each resistance gene which is often the case when single genes are deployed in circumstances of high disease pressure and favorable cultural practices. The option thus would be to generate disruptive selection by rotating major gene resistance for example Sr26 and Sr36 through time and space and by growing mixtures of cultivars with different resistance genes (Bruce *et al.*, 2002). However, selecting for slow rusting, horizontal resistance could be explored and exploited as a better promise to durable and stable resistance.

The confirmation of Sr26 and Sr36 in the F_1, F_2 and BC_1F_1 populations in the greenhouse point out that reaffirms independent assortment. Both of these genes were readily transmitted and conferred resistance in their new backgrounds.

Singh *et al.* (2006) proposes the use of molecular markers to identify plants that carry combinations of resistance genes once crosses have been made and hence expedite the process of selection of desirable recombinants. It would be an attractive strategy to incorporate Sr26 and Sr36 or other effective major genes in Sr2 backgrounds. The gene Sr2 derived from 'Yaroslav emmer' confers slow rusting resistance of adult-plant nature which is however not adequate under heavy disease pressure.

3.5.2 Inheritance of grass clump character

The 'grass clump' trait was first reported by Farrer (1898) and explored by Hermsen (1967), Moore (1968) and Canvin and McVetty (1975). Farrer (1898) found 'grass clumps' wheat crosses having high tillering, restricted height and lacking reproductive growth'. Hermsen (1967) established that inheritance of the 'dwarfism' as dependent on three complimentary dominant genes and that there are three typical types of hybrid dwarfs which differed in the severity of dwarfing under field conditions. Types 1 dwarfs were short, dark green grass-clumps that never became productive-this description closely fits the Nj Bw II/Eagle F_1. This population had the least growth with a relative height (*r*Height) of 1.12 both in the straight and the reciprocal cross (Table 8). Even after 4 months of growth, at what time the parental types-Nj Bw II and Eagle had grain filled, the Nj Bw II/Eagle F_1 remained short and clumped without any sign of reproductive growth (Plate 1A). Type 2 dwarfs developed greater than normal number of tillers, were shorter than the parents and occasionally produced seed. In this study Type 2 dwarfs would be represented in the Kwale/ Eagle F_1 (Plate 1E). The relative height (*r*Height) of this cross at 10 weeks was higher than in the Nj Bw II/Eagle F_1(Table 8).Type 3 dwarfs developed greater than normal numbers of tillers early in the growing season but were essentially normal later in the growing season perhaps characteristic of the Duma/ Eagle F_1 in this study(Plate 1D). The Duma/Eagle hybrid in this instance produced sterile spikes.

By adopting Hermsen's (1967) model of three complimentary genes D_1, D_2, D_3 and by supplying Moore's (1968) argument that grass-dwarf F_1 results from the interaction of at least three genes which cause a breakdown in the normal reproductive development in wheat, the observations in this study offer preliminary evidence of the presence of dwarfing genes in cultivar Eagle but probably in the Kenyan cultivars too. This suggestion though needs further validation.

Hermsen (1967) categorized six classes of common wheat cultivars: I ($D_1d_2D_3$), II ($d_1D_2D_3$), III ($D_1d_2d_3$), IV ($d_1D_2d_3$), V ($d_1d_2D_3$) and VI (d_1d_2 d_3). The expressivities and dominance of relations of D_1, D_2, and D3 are different:

D_1 is the strongest gene and completely dominant over its recessive allele;

D_2 is the next strongest gene and partially dominant;

D_3 is the weakest gene and perhaps also partially dominant.

Dwarfness may occur without D_3 being present but D_1 and D_2 are indispensable.

By fitting the possible combination of phenotypes following inter-variety crosses, results in Table 9 would be obtained. Furthermore by fitting the phenotypic observations obtained in this investigation, a very conceivable genetic identity of the varieties would be:

Eagle; $D_1d_2D_3$

Kwale; $d_1D_2D_3$

NJ Bw II; $d_1\ D_2d_3$

Chozi; $d_1\ D_2d_3$

Duma; $d_1d_2D_3$ or $d_1d_2d_3$

Table 9. All cross combinations among dwarf and non-dwarf genotypes and their tentative dwarfing genes.

Parent		Eagle	Kwale	(NJ Bw II or Chozi)	Duma	Duma	Other
		$D_1d_2D_3$	**$d_1D_2D_3$**	**$d_1D_2d_3$**	**$d_1d_2D_3$**	**$d_1d_2d_3$**	**$D_1d_2d_3$**
Eagle	**$D_1d_2D_3$**	$D_1d_2D_3$ (-)	$D_1D_2D_3$(+) $D_1d_2D_3$ (-)	$D_1D_2D_3$(+) $D_1d_2D_3$ (-)	$D_1d_2D_3$ (-)	$D_1d_2D_3$ (-)	$D_1d_2D_3$(-)
Kwale	**$d_1D_2D_3$**	$D_1D_2D_3$(+) $D_1\ d_2D_3$ (-)	$d_1D_2D_3$ (-)	$d_1D_2D_3$ (-)	$d_1D_2D_3$ (-) $d_1d_2D_3$ (-)	$d_1D_2D_3$ (-) $d_1d_2D_3$ (-)	$D_1D_2D_3$(+) $D_1d_2D_3$ (-)
Chozi	**$d_1D_2d_3$**	$D_1D_2D_3$(+) $D_1d_2D_3$ (-)	$d_1D_2D_3$ (-)	$d_1D_2d_3$ (-)	$d_1D_2D_3$ (-)	$d_1D_2d_3$ (-) $d_1d_2d_3$ (-)	$D_1D_2d_3$(+) $D_1d_2d_3$ (+)
Duma	**$d_1d_2D_3$**	$D_1d_2D_3$ (-)	$d_1D_2D_3$ (-) $d_1d_2D_3$ (-)	$d_1D_2D_3$ (-) $d_1d_2D_3$ (-)	$d_1d_2D_3$ (-)	$d_1d_2D_3$ (-)	$D_1d_2D_3$ (-)
Duma	**$d_1d_2d_3$**	$D_1d_2D_3$ (-)	$d_1D_2D_3$ (-) $d_1d_2D_3$ (-)	$d_1D_2d_3$ (-) $d_1d_2d_3$ (-)	$d_1d_2D_3$ (-)	$d_1d_2d_3$ (-)	$D_1d_2d_3$ (-)
Other	**$D_1d_2d_3$**	$D_1d_2d_3$ (-)	$D_1D_2D_3$(+) $D_1d_2D_3$ (-)	$D_1D_2d_3$ (+) $D_1d_2d_3$ (-)	$D_1d_2D_3$ (-)	$D_1d_2d_3$ (-)	$D_1d_2d_3$ (-)

(+) Grass dwarf plant (-) Normal plant

The occurrence of the 'grass clumps' arising from the Eagle derived crosses is a pointer to need to objectively select parental cultivars to use as sources of intended rust resistance. The same gene may be present in genetic backgrounds of different status – parents that are more widely adopted may present better results when transferring resistance. It is imperative to consider deployment of such parental types first in any planned hybridization work.

The development of molecular markers for the 'grass clump' loci should be explored. These markers would facilitate rapid screening of intended parental plants in a wheat breeding program.

CHAPTER IV

EVALUATION OF THE EFFECTIVENESS OF MAJOR AND SLOW RUSTING GENE AGAINST RACE PGTUG99 OF WHEAT STEM RUST

4.1 Introduction

One remarkable achievement of modern plant breeding is the development of improved cultivars and hybrids resistant to pathogens, insect pests and nematodes (Leppik, 1970). The primary objective in breeding for disease resistance is to reduce the mean fitness of parasite populations (Clayton *et al.*, 1976). In epidemiological terms, this is to reduce the extent to which parasite fitness contributes to *r*, the parameter Vanderplank (1963) described as the rate of increase of disease per unit of infectious tissue per unit time (Stubbs *et al.*, 1986)

As the case of Australia exemplifies, when resistant cultivars are first released to farmers, they are often grown next to susceptible cultivars (McIntosh and Brown, 1997). Therefore, the resistant cultivars are subjected to substantial uredial populations produced by susceptible wheats. Consequently they act as screening populations for any mutant uredospore or infrequent pathotype that possess virulence gene(s) corresponding to the resistance gene(s) deployed in the resistant cultivar. Rowell and Roelfs (1971) estimated that 0.4 ha of susceptible wheat at 10% stem-rust severity could produce 10^{12} uredospores per day. Similarly, Parlevliet and Zadok (1977) estimated that 1 ha of susceptible wheat with 1% infection of leaf rust could produce 10^{11} uredospores per day.

It follows that if a wheat field planted to a new cultivar carrying a single gene for resistance is surrounded by fields of susceptible cultivars, large numbers of spores will be blown into it and a few will be virulent mutants. If only the mutants can infect the plants and sporulate, the extreme selection pressure will result in their rapid increase (Knott, 1989).As such, wheats with single resistance genes gave only ephemeral protection that lasted until the occurrence and selection of a new mutant pathotype, or the increase of a rare virulent pathotype that was already present in the pathogen populations (McIntosh and Brown, 1997).

4.2 Literature review

The genetic background of the host can affect the expression of specific genes for resistance in that host (Roelfs, 1988). When a stem rust resistance gene is transferred into several different but susceptible host backgrounds, varying infection types often result (Luig and Rajaram, 1972). Resistance not only occurs in a variety of mechanisms, it also varies in intensity, from complete

to almost imperceptible. In 'complete resistance', the growth of the pathogen is reduced to zero. In many cases the reduction of the growth and the development of the pathogen are restricted. Compared with a highly susceptible host genotype resistance is often expressed as being less affected, or less diseased.

Resistance is said to exist whenever there is a reduction in the level of infection- however small it may be. Resistance and susceptibility are each other's complement. Quantitative resistance in the host genotypes characteristically shows continuous range of variation- from no resistance at all (extreme susceptibility) to good levels of resistance. The ability of certain wheat cultivars to slow down the development of stem rust was recognized many years ago as a form of resistance, even though the infection types on the plants indicated they were susceptible to rust (Farrer, 1898; Stakman, 1968; Skovmand *et al.*, 1976).

McIntosh (1992) describes rust epidemics as being analogous to bush or scrub fires-excessive amounts of dry fuel over large areas (susceptible hosts) combined with hot weather and dry winds (favorable environment).

To compare disease epidemics, Van der plank (1963) suggested the use of the area under disease progress curve (AUDPC).This parameter combines both the intensity and duration of an epidemic into a single index (Rees *et al.*, 1979).

Van der plank (1963) postulated that resistance present in cultivars can be evaluated by the relative slope and position of disease progress curves. The average assessment over all dates and the area below the disease progress curve both permit reasonable comparison of the epidemics and are easy to apply (Rees *et al.*, 1979).

In a few cases the difference in AUDPC may be related to host architecture, for example, a dense compact plant will often have more disease, perhaps partly because of heavier dew formation and duration (Roelfs, 1988).

The utility of the AUDPC character in breeding can be determined (Wilcoxson *et al.*, 1975). Data on stem rust can be summarized by means of area under stem rust progress curve. Slow rusting cultivars have low areas under the progress curve whereas fast rusting cultivars have high areas under the curve. Skovmand *et al.*, 1976, demonstrated that selecting for the slow stem rusting character should be possible since genetic control was predominantly additive and the trait appeared to be highly heritable. Their data established that slow rusting was controlled by 2 to 12 gene pairs.

Only Sr2 of the genes for specific stem rust resistance is effective only in the adult plant stage. Seedling tissue is susceptible, but as the plant grows the number and size of uredia decrease until at near maturity successful penetrants are limited to the immediate area of the nodes and awns (Hare and McIntosh, 1979; Sunderwith and Roelfs, 1980).

In the adult plant stage, Sr36 has been reported to elicit a response often deemed slow rusting. Sr25 is primarily a seedling resistance that is generally ineffective after host anthesis. The other specific resistance genes are generally effective throughout the host life cycle.

The widespread use of monogenic resistance in a crop poses extreme selection pressure on the pathogen population that can lead to rapid build-up of pathogenic races with genes for virulence that match the resistance genes used in the crops.

Disease development rate is slower in a 'slow rusting' cultivar compared to susceptible cultivars. Kuhn *et al.*, (1978) and Rajaram *et al.*, (1984) reported that slow rusting of wheat confers a more durable type of resistance. Slow rusting is the product of an interaction between the host and the pathogen at different stages of pathogenesis.

Cultivars may differ in their ability to retard disease development due to different combinations and degrees of expression of their various components that ultimately lead to the expression of slow rusting. The important components of slow rusting are: latent period, receptivity (number of uredinia per unit area of stem), uredinium size, and spore production (Parlevliet and Kuiper, 1977).

4.3 Materials and Methods

4.3.1 The test lines and cultivars

Thirty cultivars were evaluated for resistance in greenhouse and field experiments (Table 10). These were selected from preliminary PgtUg99 screening experiments -through the Borlaug Rust Initiative (BGRI; http://www.globalrust.org) at K.A.R.I. Twenty of these materials were sourced from CIMMYT, Mexico. The remaining ten cultivars, Njoro Bw II, Kwale, Duma, Chozi, K.Ibis, K.Heroe and Mwamba are popular under commercial production in Kenya; R960 is in advanced stages in the National wheat breeding program. Varieties Eagle and Cook are Australian.

The 30 bread wheat cultivars were categorized as race- specific resistant, slow rusting or susceptible (Ravi Singh pers. comm.) based on their reaction to stem rust in the field.

The criterion described by Roelfs *et al.*, (1992) was adopted in creating these categories of resistance and their associated phenotypes. A race- specific resistant cultivar was identified by incompatible reaction to *P. graminis* race PgtUg99, displaying immune to moderately resistant host response to infection. A slow rusting cultivar was recognized by a reduced disease progress compared to a susceptible check cultivar despite its compatible (or susceptible) reaction; similar to the finding of Caldwell (1968).

4.3.1.1 Greenhouse evaluation for seedling resistance

The thirty cultivars were planted in 15cm- diameter pots. Ten day old seedlings were inoculated with PgtUg99 inoculum. 3grams of the spores were weighed and then suspended in 500ml of distilled water in which 5-7 drops of the surfactant tween 20 were added, followed by thorough agitation to produce the desired inoculum mixture. This mixture was applied on the seedlings through an atomizer with a fine nozzle and the seedlings were left overnight in the growth chamber at a temperature of 16-20^{0}c. These materials were transferred to the green house at day temperatures of 24-26^{0}c. Seedling infections were recorded for each genotype after 13 days of inoculation.

Table 10. Pedigree and identified stem rust resistance gene of 30 bread wheat cultivars

Cultivar	[a] Pedigree	[b]Identified[±] resistance gene
Susceptible		
Nj Bw II	IAS-58/4/KALYANSONA/BLUEBIRD//CAJEME-F /3/ALONDRA/5/BOBWHITE	Sr2, Sr31
Kwale	KAVKAZ/TANORI F- 71/3/MAYA74//BLUEBIRD/INIA	Sr2,Sr31
Duma	AURORA/UP301//GALLO/SUPER3/PEWEE/4/MAIPO/MAYA74//PEWE	Sr31
Chozi	F-12-7/COCORAQUE 75//GENARO 81	Sr31
K.Ibis	KWALE/DUMA	Sr31
R960	-	Sr31
K.Heroe	MBUNI/SRPC64//YRPC1	Sr31
Race specific		
Mwamba	UNKNOWN	Sr24
6319	OASIS/SKAUZ//4*BCN/3/2*PASTOR	Sr25
3879	WHEAR/TUKURU//WHEAR	Sr25
Cook	SCOUT PURELINE SELECTION	Sr36
Eagle	TIMGALEN/CONDOR/2/CONDOR	Sr26
6361	CHIL/CHUM18/4/BUC/BJY/3/CNDR/ANA//CNDR/MUS	Sr Sha7
6363	MILAN/SHA7/3/THB/CEP7780//SHA4/LIRA/4/SHA4/CHIL	Sr Sha7
3910	ND643/2*WBLL1	Sr ND643
3696	BABAX/LR42//BABAX*2/3/TUKURU	Sr Tmp
3684	BABAX/LR42//BABAX*2/3/VIVITSI	Sr Tmp
6375	BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	Sr Tmp+Sr24
6404	CROC_1/AE.SQUARROSA (205)//FCT/3/PASTOR	Synthetic gene
Slow rusting		
6336	HPO/TAN//VEE/3/2*PGO/4/MILAN/5/SSERI1	APR
6338	KAMB1*2/KHVAKI	APR
6348	PFAU/WEAVER//KIRITATI	APR
6354	PGO/SERI//BAV92	APR
6356	TAM200/TUI/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	APR
6360	WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/IAS55*4/EG,AUS//IAS55*4/ALD/6/OASIS/5*BORL95/7/BORL95	Excellent APR
3751	WAXWING*2/KIRITATI	APR
3755	WAXWING*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	APR
6329	BABAX/3/OASIS/SKAUZ//4*BCN/4/PASTOR	APR
3714	GALVEZ/FRET2	APR
3716	THELIN/2*WAXWING	APR

[a] Pedigree represented following Purdy nomenclature (Purdy *et al.* 1962, Skovmand *et al.* 1997)
[b] Gene identity from Ravi P. Singh, personal communication.
[±] APR=Adult Plant Resistance.

4.3.2 Field Experiments

4.3.2.1 Test sites and cultural practices

The 30 cultivars were planted at K.A.R.I-Njoro (at 2160 m a.s.l, 00 200S, and 350 56'E) on two dates: early (17 November, 2007) and late (11 December, 2007). These dates fall on the second crop season for Njoro. A basal dose of D.A.P fertilizer at the recommended rate of 50kg/ha was applied at planting and followed with a top dress of C.A.N at 25kg/ha a month later. The trials were maintained at optimal growing conditions: 2 hours of overhead irrigation, 3 times a week were provided during the growing season to supplement for the little rainfall received during this season (Appendix F). Weed control was done by the use of the post emergence herbicide -Puma komplete® applied at tillering. Minimal mechanical weeding was also carried out later at booting to flowering. A bird watcher was employed to scare birds away and check against potential bird damage. Rodenticides were applied at the edges of the trial as baits to minimize unwarranted damage from mice.

4.3.2.2 Experimental design

Planting for each trial (differing in planting date) followed a paired- split in randomized complete block design. The fungicide treatments (either protected or unprotected) formed the main plots and the bread wheat genotypes the subplots. Four replicates were used for each trial (Appendix E). Each subplot consisted of three rows, 2.5m long planted 18cm apart. Four rows of oat variety Suregrain were planted round the trials as a buffer to test materials against exogenous inoculum from neighboring rust screening fields. A continuous row of a mixture (1:1:1 by volume) of the susceptible cultivars Duma, Chozi and K- fahari was sown perpendicular to the test plots in the middle of the 1-m pathways on both sides of the experimental plots. Furrows were cut by machine and planting done by hand.

4.3.2.3 Inoculum production and development of stem rust epidemics

The inoculum used was sourced from the plant pathology department of K.A.R.I, National plant breeding station Njoro. It was obtained from the cultivar Chozi, which bears Sr31. This inoculum was pre-tested on the differential hosts (Appendix C) in the greenhouse according to Roelfs *et al.*, (1989) and confirmed to be that of PgtUg99.

Stem rust epidemic was initiated by spraying the spreader rows twice, on 15th and 20th January 2008 with the uredeniospores of race PgtUg99. A hand held inoculator was used for this purpose. By turning through its handle, this appliance conveniently spread the inoculum suspended in talcum powder, on the leaf blades of the wheat plants. Inoculation was done in the evening in anticipation of overnight dew. However the plants were splinkler-irrigated an hour earlier to increase presence of free water necessary for spore germination and successful infection events.

To keep the protected plots free of stem rust, the fungicide tebuconazole (Folicur 125g a.i/ha) was applied on 5 February 2008 and thrice fortnightly.

4.3.2.4 Adult plant assessment and post-harvest analyses

4.3.2.4.1 Assessments of rust severity

In both trials, four recordings were made at 7 days interval beginning at heading stage until the most susceptible variety Chozi showed 90% stem rust severity and the spreader rows 100%. By the forth reading most of the bread wheat lines including the susceptible were past ripening. All rust assessments were on the basis of the modified cobb scale (Peterson *et al.*, 1948).

4.3.2.4.2 Yield measurements

Both experiments, early and late planted were hand harvested. An area of 3rows x 1m (0.8 m^2) was harvested to obtain an estimate of grain yield per plot. The weight of the grain obtained from this area was obtained after oven drying the grain samples at 70^0c for 2 days to 12% moisture content.

To get an estimate of yield traits, 50 productive culms from each plot were cut at ground level before harvesting as suggested by Herrera, (2006); Sayre *et al.*, (1998) and Rees *et al.*, (1979), placed in paper bags, and weighed after drying in an oven for 2days at 70^0c. The 50 culms were then threshed carefully and kernels dried back for 2 more days at 70^0c to 12% moisture content and then weighed. The following parameters were deduced based on Sayre *et al.*, (1998):

a) Kernel weight: A 1000 unbroken grains per plot were counted using a grain counter (contador pfeuffer®) and then weighed as a record of Kernel weight.
b) Harvest index (HI): derived from data of the 50 culms as follows:

   HI=Kernel weight/Culm weight.

c) Biomass= Grain yield/harvest index.

d) Kernels^{-1}spike= Total kernels in all 50 spikes/50.

e) Test weight (Hectoliter weight): determined using a test weight machine.

4.3.2.4.3 Evaluation and Analyses of Epidemic

4.3.2.4.3.1 Area Under Disease Progress Curve (AUDPC): AUDPC was in this study employed as a measure of the epidemiology of PgtUg99 under high inoculum pressure. For each plot, the rust severity data was organized into an Exel file. Values for AUDPC were then calculated using a computer program sourced from CIMMYT, based on the formula:

$$\sum_{i-1}^{k} \frac{1}{2}(S_i + S_{i-1})$$

Where;
S_i=rust severity at end of week 1
k=number of successive evaluations of rust

The *r*AUDPC was computed in both trials as the AUDPC relative to that of the most susceptible check cultivar Chozi. To supplement this parameter(s), the Final Disease Score (FDS) was considered to be the severity recorded on the last date of assessment disregarding infection response.

4.3.2.4.3.2 Rust data transformation:

In order to straighten the disease progress curve, the rust data was first taken through logit transformation as suggested by Rees *et al.*, (1979) and Van der plank, (1968). The following formula was applied:

$$X_t = \text{Log}_e [x/ (100-x)],$$

Where X_t is the transformed value and x is the percentage of leaf sheath rusted at a given date. Zero rust severities were replaced by 0.001(Rees et al., 1979). The X_t value for each genotype through four assessment dates gave the logit line. The slope of this line was derived as an estimate of the apparent infection rate, r (Van der plank 1968).

4.3.2.5 Data analysis

4.3.2.5.1 Analysis of variance

Individual split plot analyses for each of the two trials differing in planting date (season 1 and season 2) and a combined split-plot analysis across trials were performed for grain yield, 1000 kernel weight, biomass, test weight, Area Under Disease Progress Curve (AUDPC), Final Disease Score (FDS) based on Proc ANOVA of SAS (SAS Institute, 1999).

4.3.2.5.2 Correlation analysis

Correlation coefficients were calculated for total yield, yield loss, 50 spike-yields, biomass, harvest index, kernel weight, kernels/spike, test weight, Area Under Disease Progress Curve (AUDPC) and Final Disease Score (FDS).

4.3.2.5.3 Cluster analysis

Cluster analysis technique of SAS (Proc Cluster), was applied to the Area Under Disease Progress Curve (AUDPC) and Final Disease Score (FDS) for each trial. These data were used to classify and ordinate the cultivars on their responses to the stem rust epidemics. Groups of cultivars which responded similarly to the rust were projected on a dendrogram in each season which emphasized the more resistant cultivars.

4.4 Results

4.4.1 Stem rust development and expression of resistance

The parameters; days to heading, seedling infection type and adult plant reaction to race PgtUg99 of *P. graminis tritici,* for the 30 cultivars this study are presented in Table 11.

Table 11. Heading days, seedling infection type, adult plant reaction and identified stem rust resistance of 30 bread wheat cultivars against PgtUg99

Group/Cultivar	Heading days	Seedling infection type[a]	Adult plant reaction[b]
Susceptible			
Nj Bw II	75	4-	S-MS
Kwale	81	3+;	S-MS
Duma	68	4	S
Chozi	71	3+	S
K.Ibis	73	3-	S-MS
R960	73	3+	S
K.Heroe	76	3+	S
Race specific			
Mwamba	74	2	S
6319	71	2-	R-MR
3879	76	1+	MS-MR
Cook	74	0;	MS
Eagle	71	0	R-MR
6361	74	1+3C	S
6363	71	2+	S-MS
3910	72	2=	MS-MR
3696	71	1+	MS-MR
3684	70	1-	MS-MR
6375	69	2+	MS-MR
6404	75	2-	S
Slow rusting			
6336	72	3+	S
6338	72	3+	S-MS
6348	74	3+	S-MS
6354	72	2-	S
6356	63	3+	S-MS
6360	63	3-	MS
3751	67	3	S
3755	69	2=	MS-MR
6329	70	3+	S-MS
3714	76	2+	S-MS
3716	61	2	S

[a] Seedling infection type based on Stakman *et al.*, 1962.

[b] Adult plant reaction reaction based on modified cobb scale: R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

4.4.1.1 Stem rust severity

The drought tolerant cultivars Duma and Chozi succumbed to high severities (Table 12). The cultivar K Ibis had the lowest severity among the susceptible group. K Heroe, a cultivar released in 1999, the same year PgtUg99 was reported, and which has a line selected for résistance to earlier races of stem rust in its pedigree suffered high rust severity (Table 12). All the cultivars grouped in 'susceptible' category were associated with clear susceptible (S) host response, on the modified cobb scale, though this was markedly reduced to moderately resistant (MR) in varieties Njoro Bw II and Kwale, demonstrating the usefulness of Sr2 complex in the background of other genes including Sr31. Even within the 'resistant' group, Sr24, SrSha7 and Synthetic genes (Table 10) showed susceptible responses perhaps due to high disease pressure (Table 12). The fungicide-protected plots remained free from stem rust during the entire crop season. The epidemic especially on the susceptible category of cultivars was typically high at the time of the initial assessment (data not shown).

4.4.1.2 The Area Under Disease Progress Curve

There was a wide variation in epidemic progress rates between groups of cultivars as well as cultivars within groups. Accordingly, the mean area under disease progress curve (AUDPC) for the susceptible set of cultivars was 232.6, slightly higher than that of the slow rusting group (223.7) but significantly ($P \leq 0.05$) higher than that of the race specific cultivars (37.8) (Table 12). The rapid disease progress for the stem rust susceptible cultivars was further demonstrated in season 2 (Figure 8). Disease progress was restricted to zero in cultivars 6319, Cook and Eagle in season 1(Table 12).

4.4.1.3 Infection rate per day

The infection rates calculated for the race specific group of cultivars especially 3879, Cook, Eagle was higher than would be expected and inconsistent with the corresponding values for average severity and area under disease progress. This divergence perhaps is a result of uniform application of log transformation and the substitution of zero values with 0.001.

Table 12. Measurements of epidemic of race PgtUg99 of *P. graminis tritici* in 30 wheat cultivars during 2 seasons of 2008 at Njoro

	Season 1				**Season 2**			
Group /Cultivar	Average disease severity (%)	Area under disease Progress Curve	Infection rate/day	Logit-line intercept	Average disease Severity (%)	Area under disease progress curve	Infection rate /day	Logit-line intercept
Susceptible								
Nj Bw II	44.0	102.5	0.151	-3.357	50.0	566.9	0.178	-1.257
Kwale	43.0	55.3	0.194	-4.183	52.5	626.3	0.161	-1.063
Duma	70.0	249.0	0.157	-2.268	57.5	758.1	0.162	-0.739
Chozi	85.0	440.3	0.044	-1.863	75.0	1020.0	0.173	-0.120
K.Ibis	38.0	123.0	0.142	-3.357	42.5	553.8	0.120	-1.175
R960	63.0	308.6	0.099	-1.879	62.5	830.0	0.152	-0.548
K.Heroe	65.0	349.5	0.126	-1.889	70.0	867.5	0.183	-0.470
Mean	**58.3**	**232.6**	**0.130**	**-2.685**	**58.6**	**746.09**	**0.161**	**-0.767**
Race specific								
Mwamba	30.0	26.5	0.192	-4.816	40	543.1	0.164	-1.341
6319	8.0	0	0.217	-6.908	15	136.5	0.159	-3.110
3879	19.0	3.3	0.288	-6.908	10	136.3	0.186	-3.572
Cook	6.0	0	0.208	-6.908	1.75	11.4	0.105	-6.000
Eagle	10.0	0	0.236	-6.908	2.5	16.3	0.094	-6.055
6361	60.0	187.3	0.149	-2.571	42.5	553.8	0.122	-1.185
6363	28.0	15.5	0.210	-5.173	6.25	53.0	0.200	-4.617
3910	11.0	6.3	0.207	-6.330	10	104.8	0.194	-3.463
3696	40.0	24.5	0.150	-3.396	21.25	275.0	0.109	-2.064
3684	25.0	27.0	0.119	-3.583	12.5	158.1	0.224	-3.226
6375	33.0	4.3	0.188	-6.33	13.75	144.9	0.192	-3.232
6404	48.0	125.8	0.163	-3.357	22.5	273.8	0.115	-2.130
Mean	**25.91**	**37.8**	**0.194**	**-5.169**	**16.5**	**200.58**	**0.155**	**-3.333**
Slow rusting								
6336	63.0	263.3	0.130	-2.082	55	845.0	0.109	-0.513
6338	55.0	226.5	0.120	-2.197	65	902.5	0.157	-0.402
6348	58.0	218.3	0.135	-2.384	40	479.4	0.145	-1.430
6354	45.0	153.5	0.118	-2.571	62.5	837.5	0.159	-0.551
6356	30.0	61.8	0.195	-4.761	20	265.0	0.187	-2.592
6360	50.0	144.0	0.147	-2.944	42.5	570.6	0.168	-1.298
3751	45.0	343.5	0.078	-1.763	67.5	900.0	0.165	-0.402
3755	45.0	245.0	0.130	-2.797	57.5	947.5	0.115	-0.340
6329	63.0	226.5	0.175	-2.984	65	951.3	0.155	-0.324
3714	45.0	332.5	0.078	-1.763	57.5	711.3	0.190	-0.918
3716	53.0	246.3	0.115	-2.197	47.5	616.3	0.127	-1.036
Mean	**50.2**	**223.7**	**0.130**	**-2.586**	**52.73**	**729.7**	**0.152**	**-0.891**
Grand mean	**44.8**	**164.7**	**0.151**	**-3.48**	**42.6**	**559.1**	**0.156**	**-1.66**

4.4.1.4 The logit line intercepts

The lowest mean logit line intercept was observed in the race specific group of cultivars, -5.169 and -3.333 for season 1 and 2, respectively. This parameter was remarkably low in cultivars 6319, 3879, Cook, Eagle, 3910 and 6375 (Table12). The logit line intercept was highest in the slow rusting and susceptible categories and appeared related to the high average disease score.

Lower logit-line intercept for the race specific cultivars was an indication of a delay in the onset of epidemic. Similarly, it was apparent that the rust epidemic was earlier in season 1 (mean logit line intercept= -1.66) than in season 2 (mean logit line intercept= -3.48) (Table 12).

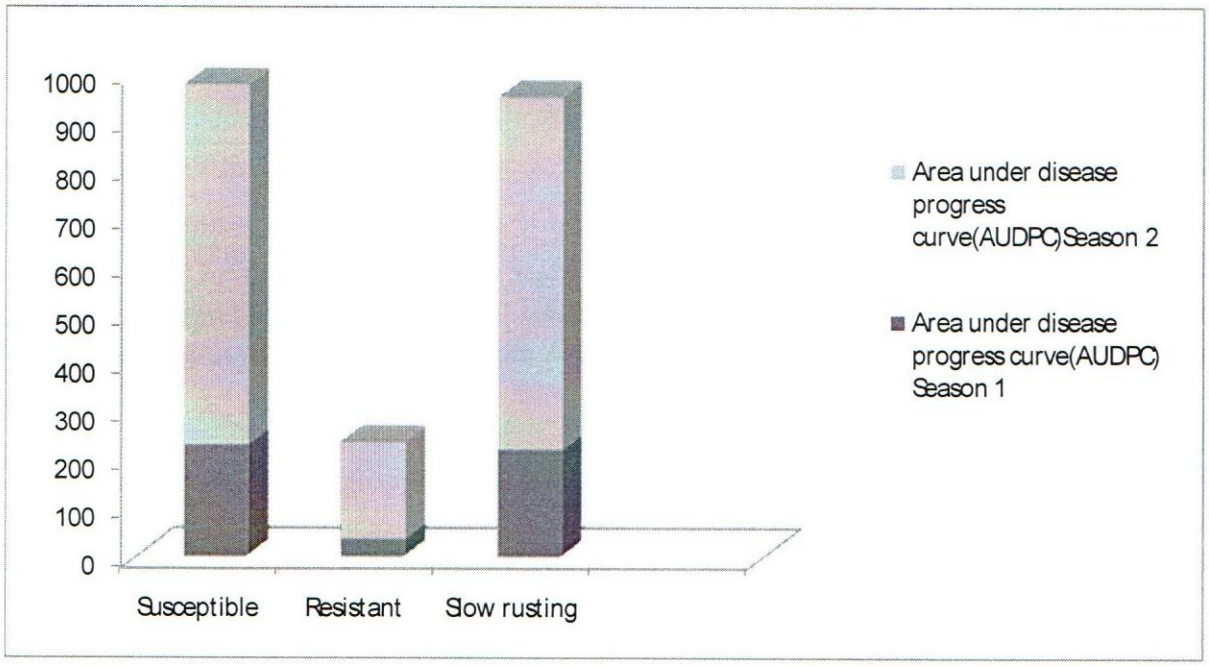


Figure 8. The area under disease progress curves for three categories of cultivars between two seasons

An ordination of the cultivars through cluster analysis and the subsequent generation of dendrogram, hierarchically classified the cultivars based on the stem rust assessment data in season 1 as shown in Figure 9.

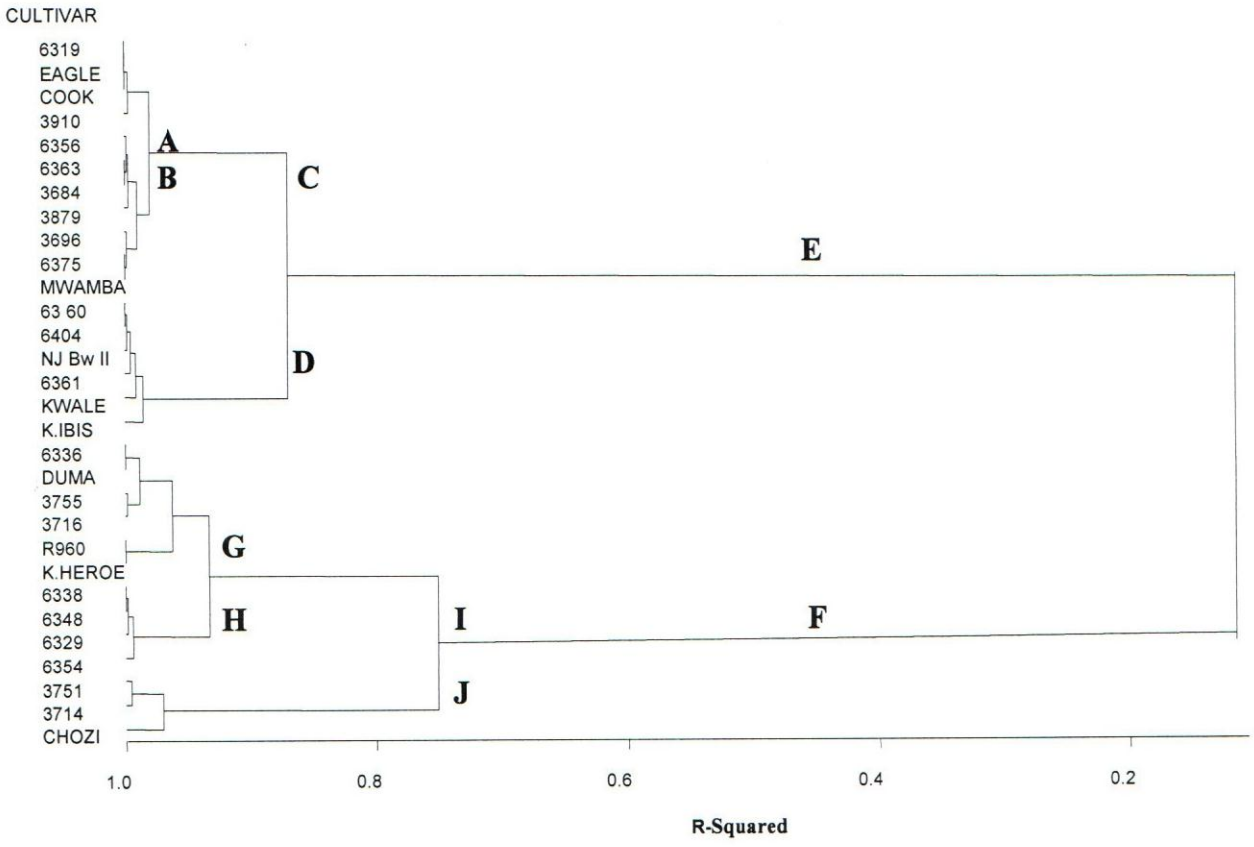


Figure 9. Hierarchical ordination of 30 wheat cultivars based on stem rust assessment in Season 1. Ten major groups are designated from A to J. At each fusion level the upper arm is the more resistant

Ten major resistance groups were identified from the dendrogram (Figure 9). Group A, consisted of the most resistant cultivars 6319 (Sr25), Eagle (Sr26), Cook (Sr36) and 3910 (SrND643). Cultivar 3879 (Sr25) was ranked much lower than genotype 6319 bearing the same resistance gene. The slow rusting cultivars were distributed through the whole spectrum suggesting quantitative response; as would be expected of an additively inherited trait. While the slow rusting cultivar 6356 was ranked among the most resistant (actually having excellent' Adult Plant Resistance (APR)'), cultivars 3751 and 3714 with slow rusting resistance were at the bottom of the hierarchical classification (Group J). Cultivar Chozi proved to be the most susceptible (Figure 9).

Though disease pressure was apparently higher in season 2 than in season 1, hierarchical clustering of the cultivars produced similar results with cultivars bearing monogenic resistance ranking high (Figure 10). SrTmp and the Synthetic genes in cultivars 3696 and 6404 respectively

appeared to be of intermediate effect in both seasons. The gene Sr Sha7 proved more effective while in the background of cultivar 6363 than in 6361.

The cultivars were delineated into more clusters in season 2 than in season 1. This observation was attributable to the increased selection pressure associated with higher disease incidence in season 2. The differences in the relative AUDPC (Table 13) between season 1 and season 2 was highly significant ($P \leq 0.01$) for Nj BwII, Kwale, Duma, K.Ibis, Mwamba, 3696, 6336, 6338,6354,6360,3755 and 6329. This difference was also high ($P \leq 0.05$) in R960, 6319, 3879, 6361 and 6375.

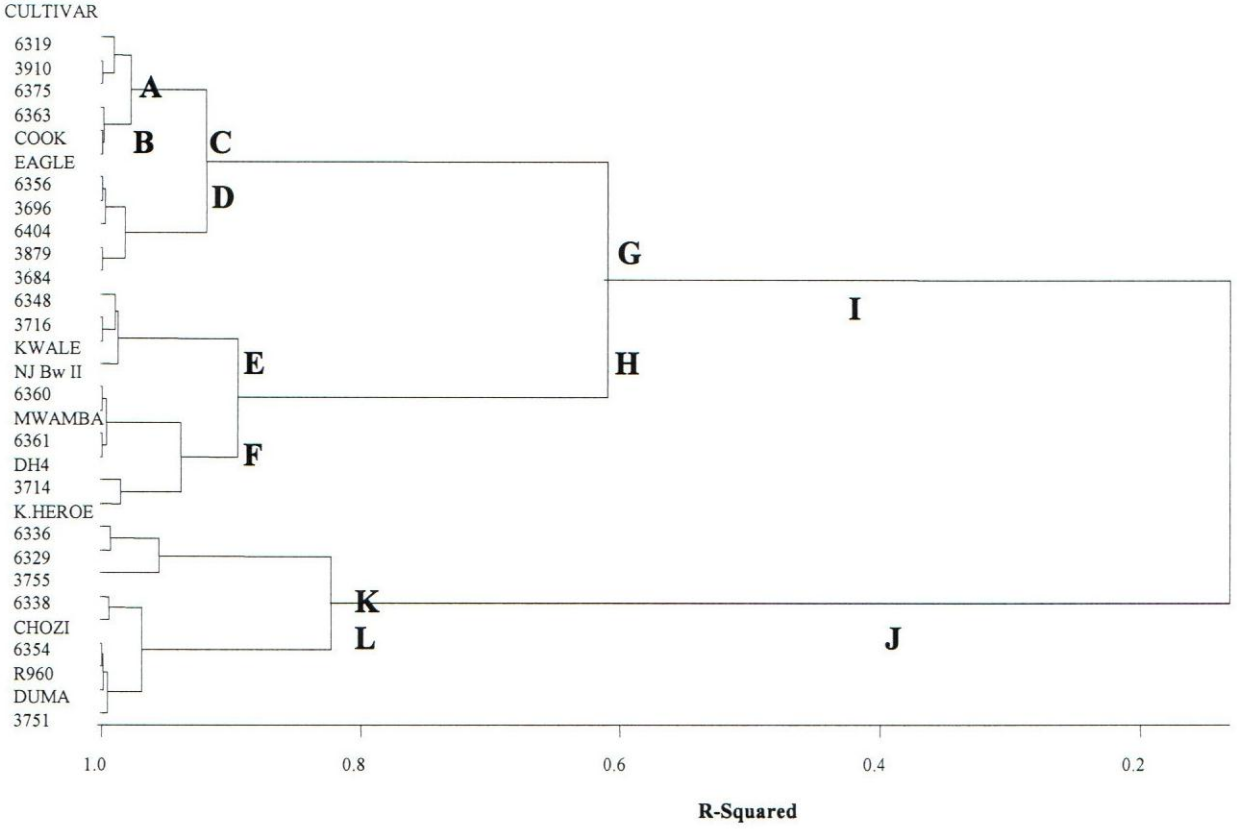


Figure 10. Hierarchical ordination of 30 wheat cultivars based on stem rust assessment in Season 2: Twelve major groups are designated from A to L. At each fusion level the upper arm is the more resistant

Differences in final disease score were not significant between the two seasons for any of the thirty cultivars. Under high disease pressure, would be resistant cultivars especially those of intermediate value and slow rusting ones, rust as fast as the susceptible cultivars though the final disease score is stable. This possibly results from early disease onset and perhaps an element of greater pathogen aggressiveness, or increased infectivity (Van der plank 1963).

Table 13. Relative area under disease progress curve (rAUDPC) and final disease score for 30 bread wheat cultivars evaluated in two dates in Njoro -Kenya

	rAUDPC (%)[a]			Final disease score (%)		
GROUP, CULTIVAR	Season1	Season2	Difference†	Season1	Season2	Difference
Susceptible						
Nj Bw 2	23.26	55.97	32.7**	43.8	50	6.2
Kwale	12.44	61.76	49.3**	42.0	52	10.0
Duma	56.14	74.23	18.1**	65.0	70	-12.5
Chozi	100.0	100.0	0.0	92.5	75	-17.5
K.Ibis	28.08	54.68	26.6**	37.5	42.5	5.0
R960	70.44	82	11.6*	62.5	62.5	0.0
K.Heroe	79.96	85.54	5.6	65.0	70	5.0
Mean	**52.9**	**73.5**	**20.6**	**58.3**	**60.3**	**-0.54**
Race specific						
Mwamba	6.24	53.7	47.5**	30.0	40.0	10.0
6319	0.0	13.45	13.5*	7.5	15.0	7.5
3879	0.70	13.29	12.6*	26	10.0	-16.0
Cook	0.0	1.1	1.1	6.25	1.25	-4.5
Eagle	0.0	1.6	1.6	10.0	2.5	-7.5
6361	42.43	54.75	12.3*	60.0	42.0	-18.0
6363	3.47	5.15	1.7	27.5	6.25	-21.3
3910	1.41	10.26	8.9	11.3	10.0	-1.3
3696	5.64	27.03	21.4**	40.0	21.3	-18.7
3684	6.13	15.53	9.4	25.0	12.0	-13.0
6375	1.00	14.2	13.2*	7.5	13.8	6.25
6404	28.86	26.94	-1.9	47.5	22.5	-25.0
Mean	**7.99**	**19.8**	**11.8**	**24.9**	**16.4**	**-8.5**
Slow rusting						
6336	60.08	82.99	22.9**	62.5	55	-7.5
6338	51.66	88.8	37.1**	55	65	10.0
6348	49.68	47	-2.68	57	40	-17.0
6354	34.53	82.57	48**	45	62	17.0**
6356	14.19	25.85	11.7	30	20	-10.0
6360	32.90	56.1	23.2**	50	42	-8.0
3751	78.49	88.41	9.9	45	67	22.0**
3755	55.71	93.38	37.7**	45	57	12.0*
6329	51.60	93.36	41.8**	62	65	3.0
3714	75.75	70.11	-5.6	45	57	12.0*
3716	56.01	60.93	4.9	52	47	-5.0
Mean	**51.0**	**71.8**	**20.8**	**49.9**	**52.5**	**2.6**

[a] rAUDPC of the cultivars with respect to the most susceptible check within each season.
LSD from combined analysis (a) rAUDPC, 10.59 (P≤0.05) and 13.98 (P=0.01) (b) FDS, 10.28 (P≤0.05) and 13.56 (P=0.01)
† * and**=significant at P≤0.05 and 0.01, respectively

4.4.2 Effects of PgtUg99 epidemics

4.4.2.1 Grain yield and associated losses

Stem rust race PgtUg99 was associated with differential losses in yield. Significantly high yield losses (P=0.01) were observed with the susceptible group (Table 14).

Table 14. Grain yields (t/ha) and losses (%) for 30 bread wheat cultivars under fungicide protected (Prot) and non protected (Non Prot.) treatments through season 1 and season 2

	Season 1			Season 2			Seasons mean		
Group, genotype	Prot	Non prot	Loss[a] (%)	Prot	Non prot	Loss[b] (%)	Prot	Non Prot	Loss[c] (%)
Susceptible									
Nj Bw II	4.07	2.16	46.8**	3.04	1.89	37.7*	3.11	2.46	20.9*
Kwale	4.24	2.4	43.5**	3.21	2.58	19.5	3.32	2.89	12.8*
Duma	3.64	1.46	59.9**	3.1	1.91	38.6*	2.55	2.73	1.6
Chozi	4.51	0.85	81.1**	2.91	1.44	50.0*	2.68	2.17	18.9*
K.Ibis	4.29	2.70	37.1**	3.59	2.49	30.7	3.49	3.04	12.9*
R960	4.12	1.57	61.9**	3.24	1.87	42.2*	2.84	2.56	10.1*
K.Heroe	4.1	1.5	63.5**	2.55	1.54	39.6	2.8	2.05	26.8*
Mean	**4.14**	**1.81**	**56.23**	**3.09**	**1.96**	**36.9**	**2.97**	**2.56**	**14.87**
Race specific									
Mwamba	2.8	2.65	5.4	2.93	2.24	23.7	2.73	2.59	5.2
6319	4.38	4.1	6.5	3.42	3.16	7.7	4.24	3.29	22.5*
3879	3.81	3.48	8.8	2.45	2.45	0.3	3.65	2.45	32.8*
Cook	3.25	3.12	0.13	2.66	1.79	32.5	3.19	2.23	29.9*
Eagle	4.22	3.84	9	2.38	2.34	1.7	4.03	2.36	41.4*
6361	3.44	2.81	18.2	3.27	2.45	25.6	3.13	2.86	8.5*
6363	2.9	2.6	10.4	2.14	1.85	13.3	2.75	1.99	27.5*
3910	3.58	2.75	23.2	3.15	2.33	25.9	3.16	2.74	13.5*
3696	4.54	3.32	26.8	3.68	2.27	38.2*	3.93	2.98	24.2*
3684	4.49	3.53	21.4	3.89	3.13	19.6	4.01	3.51	12.4*
6375	4.21	2.9	31	2.87	2.5	12.9	4.61	2.69	41.6*
6404	3.2	2.22	30.6	1.78	1.73	2.6	2.71	1.76	35*
Mean	**3.84**	**3.39**	**16.27**	**2.88**	**2.35**	**16.99**	**3.52**	**2.62**	**26.77**
Slow rusting									
6336	5.06	2.85	43.6**	3.09	2.57	16.8	3.96	2.83	28.3*
6338	3.07	2.15	29.8	2.73	2.27	17	2.62	2.5	4.5
6348	3.83	2.34	38.8*	2.88	1.49	48.5**	3.09	2.18	29.2*
6354	5.11	2.91	43.1**	3.13	1.99	36.2*	4.01	2.57	36*
6356	3.65	2.79	23.5	3.09	2.55	17.6	3.22	2.82	12.4*
6360	4.17	2.42	42*	3.07	2.37	23.1	3.29	2.72	17.3*
3751	3.4	2.84	16.3	2.3	2.09	8.7	3.12	1.92	38.4*
3755	4.37	1.8	58.7**	3.02	2.3	23.8	3.09	2.66	13.9*
3716	4.83	2.59	46.4**	2.91	2.20	24.2	3.71	2.56	31.1*
6329	4.41	2.03	54**	4.06	2.17	46.6**	3.22	3.12	3.2
3714	3.5	2.07	40.7*	3.27	2.48	24.2	2.79	2.87	-3.1
Mean	**4.12**	**2.43**	**39.67**	**3.05**	**2.34**	**26.1**	**3.28**	**2.60**	**19.2**

*, **=significant at P=0.05 and 0.01, respectively: [a] LSD= 1.3309, 1.7388; [b]LSD =1.1247,1.4842; [c] LSD =0.2266

Yield losses in cultivars with 'race specific gene' 'resistance was insignificant in both seasons, except in cultivar 3696 where the loss was 38.2% in season 2. The mean yield was significantly (P=0.01) higher in protected trial (4.03t/ha) than in the non-protected (2.47t/ha) trial in season 1. The mean yield differential was however not significantly different for the protected and non-protected trial in season 2. Given that there was an early onset of stem rust in season 2 than in season 1, the average yield in the second season (2.35t/ha) was lower than that in the first season 1 (3.3t/ha). The average yield loss in season1 was 56.23%, 38.34% and15.65%, for the susceptible, slow rusting and race-specific categories (Table 14, Figure 11a) respectively compared to 36.9%, 26.1% and16.19% for the same categories in season 2 (Figure 11b). Though the epidemic set in early in season 2 and was generally associated with tremendous increase in AUDPC (Table 12), the yield losses were interestingly lower in season 2 perhaps due to sub-optimal temperature for proliferation of the pathogen in the later and critical grain filling stages in season 2. This observation was further validated by the fact that the final disease score (FDS), the last rust severity reading taken on the last date in the non- protected trial, was not significantly different between the two seasons (Table 13).

Though individual losses within the susceptible group were all highly significant (P=0.01) in season 1, the gap between the least loss (37.1%) in K-Ibis and most loss (81.1%) in Chozi was remarkably wide. K-Ibis, the first cultivar developed from 'doubled haploids' and released by K.A.R.I-Njoro has cultivar Kwale as one of its parents. In this study Kwale alongside Nj Bw II were associated with lower losses of 43.5% and 46.8% respectively, in season 1 and 19.5% and 37.7% in season 2. The cultivars Kwale and Nj Bw II show the pseudo black chaff phenotype associated with the slow rusting gene Sr2 (alongside Sr31) which predictably could be the gene cushioning the two from high yield losses. The pseudo black chaff marker was not discerned in cultivar K- Ibis. However, perhaps it may have acquired Sr2 from its progenitor cultivar Kwale, and hence lower yield losses.

In the slow rusting group significant (P=0.01) yield losses were observed in both seasons for most cultivars. There was a wide variation in yield loss among the slow rusting cultivars; 16-58.7 % and 8.7- 48.5% in season 1 and season 2 respectively. Cultivars 6338, 6356, 3751 and 3910 though were able to withstand the epidemic and as such yield losses were not significant. The genotype 3751 was in both seasons clustered in the most susceptible hierarchical group of the

dendrograms (Figure 9 and Figure 10). This result was inconsistent with the low yield loss for this line perhaps pointing towards tolerance. Similar observation was made in cultivar 6338.

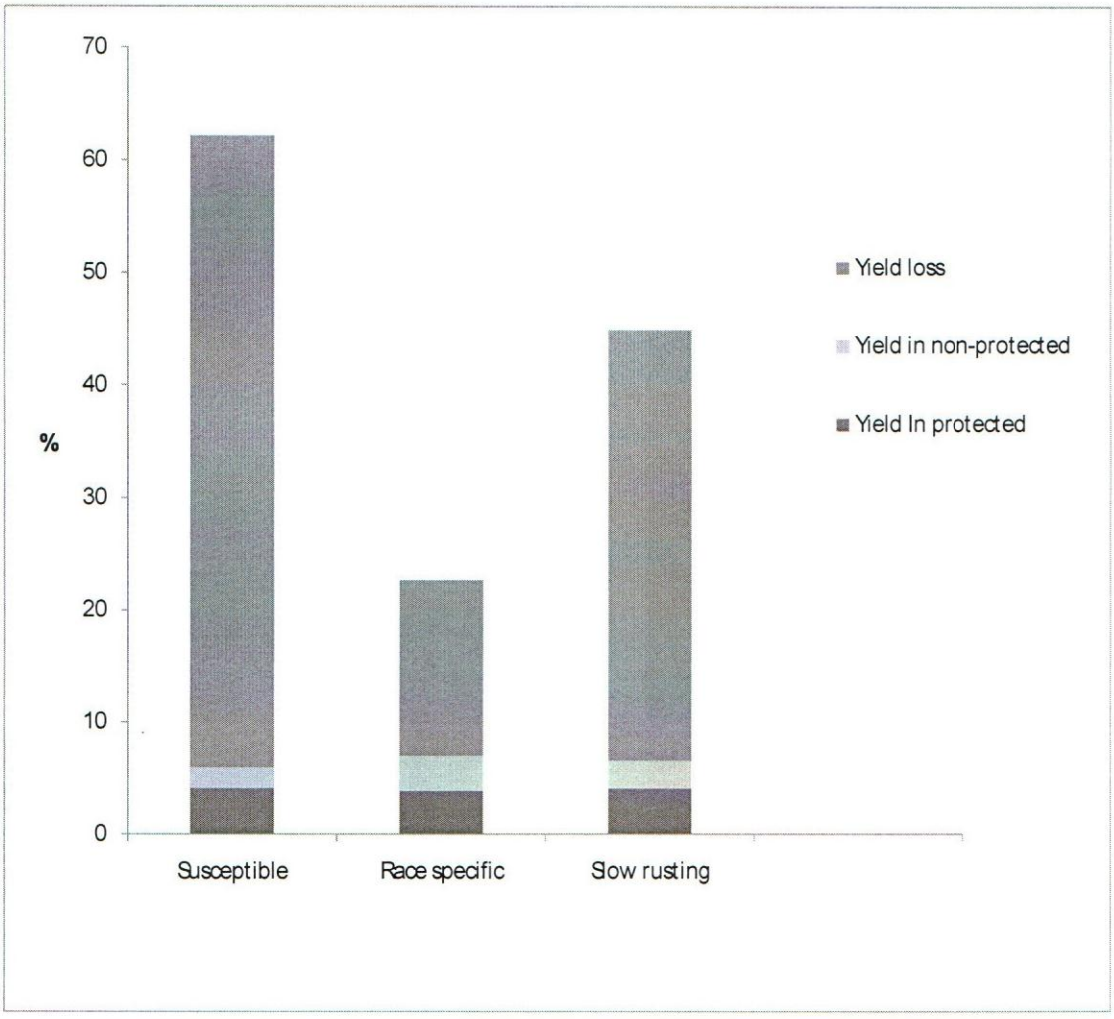


Figure 11a. Yield and yield loss (%) in season 1 in different cultivar groups

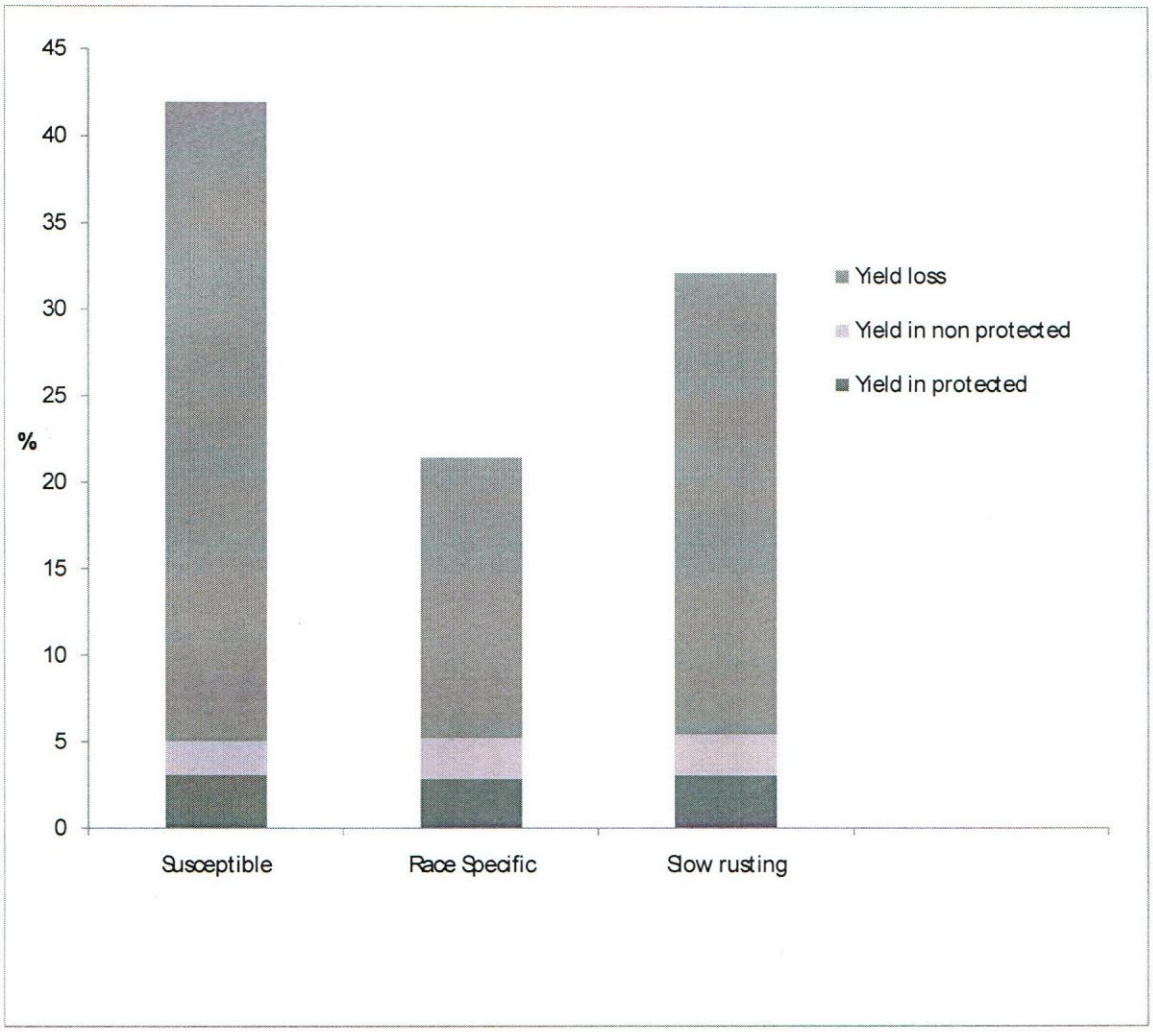


Figure 11b. Yield and yield loss (%) in season 2 in different cultivar groups

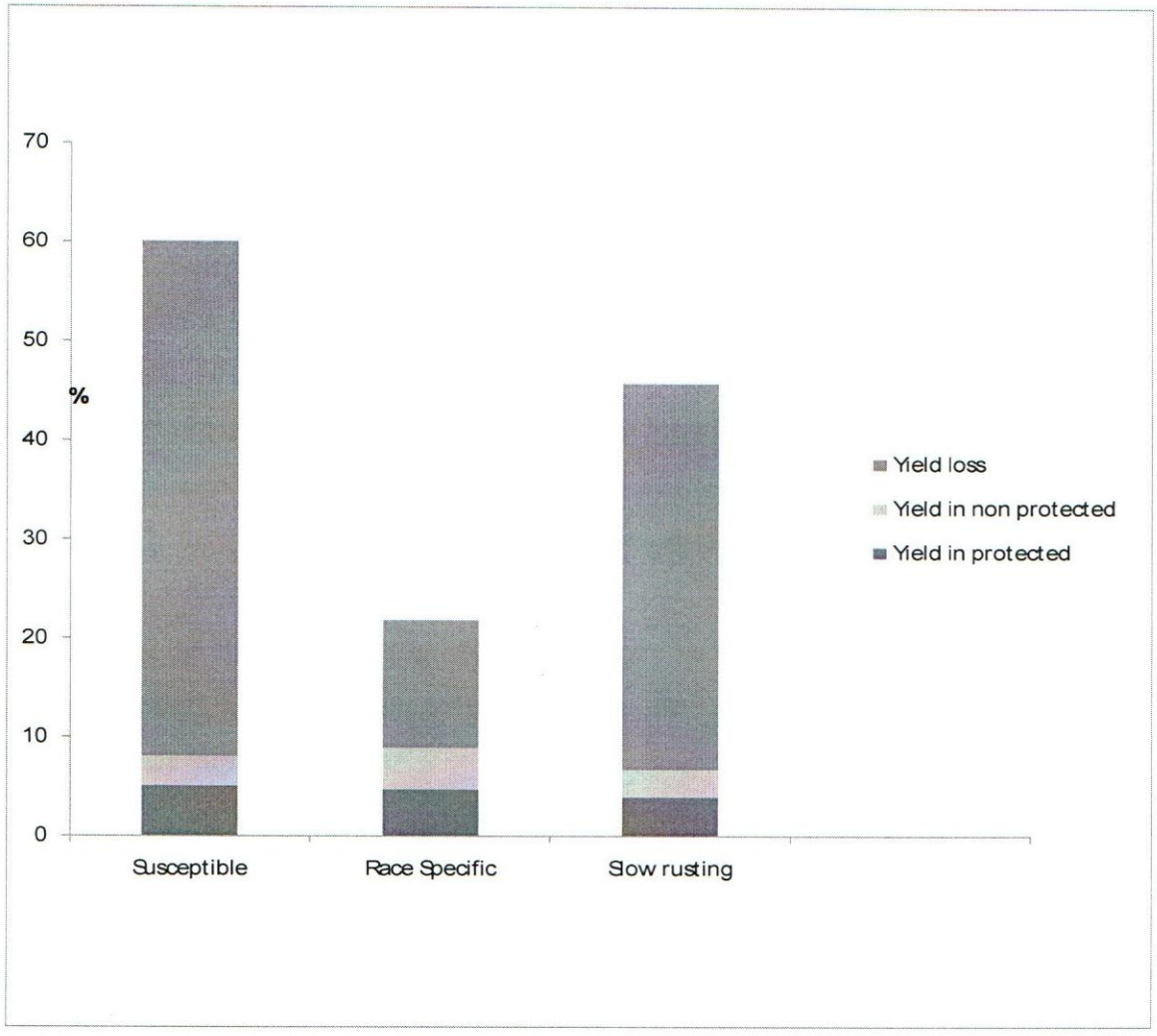


Figure11c. Yield and yield loss (%) in season1 and season2 in different cultivar groups

4.4.2.2 Kernel weight

Table 15 summarizes results for 1000-grain weight (g) and its losses between the two seasons. Tolerance indices are represented as ratios of Nonprot: Prot in both seasons. The 1000 grain weight was on average higher in the protected (40.53%) than in the non protected (32.92%) trials across the two seasons. The average loss of 19.67% observed in season2 for this trait was higher than 17.5% in season1.

Among the susceptible group, cultivar Chozi and Duma had the highest loss of 30.2% (P=0.01) in season 1 compared to the insignificant loss in variety K.Ibis at 7% (Table 15). However, significant losses (P=0.01) were observed in cultivars 6361, 6375 and 6404. Losses in Mwamba, Cook, Eagle, 6363, 3910 and 3684 were insignificant. In the slow rusting category, losses in 1000-kernel weight were generally highly significant (P=0.01). However, in cultivar 6356 this was insignificant.

In season 2, losses in 1000-grain weight were significantly high (P=0.01) in cultivars Cook and Eagle.

With regard to tolerance, on average the race specific cultivars had the highest tolerances in 1000- grain weight followed by the slow rusting and the susceptible group.

4.4.2.3 Biomass and test weight

Figure 12 is illustrates loses in biomass associated with the three categories of cultivars. In season 1, significantly higher ($P \leq 0.05$) loses were recorded in the PgtUg99 susceptible cultivars Chozi, R960 and 6336 (Table 16). Though individual cultivar losses in test weight appeared insignificant, variation in the mean tolerance indices for this parameter in the three groups of cultivars was characteristic.

Table 15. 1000-grain weight of 30 cultivars in protected and non protected trials, the attendant losses and ratios through season 1 and season 2

Group/ Cultivar	Season 1 1000-grain wt(g)				Season 2 1000-grain wt (g)			
	Prot[a]	Non Prot[b]	Loss† (%)	Ratio[¶]	Prot[a]	Non Prot[b]	Loss† (%)	Ratio[¶]
Susceptible								
Nj Bw II	36.84	30.39	17.5*	0.82	38.7	29.34	24.2**	0.79
Kwale	41.85	31.89	23.8**	0.76	44.8	34.72	22.5**	0.78
Duma	40.41	28.21	30.2**	0.7	39.93	24.27	39.2**	0.61
Chozi	43.85	30.6	30.2**	0.7	48.77	31.14	36.1**	0.64
K.Ibis	36.31	33.77	7	0.93	36.64	32.92	10.2**	0.9
R960	39.82	30.24	24.1**	0.76	44.45	32.17	27.6**	0.72
K.Heroe	39.23	29.4	25.1**	0.75	37.21	25.57	31.3**	0.69
Mean	**39.75**	**30.64**	**22.6**	**0.77**	**41.5**	**30.02**	**27.3**	**0.73**
Race specific								
Mwamba	37.05	34.44	7	0.92	37.48	32.49	13.3**	0.87
6319	40.27	36.85	8.5	0.92	40.23	36.39	9.5**	0.9
3879	41.79	37.14	11.1*	0.88	43.91	38.07	13.3**	0.87
Cook	33.17	31.98	3.9	0.96	34.94	33.67	3.6	0.96
Eagle	37.98	34.2	9.9	0.9	35.56	33.84	4.8	0.95
6361	38.84	31.74	18.3**	0.82	41.1	32.25	21.5**	0.78
6363	43.15	39.77	7.8	0.92	43.94	40.09	8.8**	0.91
3910	34.29	29.76	13.2	0.87	39.12	34.69	11.3**	0.89
3696	41.79	36.86	11.8*	0.88	43.22	37.67	12.8**	0.87
3684	40.19	35.94	10.6	0.89	42.49	36	15.3**	0.85
6375	43.53	36.12	17**	0.83	43.09	36.94	14.3**	0.86
6404	41.54	35.27	15.1**	0.85	42.1	34.16	18.9**	0.81
Mean	**39.47**	**35.0**	**11.2**	**0.88**	**40.59**	**35.52**	**12.3**	**0.88**
Slow rusting								
6336	42.42	33.94	19.9**	0.8	47.41	37.03	21.9**	0.78
6338	38.55	33.86	12.2*	0.88	42.19	37.39	11.4**	0.89
6348	42.84	30.77	28.2**	0.72	38.93	27.3	29.9**	0.7
6354	46.26	40.68	12.1*	0.9	48.68	42.41	12.9**	0.87
6356	38.33	33.85	11.7	0.88	39.11	34.95	10.6**	0.89
6360	40.19	32.49	19.2**	0.81	38.95	32.14	17.5**	0.83
3751	38.69	29.79	23**	0.77	37.52	28.14	25**	0.75
3755	40.65	33.65	17.2**	0.83	37.27	30.58	18**	0.82
6329	36.41	27.21	25.4**	0.77	43.32	31.77	26.7**	0.73
3714	43.13	34.4	20.2**	0.8	40.3	32	20.6**	0.79
3716	37.67	31.57	16.2**	0.84	41.8	33.97	18.7**	0.81
Mean	**40.47**	**32.93**	**18.7**	**0.81**	**41.41**	**33.43**	**19.4**	**0.81**
Grand mean	**39.89**	**32.86**	**17.5**	**0.82**	**41.17**	**32.99**	**19.67**	**0.81**

[a] Prot= Protected

[b] Non Prot= Non protected

[¶] Ratio =1000 grain wt non prot/ 1000 grain wt prot; a measure for tolerance

† *, **=significant at P≤0.05 (LSD: Season 1=4.622 Season 2=2.45) P=0.01 (LSD: Season 1=6.099 Season 2= 3.23), respectively

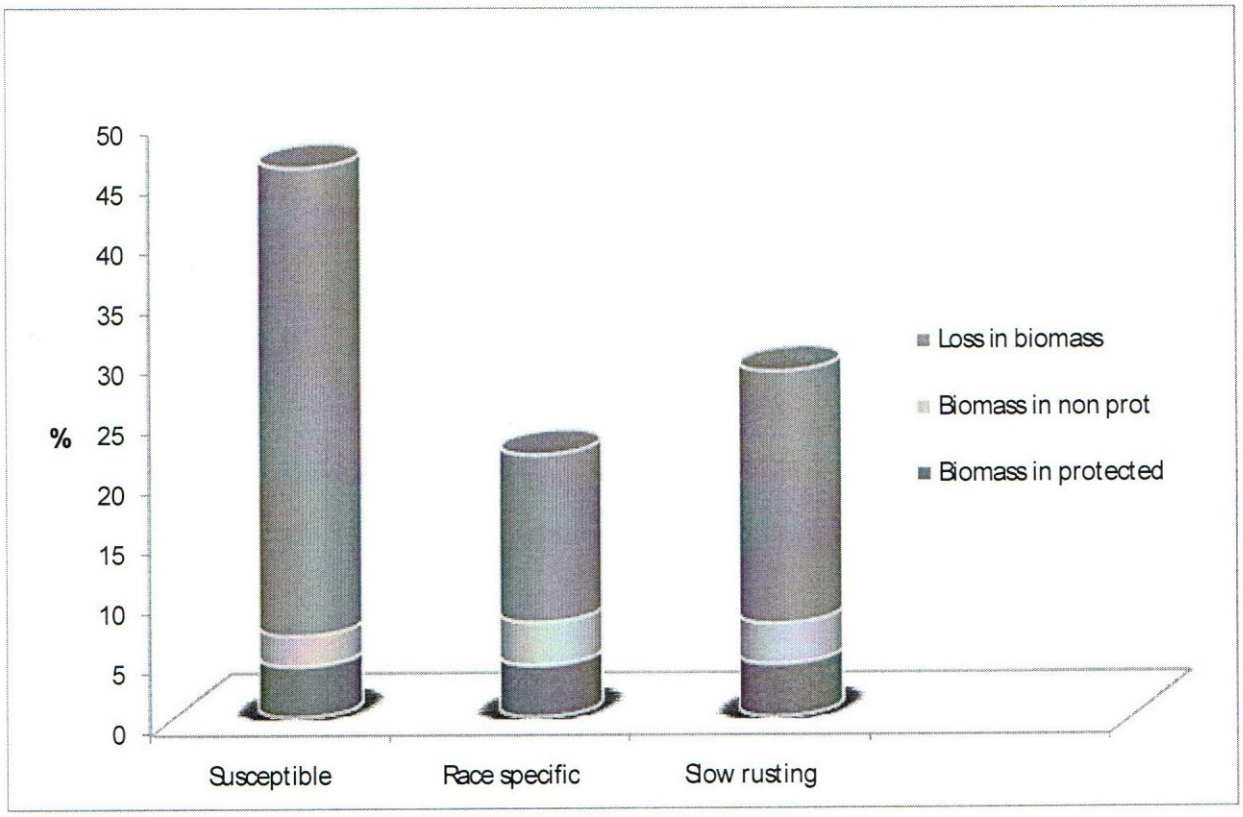


Figure 12. Loss in biomass following 'PgtUg99' epidemic in season 1.

The high loss in biomass in Chozi and R960 was consistent to their high susceptibility to PgtUg99. The 46.5% biomass loss in the cultivar 3910 within the race specific group and certainly resistant to stem rust could be attributed to an early attack by the 'Russian wheat aphid' on this cultivar in the early growth stages.

Table 16. Loss in biomass and Test weight associated with PgtUg99 epidemic in season 1.

Cultivar	Season 1				Season 1			
	Biomass				Test weight (g)			
	Prot	Non Prot	Loss (%)¶	Ratio	Prot	Non Prot	Loss (%) ¶	Ratio
Susceptible								
Nj bw2	3.86	1.97	48.8*	0.51	474	429.4	9.4	0.91
Kwale	4.46	3.77	15.3	0.85	468.9	421.2	10.2	0.9
Duma	3.64	3.20	12.1	0.88	462.5	434.3	6.1	0.94
Chozi	4.51	1.49	66.9**	0.33	478.8	359.8	24.9	0.75
K.Ibis	4.92	3.23	34.3	0.66	468.2	420.8	10.1*	0.9
R960	4.12	1.84	55.3**	0.45	478.8	416.9	12.9	0.87
K.Heroe	4.10	2.45	40.2	0.6	464	384.9	17.1	0.83
Mean	**4.23**	**2.56**	**38.99**	**0.61**	**470.74**	**409.61**	**12.95**	**0.87**
Race specific								
Mwamba	4.00	3.58	10.5	0.9	465.9	447.1	4.04	0.96
6319	3.89	4.01	-3.2	1.03	462.3	454.1	1.8	0.98
3879	4.31	3.22	25.4	0.75	465	451	3	0.97
Cook	4.09	2.46	39.9	0.6	473.6	460	2.9	0.97
Eagle	4.22	3.48	-0.3	0.82	484	469.1	3.1	0.97
6361	5.44	4.23	22.2	0.78	462.8	456.9	1.3	0.99
6363	2.90	3.18	-9.4	1.1	468.5	422.1	9.9	0.9
3910	4.33	2.32	46.5*	0.54	480.3	462.3	3.7	0.96
3696	5.04	4.63	8	0.92	467.2	446.3	4.5	0.96
3684	4.34	4.62	-6.3	1.06	462.7	439.1	5.1	0.95
6375	4.72	4.09	13.3	0.87	476.9	460.2	3.5	0.96
6404	4.09	3.22	21.3	0.79	470.9	409.6	13	0.87
Mean	**4.28**	**3.59**	**13.99**	**0.84**	**470**	**448.15**	**4.65**	**0.95**
Slow rusting								
6336	5.06	2.59	48.7**	0.51	469.3	451.6	3.8	0.96
6338	3.57	3.64	-2.1	1	448.9	409.7	8.7	0.91
6348	3.79	3.11	18.2	0.82	466.7	432.1	7.4	0.93
6354	5.11	4.19	17.9	0.82	477.2	418	12.4	0.88
6356	3.65	3.43	6	0.94	473.4	444.2	6.2	0.94
6360	4.92	2.91	40.8*	0.59	475.3	444.2	6.5	0.93
3751	4.25	3.73	12.1	0.88	481.4	447.3	7.1	0.93
3755	4.79	3.07	36	0.64	473.5	431	9	0.91
6329	4.43	3.02	31.8	0.68	472.4	436.1	7.7	0.92
3714	3.50	2.76	21	0.79	454.9	453.8	0.2	1
3716	4.83	4.79	0.8	0.99	476.2	433.9	8.9	0.91
Mean	**4.35**	**3.39**	**21.02**	**0.79**	**469.93**	**436.54**	**7.08**	**0.92**

¶ *, ** significant at P=0.01 and P≤0.05 respectively.

4.4.3 Correlations

Generally, the correlations among the grain yield and yield component traits in the non protected treatment were higher than those in the fungicide-protected treatment (Table 17).

In the non protected plots, high and negative correlations were observed between yield and yield loss ($P<0.01$); yield and Kernel weight ($P<0.05$); yield and AUDPC ($P<0.01$); yield and FDS ($P<0.01$); yield loss and 50 spikes yield ($P<0.05$); 50spike yield and AUDPC ($P<0.05$); 50 spike yield and FDS ($P<0.05$). High and positive correlations in the non protected plots were evident between yield and 50 spike yield ($P<0.05$); yield and kernels/spike ($P<0.05$); yield loss and AUDPC ($P<0.01$); yield loss and FDS ($P<0.01$); 50 spike yield and kernel weight ($P<0.01$); 50 spike yield and kernel/spike ($P<0.01$); biomass and harvest index ($P<0.01$); biomass and test weight ($P<0.01$); kernel weight and kernels/spike ($P<0.05$); AUDPC and FDS ($P<0.01$).

In the fungicide protected plots, there was high negative correlation between biomass and harvest index ($P<0.05$). High positive correlations were recorded between 50 spikes yield and harvest index ($P<0.01$); 50 spikes yield and kernels/spike ($P<0.01$); harvest index and kernels/spike ($P<0.01$); kernel weight and kernels/spike ($P<0.05$).

Table 17. Correlations among grain yield, yield loss, various yield traits, Area Under Disease Progress Curve (AUDPC), and Final Disease Score (FDS) of 30 bread wheat cultivars in fungicide-protected (Prot) and nonprotected (Nonprot) plots

Variable	Yield	Yield Loss	50 spike yield	Bio Mass	Har vest index	Kernel weight	Kernels /spike	Test wght	AUDPC
Yield loss (Nonprotected)	-0.73**								
Yield loss (Protected)	0.54								
50 spikes yield (Non protected)	0.54*	-0.37*							
50 spikes yield (Protected)	0.18	0.13							
Biomass (Non protected)	0.48	-0.31	0.20						
Biomass (Protected)	0.66	0.33	-0.21						
Harvest index (Non protected)	0.49	-0.15	0.22	0.97**					
Harvest index (Protected)	0.07	0.09	0.72**	-0.52*					
Kernel weight (Non protected)	-0.57*	-0.42	0.74**	0.20	0.21				
Kernel weight (Protected)	0.34	0.27	0.52*	-0.08	0.33				
Kernels/spike (Non protected)	0.41*	-0.35	0.74**	0.06	0.05	0.40*			
Kernels/spike (Protected)	0.20	0.09	0.75**	-0.18	0.67**	0.51*			
Test weight (Non protected)	0.49	-0.15	0.22	0.97**	1.0	0.21	0.05		
Test weight (Protected)	0.33	0.17	-0.14	0.45	-0.01	-0.13	-0.13		
AUDPC (Non protected)	-0.76**	0.72**	-0.51*	-0.21	-0.16	-0.51	-0.35	-0.17	
FDS (Non Protected)	-0.75**	0.72**	-0.51*	-0.18	-0.14	-0.51	-0.35	-0.14	0.99**

*, ** significant at P=0.05 and 0.01, respectively

4.5 Discussion

This study found that yield losses in the nonprotected plots compared with those of fungicide-protected plots were insignificant for most of the cultivars with race-specific resistance. The susceptible cultivars Njoro Bw II, Kwale, Duma, Chozi, Kenya Ibis, R960 and K Heroe that carry the resistant gene Sr31 had generally highly significant losses in the presence of PgtUg99. The Pseudo black chaff phenotype associated with the adult plant resistance gene Sr2 (Brown, 1997) was discerned in cultivars Njoro Bw II and Kwale perhaps the source of reduced disease severity on these cultivars (Table 12). Herrrera (2006) reported similar results in durum wheat inoculated with the highly virulent race BBG/BN of leaf rust in Mexico. According to Samboorski and Peturson (1960) plants once inoculated respond with energy-demanding physiological processes, probably defense reactions, using stored energy that otherwise would go to growth and seed production. That a reduction in the photosynthetic leaf area due to flecking also can reduce yield. Here too, cultivars that had extensive pustule cover on the stems invariably had heavy rust on the leaves thus reducing leaf surface available for light interception and optimal photosynthesis.

From the correlation analysis, it is evident that high stem rust pressure results in far greater yield loss in cultivars that are potentially higher yielding. Predictably, all breeding programs aim at releasing high yielding cultivars and where such materials are not protected with suitable resistance genes, they are likely to suffer phenomenal yield losses.

The high correlation between area under disease progress curve and final disease score implies that following the onset of an epidemic, one can readily predict the final disease rating and possible yield losses. Rapid disease progression on wheat cultivars especially those with ineffective genes is accompanied with extensive losses in yield and related traits. When ideal environmental conditions are present for proliferation and rapid progression of stem rust, it is predictable that low yields would be realized in susceptible cultivars. Cultivars that are fast rusters would be associated with high disease progress and final disease rating.

The measurement of epidemics in small adjacent plots of selected wheat cultivars enabled comparison of rust epidemics in the various cultivars. Interaction of one plot with another and the use of external spreader rows in a nursery situation reduce the expression of slow rusting. These levels of slow rusting determined under these conditions of high disease pressure would predictably be more effective in isolated plots or a crop situation.

It was expected that infection rate (r) would be a valuable measure for comparison of rust epidemics in a range of wheat cultivars as suggested by Van der plank (1968). Infection rate proved not a very useful measure of disease progression through, probably as a result of the uniform application of the logit transformation and the inclusion of zeroes and small values in the analyses.

The logit- line intercept-*a,* proved more valuable for comparing epidemics in different cultivars with lower *a* value obtained for the more resistant cultivars interpreted as an indication of greater delay on the onset of the epidemics in these cultivars, that is they are late rusters. Van der plank (1968) suggested a delay in epidemic onset was a result of vertical resistant in the host. This vertical resistance necessitated a preferential increase in the virulent component of the rust population before the epidemic developed and hence the delay.

Hierachical ordination of the cultivars in different groups provided an immediate criterion to compare the impact of epidemics on different resistance genes. The fast rusting nature of most of the Kenyan commercial cultivars reconfirms the aggressiveness of PgtUg99 and the threat it poses to wheat production if definite interventions are not implemented to circumvent it.

The classification of the cultivars through the dendrogams has helped identify resistance genes Sr25, Sr26, Sr36 and SrND643 as the most useful under the Kenyan local circumstances with PgtUg99 and pyramiding them into high yielding backgrounds would be a good tactic.

In these experiments though slow rusting lines having additively inherited resistance spanned through the whole length of the resistance spectrum, some cultivars for example 6356 were highly resistant in both seasons and could not be separated from effects of vertical resistance. Such sources would be very useful and where they are readily available in high yielding introduced germplasm can be recommended for release as cultivars and their seed multiplied without delay.

Insignificant yield losses in the race specific group implied that all the genes in this background would be useful in ameliorating field losses due to PgtUg99, though perhaps in a practical situation, these cultivars need be employed in mixtures or as has been proposed by Borlaug (1958), Browning and Frey (1969) and Marshall and Weir (1985) as multilines.

Several authors have described tolerance as the ability of plants to maintain desirable 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; Gaunt 1981). Yield loss, rather than yield

performance or disease development has been suggested to more accurately reflect tolerance of a cultivar. Seen in this way, the lines 6356 and 3751 were found more tolerant despite the fact that the latter yielded lowest in the protected treatment among the slow rusters in both seasons.

Conclusions

In the present study the pathogenic nature of PgtUg99 has been demonstrated. The study provides beneficial information to both wheat researchers and producers in confronting a pathogen with economically devastating implications, for which chemical control though feasible is expensive. All the hypotheses tested were rejected and the following key inferences made:

1. Genes Sr26 (Cook) and Sr36 (Eagle) proved resistant to race PgtUg99. Both genes expressed dominant monogenic inheritance. They can be easily introgressed to the susceptible Kenyan cultivars through backcrossing breeding.
2. Cultivar Eagle used in the inheritance study is a carrier of the dwarfing gene(s).
3. All Kenyan wheat cultivars tested proved susceptible to the new race PgtUg99 of stem rust. The vulnerability of hitherto resistant gene Sr31 present in many contemporary wheat cultivars e.g. Njoro Bw II, Chozi revealed.
4. Race PgtUg99 of stem rust has highly negative impact in limiting grain production potential of most of the commercially popular Kenya bread wheat cultivars with losses as high as 80% recorded.
5. Genes Sr24, Sr25, SrSha7, SrTmp studied, proved highly variable in providing protection to stem rust.
6. Resistance due to 'slow rusting' genes appear to be useful especially under high disease pressure with some cultivars e.g. 6356 posting very high resistance to PgtUg99.
7. A single measurement of rust severity measured towards the end of the epidemic can be used to select for slow rusting cultivars, if resources are limited and several disease readings are not available to complete AUDPC, given the very high positive correlation between AUDPC and FDS.

Recommendations

The present study draws the following recommendations:

1. All the Kenyan wheat cultivars released since the introduction of the crop in the country should be characterized and novel genes of resistance identified.
2. The genes Sr24, Sr25, SrSha7, SrTmp should be pyramided in combinations into the high yielding susceptible Kenyan cultivars through backcrossing (marker-mediated).
3. 'Slow rusting' cultivars (e.g. 6354, 6356) that show desirable yield potential should be exploited. They should be included in the national performance trials and their seed subsequently multiplied and distributed.
4. There should be concerted effort in planned retrieval of the extremely susceptible cultivars with the farmers to reduce pathogen proliferation and inoculum load.

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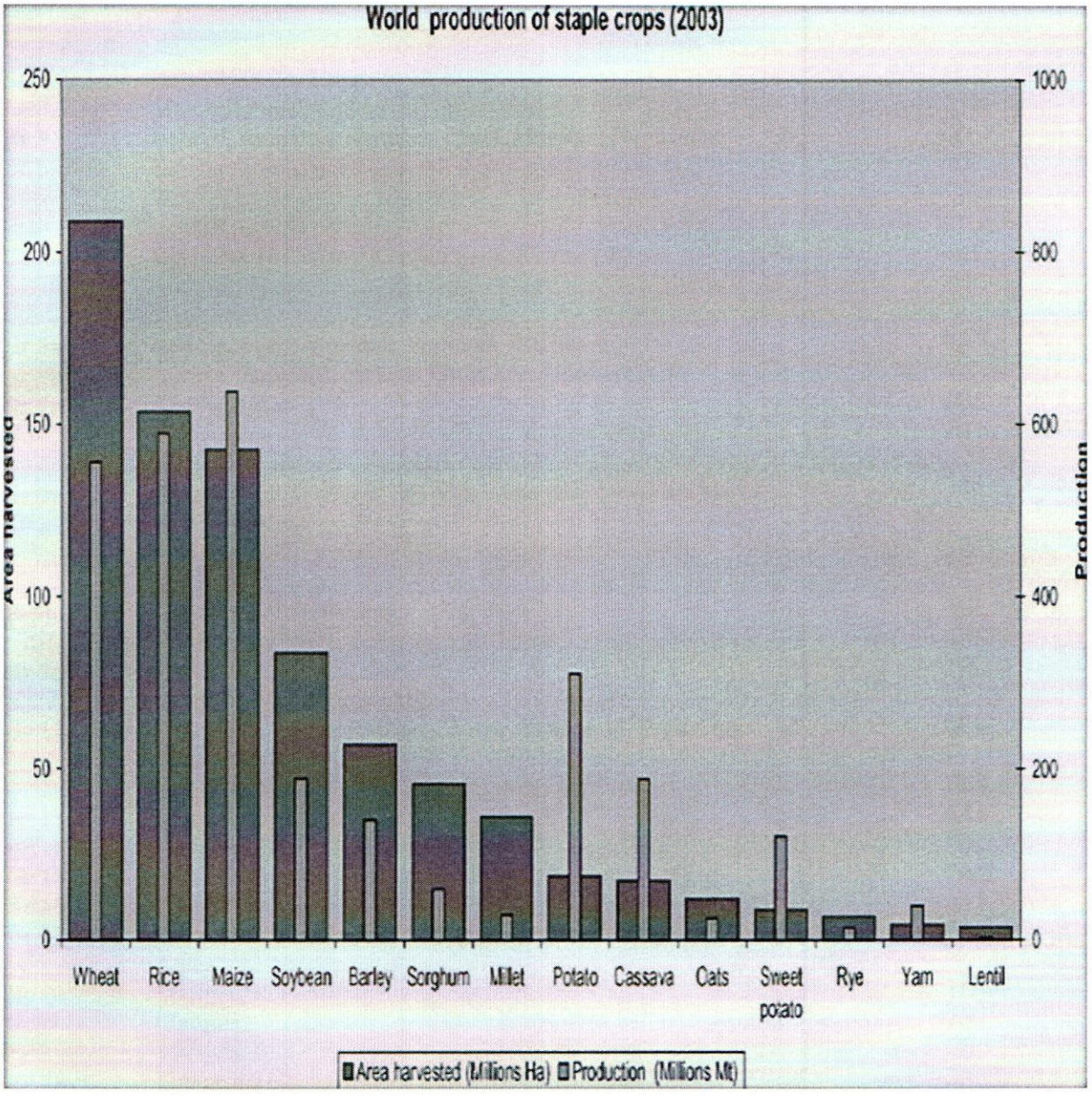
APPENDICES

Appendix A . World production of staple food crops (FAO Stastistical databases, 2004, Rome Italy: FAO).



Appendix B. List of the identified genes for stem rust resistance, (McIntosh, 1985).

Gene	Common sources	Typical seedling IT	Chromosome location
Sr 1=Sr9d			
Sr 2	Hope, H44-24, Hopps	4	3BS
Sr 3, Sr4	Marquillo-not available in separate lines	_	_
Sr 5	Kanred, Reliance, Thatcher, Chris, Manitou, Hochzucht	00;	6D
Sr 6	Kenyan lines(e.g. Kenya 58), Red Egyptian, Africa 43, Eureka, McMurachy, Kentana 52, Chris, Manitou, Selkirk, Gamut	0;	2D
Sr 7a	Egypt Na 101, many Kenyan lines, Kentana 52	13	4BL
Sr 7b	Marquis, Hope, Spica, Renown, Selkirk, Chris, Manitou, Khapstein	2	4BL
Sr 8a	Red Egpytian, Mentana, Frontana, Rio negro	2	6A
Sr 8b	Barleta Benvenuto, Klein Titan, Klein Cometa	X	6A
Sr 9a	Red Egyptian	1+ 2-	2BL
Sr	Many Kenyan lines(e.g., Kenya 117a, Kenya Farmer), Frontana, Gamenya, Festival, Gamut	2	2BL
Sr 9c	Reserved for *Sr Tt1*(*Sr 36*) which was later found not to be an *Sr9* allele		
Sr9d	Hope, H-44-24, Lancer, Scout, Lawrence, Renown, Redman	0;2	2BL
Sr9e	Vernstein, Vernal emmer	0;1	2BL
Sr9f	Chinese Spring	0;2	2BL
Sr 9g	Thatcher, Kubanka, Acme	22+	2BL
Sr10	Egyptian Na95, Kenyan lines	0;X	-
Sr 11	Lee, Gabo, Kenya Farmer, Charter, Sonora 64, Tobari 66, Yalta, Mendos	12-	6BL
Sr 12	Thatcher, Windebri, Egret, Chris, Manitou	X	3BS
Sr 13	Khapstein, Mdden	2-2	6Aβ
Sr 14	Khapstein	12	1BL
Sr 15	ASII, Axminster, Festival, Norka, Thew, Normandie	$0;1^{+}$	7AL
Sr 16	Thatcher, Reliance	2	7AL
Sr 17	Hope, Renown, Redman, Lawrence, Spica, Warigo, Aotea	0;X	7BL
Sr 18	Marquis, Reliance, many wheat lines	0;2	1D
Sr19	Marquis	1^{-}	2B
Sr 20	Marquis, Reliance	2	2B
Sr 21	Einkorn(*Triticum monococcum*), tetraploid and hexaploid derivatives	12^{-}	2A
Sr22	Einkorn(*Triticum monococcum*),tetraploid and hexaploid derivatives	12^{-}	7AL
Sr 23	Exchange, Etoile de Choisy, Selkirk, Warden	23	4A
Sr 24	*Agropyron elongatum*, Agent, Blueboy II, Cloud, Fox, Sage	12^{-}	3DL
Sr25	*Agropyron elongatum*,Agatha, Sears 7D/Ag translocations	12^{-}	7DL
Sr 26	*Agropyron elongatum*, Knott's 6A/Ag translocations,	12^{-}	6Aβ

	Eagle, Kite		
Sr 27	*Secale cereale*, Wheat-rye translocation WRT 238-5	0;	3A
Sr 28	Line AD, Kota	0;	2BL
Sr 29	Etoile de Choicy	23^{-}	6Dβ
Sr 30	Webster, Festiguay	2^{-}	5DL
Sr 31	*Secale cereale*, 1B/1R translocations, Aurora, Kavkaz, Lovrin,Neuzucht, Veery, Weique	0;1	1BL/ 1RS
Sr 32	*Triticum speltoides* derivatives	2^{-}	2A,2B,2D
Sr33	RL 5405 (Tetra Canthatch/ *Aegilops squarrosa*)	$1^{-}2$	1DL
Sr34	*Triticum comosum*, compare, various translocation lines	$1^{-}2$	2A,2D
Sr35	Triticum monococcum derivatives, Arthur	0;	3AL
Sr36	*Triticum timopheevii*, CI 12632, CI 12633, Cook, Idaed 59, Timgalen, Timvera, formerly *SrTt1*	$0;1^{+}$	2BS
Sr 37	Steinwedel/ *Triticum timopheevii* derivative	0;	4Aβ

Appendix C. Possible hosts with the required genes for resistance to *Puccinia graminis* f. sp. *tritici* for the Pgt differential set (Roelfs, 1989)

Cultivar	**Stem rust gene**	**Single gene-line**
Summit	**Sr5**	Isr 5-Ra
Einkorn	**Sr21**	*Triticum monococcum* derivative
Vernal	**Sr9e**	Vernstein
Red fife	**Sr7b**	Isr 7b-Ra
Gabo	**Sr11**	Isr- 11-Ra
McMurachy	**Sr6**	Isr 6-Ra
Mentana	**Sr8a**	Isr 8-Ra
Kubanka	**Sr9g**	CnSSr 9g
Idaed 59	**Sr36(Tt-1)**	W2691SrTt-1
Gamenya	**Sr9b**	W2691Sr9b
Festiguay	**Sr30**	BtSr30Wst
Regent	**Sr17**	Combination VII

Appendix D. Bread wheat cultivars released in Kenya from 1920 to 2007

CULTIVAR	YEAR	CULTIVAR	YEAR	CULTIVAR	YEAR
EQUATOR	1920	KENYA EAGLE	1959	GOBLET	1973
KENYA-324	1920	KENYA CURLEW	1959	KENYA NYATI	1973
KENYA B-256-G	1920	KENYA HAWK	1959	KENYA KIBOKO	1973
KENYA GOVERNOR.	1925	KENYA QUAIL	1959	KENYA MBWEHA.	1974
KENYA STANDARD.	1930	AFRICA MAYO	1960	KENYA KURO	1974
KENYA-112	1936	KENTANA YAQUI	1960	KENYA BATA	1974
KENYA-58	1937	KENYA JAY	1962	KENYA NUNGU	1975
KENYA-131	1939	KENYA GRANGE	1962	KENYA NYOKA	1975
KENYA-122	1939	KENYA PAGE	1963	K.TEMBO	1975
REGENT	1939	SALMAYO	1963	K.PAKA	1975
ENYA- 117-A	1939	TAMA	1963	KENYA KIFARU	1976
KENYA-291	1946	GABRINO.	1963	K.KULUNGU	?
KENYA-294-B-2 A-3	1947	LENANA.	1963	K.FAHARI	1977
KENYA-261	1949	FRONTHATCH.	1963	K.NYANGUMI	1979
KENYA-318-AJ-4 A-1	1949	MENCO.	1963	K.NGIRI	1979
KENYA-318	1949	CATCHER.	1963	KENYA ZABADI	1979
KENYA PLOUGHMAN	1950	BAILEY	1964	KENYA-4792-K-1B-4A	1979
KENYA SETTLER	1950	MORRIS	1964	KENYA PAA	1980
KENYA-350	1951	GEM.	1964	K.KONGONI	1981
KENYA-337	1951	FANFARE	1964	K.POPO	1982
KENYA-184-P	1951	FURY-KEN.	1964	KENYA NYUMBU	1982
KENYA-360-H	1951	KENYA HUNTER	1964	KENYA TUMBILI	1984
KENYA-321	1954	KENYA PLUME	1965	KWALE	1987
KENYA FARMER.	1954	KENYA KUDU	1966	K.CHIRIKU	1989
KENYA-356-A	1955	KENYA LEOPARD.	1966	PASA	1989
KENYA-354	1955	KENYA CHEETAH	?	NGAMIA	1993
KENYA-261-E	1955	KENYA CIVET.	1966	DUMA	1993
KENYA-356-B	1956	ROMANY	1966	MBEGA	1993
KENYA-362	1956	BEACON-KEN.	1966	MBUNI	1993
KENYA-358-AA	1956	BOUNTY.	1967	K.HEROE	1999
KENYA-358	1956	TOKEN-KEN.	1967	CHOZI	1999
KENYA-363	1957	BONNY.	1967	K.YOMBI	1999
KENYA-358-R	1957	MENTOR.	1967	NJBWII	2006
KENYA-358-P	1957	TROPHY.	1968	K.IBIS	2008
KENYA-358-AC	1957	KENYA SUNGURA	1969		
KENYA-362-B-1 E-4	1958	KENYA KANGA	1971		
KENYA-339	1958	KENYA BONGO	1971		
KENYA-291-J-1-I-1	1958	BREWSTER.	1971		
KENYA-6297-2	1958	K.SWARA	1972		
KENYA-362-B-1 A	1958	K.MAMBA	1972		

Appendix E. Field design utilized in both seasons for 'gene effectiveness' trial

	REPLICATE 1		REPLICATE II		REPLICATE III		REPLICATE IV		
	BORDER ROWS								
	UNPROT.	PROT.	UNPROT.	PROT.	UNPROT.	PROT.	UNPROT.	PROT.	
B	VAR1	VAR1	VAR6	VAR6	VAR21	VAR21	VAR22	VAR22	B
	VAR2	VAR2	VAR23	VAR23	VAR18	VAR18	VAR29	VAR29	
	VAR3	VAR3	VAR14	VAR14	VAR13	VAR13	VAR21	VAR21	
O	VAR4	VAR4	VAR30	VAR30	VAR24	VAR24	VAR7	VAR7	O
	VAR5	VAR5	VAR21	VAR21	VAR25	VAR25	VAR17	VAR17	
	VAR6	VAR6	VAR26	VAR26	VAR17	VAR17	VAR28	VAR28	
R	VAR7	VAR7	VAR15	VAR15	VAR29	VAR29	VAR2	VAR2	R
	VAR8	VAR8	VAR20	VAR20	VAR15	VAR15	VAR3	VAR3	
	VAR9	VAR9	VAR17	VAR17	VAR26	VAR26	VAR30	VAR30	
D	VAR10	VAR10	VAR10	VAR10	VAR30	VAR30	VAR14	VAR14	D
	VAR11	VAR11	VAR7	VAR7	VAR28	VAR28	VAR23	VAR23	
	VAR12	VAR12	VAR29	VAR29	VAR1	VAR1	VAR16	VAR16	
E	VAR13	VAR13	VAR1	VAR1	VAR20	VAR20	VAR4	VAR4	E
	VAR14	VAR14	VAR9	VAR9	VAR5	VAR5	VAR10	VAR10	
	VAR15	VAR15	VAR22	VAR22	VAR8	VAR8	VAR27	VAR27	
R	VAR16	VAR16	VAR25	VAR25	VAR4	VAR4	VAR11	VAR11	R
	VAR17	VAR17	VAR2	VAR2	VAR6	VAR6	VAR18	VAR18	
	VAR18	VAR18	VAR3	VAR3	VAR10	VAR10	VAR26	VAR26	
R	VAR19	VAR19	VAR11	VAR11	VAR27	VAR27	VAR25	VAR25	R
	VAR20	VAR20	VAR27	VAR27	VAR2	VAR2	VAR13	VAR13	
	VAR21	VAR21	VAR28	VAR28	VAR23	VAR23	VAR16	VAR16	
O	VAR22	VAR22	VAR5	VAR5	VAR7	VAR7	VAR24	VAR24	O
	VAR23	VAR23	VAR16	VAR16	VAR3	VAR3	VAR8	VAR8	
	VAR24	VAR24	VAR1	VAR1	VAR12	VAR12	VAR15	VAR15	
W	VAR25	VAR25	VAR18	VAR18	VAR11	VAR11	VAR9	VAR9	W
	VAR26	VAR26	VAR24	VAR24	VAR14	VAR14	VAR5	VAR5	
	VAR27	VAR27	VAR8	VAR8	VAR19	VAR19	VAR12	VAR12	
S	VAR28	VAR28	VAR19	VAR19	VAR22	VAR22	VAR20	VAR20	S
	VAR29	VAR29	VAR13	VAR13	VAR9	VAR9	VAR1	VAR1	
	VAR30	VAR30	VAR12	VAR12	VAR16	VAR16	VAR6	VAR6	
	BORDER ROWS								

10m

Appendix F. Critical Daily trial activities and associated rainfall and temperature data recorded at site during growth seasons 1 and II

Month	Date	Event/activity	Rainfall(mm)	Maximum(°C)	minimum(°C)
November,2007	16		0.4	22	9
	17	Planting trial 1	TR	17	8
	18		NIL	22	10
	19		NIL	22	8
	20		NIL	23	8
	21		NIL	22	8
	22		NIL	21	10
	23		TR	22	10
	24		1.6	22	8
	25		NIL	24	10
	26		NIL	25	8
	27		NIL	24	8
	28		NIL	24	7
	29		NIL	25	10
	30		NIL	22	7
December,2007	1		TR	21	8
	2		NIL	21	9
	3		NIL	24	7
	4		NIL	24	7
	5		NIL	24	7
	6		NIL	24	8
	7		3.4	24	9
	8		TR	23	7
	9	Herbicide application trial 1	0.9	23	10
	10		TR	23	8
	11	Planting trial 2	0.8	23	13
	12		NIL	18	10
	13		0.9	21	9
	14		NIL	24	12
	15		NIL	24	12
	16		NIL	24	11
	17	C.A.N application trial 1	NIL	24	11
	18		NIL	25	12
	19		NIL	21	10
	20		NIL	25	9
	21		NIL	24	7
	22		NIL	23	7
	23		NIL	24	7
	24		NIL	24	11
	25		NIL	23	9
	26		0.4	25	10
	27		NIL	24	10
	28		0.4	24	8
	29		NIL	23	9

	30		NIL	24	12
	31		NIL	24.5	6
January,2008	1		0.4	24	7
	2		NIL	24	8
	3		NIL	24	8
	4		NIL	26	12
	5		NIL	26	8
	6	Herbicide application trial 2	NIL	27	9
	7		NIL	27	6
	8		NIL	27	7
	9		NIL	27	11
	10		NIL	27	7
	11		NIL	27	6
	12	C.A.N application trial 2	NIL	27.5	7
	13		NIL	27	7
	14		6.6	27	10
	15		TR	24	9
	16		2.2	24	10
	17		TR	21	9
	18		TR	20	12
	19		TR	23	9
	20		3.6	23	12
	21		8.2	23	10
	22		TR	25	12
	23		TR	25	9
	24		NIL	24	10
	25		NIL	26	9
	26		NIL	25	8
	27		NIL	25	10
	28		NIL	26	9
	29		NIL	26	8
	30		NIL	27	9
	31		NIL	27	9
February,2008	1		NIL	27	10
	2		NIL	25	8
	3		NIL	26	7
	4		NIL	25	8
	5	Folicur applied on prot. plots trial 1	NIL	27	9
	6		NIL	27	10
	7		1.3	26	13
	8		2.5	25	13
	9		NIL	26	8
	10		NIL	26	8
	11	1st Stem rust data trial 1	NIL	26	8
	12		NIL	25	10
	13		NIL	25	8
	14		NIL	25	9

	15		0.4	25	9
	16		NIL	26	9
	17		NIL	27	10
	18	2nd Stem rust data trial 1	NIL	26	8
	19		NIL	27	7
	20	Folicur applied on prot. plots trial 1	NIL	27	8
	21		NIL	27	8
	22		NIL	25	6
	23		NIL	24	8
	24		NIL	27	7
	25	3rd Stem rust data trial 1	NIL	26	7
	26		NIL	19	9
	27		NIL	28	6
	28	Folicur applied on prot. plots trial 1, 2	NIL	27	8
	29		NIL	25	9
March,2008	1		NIL	27	8
	2		NIL	27	7
	3	4th Stem rust data trial 1	NIL	26	7
	4		NIL	27	9
	5		NIL	27	10
	6		NIL	27	9
	7	1st stem rust notes trial 2	NIL	27	7
	8		NIL	26	8
	9		NIL	26	9
	10		NIL	27	9
	11		NIL	18	8
	12	Folicur applied on prot. plots trial 1,2	NIL	27	8
	13		NIL	27	8
	14	2nd Stem rust data trial 2	NIL	27	10
	15		2.6	24	9
	16		0.6	27	8
	17		NIL	27	9
	18		NIL	28	9
	19		NIL	18	9
	20		1.7	17	11
	21	3rd Stem rust data trial 2	4.4	26	10
	22		1.1	27	10
	23		NIL	26	12
	24		10.9	25	11
	25		13.6	21	12
	26	Folicur applied on prot. plots trial 1,2	NIL	26	12

	27		NIL	24	12
	28	4th Stem rust data trial 2	13.4	23	12
	29		13.5	21	12
	30		10.8	23	11
	31		2.1	24	8
April,2008	1		5.2	24	8
	2		0.4	24	6
	3		NIL	24	6
	4		NIL	24	7
	5		NIL	24	7
	6		NIL	24	8
	7		TR	25	9
	8		NIL	25	8
	9		TR	24	9
	10	Folicur applied on prot. plots trial 2	NIL	24	12
	11		4.2	22	10
	12	Harvesting trial 1	9.5	25	9
	13		0.4	26	11
	14		4.9	23	11
	15		9.4	24	9
	16		2.5	24	12
	17		10.3	23	9
	18		2.1	23	8
	19		4.0	24	10
	20		0.5	24	10
	21		1.0	23	12
	22		2.6	21	12
	23		28.4	21	8
	24		25.6	22	8
	25		3.1	21	8
	26		NIL	22	7
	27		1.2	22	7
	28		TR	23	9
	29		TR	24	9
	30		NIL	24	9
May 2008	1st	Harvesting trial 2			

Appendix G. Typical Proc Chi-Square output. A test of observed R=28:S=35 against expected 1:1 ratio for (Cook/Kwale)/Kwale BC1F1 population. Other populations tested in a similar model

The Freq Procedure

Classes	Freq	Test freq	Percent	Cumlative Freq	Cumulative Percent
R	28	31.5	44.44	28	44.44
S	35	31.5	55.56	63	100

Chi square for specified Frequencies

Chi square	0.7778
DF	1
Pr>Chi-Square	0.3778
Sample size	63

Appendix H. Anova for difference in rAUDPC between Season 1 and Season 2

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	2075.44	7156.68	124.21	<0.0001
Treatment	1	17563.18	17563.18	304.83	<0.0001
Entry*Treatment	29	15340.09	528.97	9.18	<0.0001
Rep	3	236.63	78.88	1.37	0.2539
Error A	3	1492.68	497.56	8.64	<0.0001
Error B	174	10025.11	57.62		

CV=17.7

Appendix I. Anova for difference in Final Disease Score between Season 1 and Season 2

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	2075.44	7156.68	124.21	<0.0001
Treatment	1	17563.18	17563.18	304.83	<0.0001
Entry*Treatment	29	15340.09	528.97	9.18	<0.0001
Rep	3	236.63	78.88	1.37	0.2539
Error A	3	1492.68	497.56	8.64	<0.0001
Error B	174	10025.11	57.62		

CV=18.03

Appendix J. Anova for Yield differential between Protected and Non prot. trials in season 1

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	69.02	2.38	2.62	<0.0001
Treatment	1	117.04	117.04	128.82	<0.0001
Entry*Treatment	29	48.2	1.66	1.83	0.0096
Rep	3	6.76	2.25	2.48	0.0628
Error A	3	9.29	3.10	3.41	0.0188
Error B	174	158.08	0.91		

CV=28.88

Appendix K. Anova for Yield differential between Protected and Non pro. trials in Season 2

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	35.56	1.23	1.89	0.0065
Treatment	1	39.24	39.24	60.62	<0.0001
Entry*Treatment	29	15.52	0.54	0.83	0.7209
Rep	3	3.52	1.17	1.81	0.1468
Error A	3	1.89	0.63	0.97	0.4066
Error B	174	112.62	0.64		

CV=31.37

Appendix L. Anova for 1000-grain weight differential between Protected and Non prot. trials in Season 1

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	1698.04	58.55	5.34	<0.0001
Treatment	1	2663.93	2663.93	242.88	<0.0001
Entry*Treatment	29	549.63	18.95	1.73	0.0172
Rep	3	89.29	29.76	2.71	0.0465
Error A	3	30.07	10.02	0.91	0.4355
Error B	174	1908.48	10.97		

CV=9.1

Appendix M. Anova for 1000-grain weight differential between Protected and Non prot. trials in Season 2

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	2597.39	89.56	29.04	<0.0001
Treatment	1	3502.09	3502.09	1135.56	<0.0001
Entry*Treatment	29	852.27	29.39	9.53	<0.0001
Rep	3	7.88	2.63	0.85	0.4672
Error A	3	1.76	0.59	0.19	0.9028
Error B	174	536.62	3.08		

CV=4.7

Appendix N. Anova for biomass differential between Protected and Non prot. trials in Season 1

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	81.65	2.82	1.03	0.43
Treatment	1	28.81	28.81	10.57	0.0014
Entry*Treatment	29	75.21	2.59	0.95	0.5426
Rep	3	98.14	32.71	12.0	<0.0001
Error A	3	122.14	40.71	14.93	<0.0001
Error B	174	474.43	2.73		

CV=43.9

Appendix O. Anova for Test weight differential between Protected and Non prot. trials in Season 1

Source	DF	Sum of squares	Mean Square	F-value	Pr>F
Entry	29	53121.93	1831.79	3.76	<0.0001
Treatment	1	92239.34	92239.34	189.59	<0.0001
Entry*Treatment	29	38148.03	1315.45	2.70	<0.0001
Rep	3	4689.77	1563.26	3.21	0.0237
Error A	3	3940.14	1313.38	2.70	0.0465
Error B	174	111424.07	486.57		

CV=4.91