RESPONSE OF SELECTED BARLEY (*Hordeum vulgare* L.) GENOTYPES TO STEM RUST (*Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn) DISEASE

EGERTON UNIVERSITY LIBRARY
16 SEP 2013
P.O. BOX 536,
EGERTON
AFRICANA SECTION

EDWARD KULECHO MWANDO

EULIB
038 221

A Thesis Submitted to Graduate School in Partial Fulfillment of the Requirements for Master of Science in Agronomy (Crop Protection) of Egerton University

EGERTON UNIVERSITY

MARCH, 2011

2013/9505P

EGERTON UNIVERSITY LIBRARY

DECLARATION AND RECOMMENDATION

Declaration

This is my original work and has not been presented in this or any other university for the award of a degree

Signed ..... Date 8:3:2011

Edward Kulecho Mwando

KM12/1939/07

Recommendation

This work has been submitted with our approval as University Supervisors.

Signed ..... Date 9/3/2011

Dr. I. M. Tabu

Department of Crops Horticulture and Soil Science

Egerton University

Signed ..... Date 9/3/2011

Dr. O. D. Otaye

Department of Biological Sciences

Egerton University

2013/95052

COPYRIGHT

All rights reserved. No part of this publication may be translated, reproduced or transmitted in any means, electronic or mechanical including photocopying, recording or information storage and retrieval system or otherwise without the prior written permission of the author or Egerton University on his behalf.

Edward Kulecho Mwando © 2011

DEDICATION

This thesis is dedicated to my late father Mr. Charles P. Mwando and my inspirational sister Carol.

ACKNOWLEDGEMENT

I acknowledge all those who have assisted me at various stages of my study. My deep gratitude's go to Drs. I. M. Tabu and O. D. Otaye of Egerton University for their constructive criticism, good guidance and very useful discussions throughout the period of this study. Special thanks to Dr. Tabu for assistance with data analysis and the fatherly counsel. I also take this opportunity to thank the Board of Post Graduate Studies, Egerton University for the financial support that helped me complete the research project.

Thanks to Dr. Gethi the Director National Plant Breeding Centre Njoro, for allowing me to use the facilities at the institute. My special attention to Mr. Peter Njau, the wheat breeder at KARI Njoro for the unreserved support. Heartfelt gratitudes to Mr. Macharia G. Kamau, Mr. Kamundia and the entire staff at the National Plant Breeding Centre Njoro for making my life bearable during the research period. During the period I interacted with great scientists like Dr. Ravi Singh, Dr. Davinder Singh (CIMMYT Mexico), Dr. Yue Jin (Cereal laboratory, USDA Minnesota), and Prof. Brian Steffenson (University of Minnesota, USA) among others. I acknowledge the help, encouragement and co-operation extended to me by the members of staff, from the department of Crops, Horticulture and Soil Sciences colleagues and friends within Egerton University who are too far numerous to mention.

I am grateful to God for giving me a very supportive sister Carol Mwando who paid for my first year college fees and has been helpful throughout. I benefited immensely from words of inspiration, encouragement and prayers from my mother Margaret Mwando. God bless you.

ABSTRACT

Stem rust, caused by (*Puccinia graminis* f.sp. *tritici)* is a catastrophic disease because of its ability to cause complete annihilation of barley and wheat crops over wide areas. Previously rpg1, sr31, sr24 and sr36 genes for resistance were incorporated in genotypes of barley and wheat grown in Kenya kept it at bay. In 2001 a new race Ug99 with virulence to previously resistant genotypes was detected in Kenya. Most of the wheat varieties are susceptible to the disease hence the need to seek for alternative sources of resistance. Barley is one of the genotypes affected by stem rust, but limited work has been done to evaluate the germplasm. There was need to search for new materials that are resistant to the new physiological race of stem rust and estimate the actual yield loss caused by it. The performance of 20 barley genotypes from Kenya and CIMMYT/ICARDA were evaluated under controlled greenhouse and field in stem rust prone environments. At seedling stage, infection levels in most genotypes, ranged from 0 to 2, except CBSS99MOO349T-F-3M-IY-IM-OM and CBSW98WOOO54S-BY-2M-IY-2M-IY-OM that showed infection type 3 and 3,4 respectively. Among the 20 genotypes that were evaluated for adult plant resistance CBSS99MOO39IT-H-IM-IY-IM-IY-OM, Nguzo and Karne were moderately resistant while the rest were susceptible or moderately susceptible. The new line 1512-5 showed the highest severity of (93%) in season 1, Sabini had the highest severity of 30% in the season 2. Nguzo had the lowest (16% and 5%) disease severity in season 1 and 2 respectively. In the field, FDS on susceptible cultivars was highly positively correlated with Area Under the Disease Progress Curve (AUDPC). The highest yield loss of 53.8% was attained in CBSSOOYOO236T-E-0Y-OM-2Y-OM while the lowest loss of 6.9% was obtained in CBSW98WOOO54S-BY-2M-IY-2M-IY-OM in season 1. In season 2, the highest (32.1%) loss was noted on 1385-13, a Kenyan line from malting. Generally, in the experiment most Kenyan cultivars were susceptible to the new race of stem rust. Race Ug99 of stem rust had highly negative impact in limiting grain production potential of most of the commercially popular Kenyan barley cultivars. Resistance due to 'slow rusting' genes appears to be useful especially under high disease pressure with some cultivars posting very high resistance to PgtUg99. The study demonstrated the pathogenicity of Ug99 to barley despite the fact that it poses a great threat to wheat production. The information in this study is beneficial to both researchers, barley and wheat producers in confronting a pathogen that is devastating.

EGERTON UNIVERSITY LIBRAR

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION.....ii
COPYRIGHT.....iii
DEDICATION.....iv
ACKNOWLEDGEMENT.....v
ABSTRACT.....vi
TABLE OF CONTENTS.....vii
LIST OFTABLES.....x
LIST OF FIGURES.....xi
LIST OF PLATES.....xii
LIST OF APPENDICES.....xiii
LIST OF ABBREVIATIONS AND ACRONYMS.....xiv
DEFINITION OF TERMS.....xv
CHAPTER ONE.....1
INTRODUCTION.....1
1.1 Background Information.....1
1.2 Statement of the problem.....2
1.3 Objectives.....2
1.3.1 General objective.....2
1.3.2 Specific objectives.....2
1.4 Hypotheses.....3
1.5 Justification.....3
CHAPTER TWO.....4
LITERATURE REVIEW.....4
2.1 Barley.....4
2.2 Stem rust of barley.....5
2.3Disease distribution and taxonomy.....5
2.3.1 Distribution of race Ug99.....5
2.4 Etiology.....5

2.4.3 Evolution and dispersal of new races 10
2.5 Epidemics 12
2.5.1 Infection process 12
2.6 Disease symptoms 13
2.6.1 Rating of seedling infection 15
2.6.2 Relation between disease symptoms and amount of the pathogen 15
2.7 Crop losses due to stem rust of barley 16
2.8 Disease management 17
2.8.1 Cultural practice 17
2.8.3 Chemical control 18
2.8.4. Genetic resistance/ tolerance 18
2.8.5 Crop resistance to diseases 20
2.9 Evaluation of barley genotypes for stem rust resistance 20
2.9.1 Rust resistance genes 22
CHAPTER THREE 23
MATERIALS AND METHODS 23
3.1 General methods 23
3.1.1 Site description 23
3.1.2 Barley germplasm 23
3.1.3 Stem rust isolate preparation 23
3.2 Experiment I: Response of barley germplasm to stem rust under greenhouse conditions 25
3.2.1 The infection type at seedling stage 25
3.2.2 The reaction type to stem rust at adult plant stage 26
3.3 Experiment II: Performance of the different barley accession in a stem rust pressure in the field 27
3.3.1 Barley quality analysis 27
3.4 Parameter measured 27
3.5 Data analysis 28
CHAPTER FOUR 29
RESULTS AND DISCUSSION 29
4.1 Disease severity under varying conditions 29
4.1.1 Infection levels of stem rust in the greenhouse 29
4.1.2 Stem rust severity under field conditions 29

4.1.3 Stem rust development and expression of resistance in the field 30
4.2 Effect of stem rust Ug99 on growth and development of barley 32
4.2.1 Dry matter 32
4.2.2 Leaf area 33
4.2.3 Chlorophyll index 34
4.2.4 Tillering capacity 34
4.3.5 Plant height 35
4.3 Effect of stem rust Ug99 epidemics on yield and yield components of barley 36
4.3.1 Total grain yield 37
4.3.2 Thousand kernel weight 38
4.3.3 Grain size 39
4.4 Effect of stem rust Ug99 epidemic on the quality of barley 40
4.4.1 Germination percent 41
4.4.2 Starch content 41
4.4.3 The protein, zeleny and gluten content 42
4.5 Correlation and factor analysis 46
4.5.1 Correlations 46
4.5.2 Factor analysis 48
CHAPTER FIVE 50
CONCLUSIONS AND RECOMMENDATIONS 50
5.1 Conclusions 50
5.2 Recommendations 51
REFERENCES 52
APPENDICES 64

LIST OF TABLES

Table 1: The coding system used in the U.S to designate stem rust races and indicate their pathogenicity 9

Table 2: Races of stem rust recorded in Kenya from 1928 to 2008 10

Table 3: Type and source of barley genotypes used in the experiment 24

Table 4: Infection codes used in classifying the reaction to stem rust on seedling barley leaves 25

Table 5: Host infection response used in classifying stem rust reaction at adult stage 26

Table 6: Seedling infection type and adult plant reaction of barley to Ug99 in the greenhouse 29

Table 7: The, final disease severity (fds %) and area under disease progress curve (AUDPC) of barley genotypes 31

Table 8: Effect of stem rust infection on dry matter (kgs) of the barley genotypes 32

Table 9: Effect of stem rust Ug99 on leaf area (cm) of different germplasms of barley 33

Table 10: Effect of stem rust Ug99 on the chlorophyll index of barley genotypes 34

Table 11: Effect of stem rust Ug99 on the number of effective tillers (m^{-2}) of barley genotypes 35

Table 12: Effect of stem rust Ug99 on plant height (cm) of barley genotypes 36

Table 13: Effect of stem rust on yield (t/ha) of barley 38

Table 14: Effect of stem rust on the 1000 kernel weight (gm) of barley 39

Table 15: Effect of stem rust on grain size of barley 40

Table 16: Effect of stem rust disease on germination percent of barley genotypes 41

Table 17: Effect of stem rust Ug99 on starch content of barley grains 42

Table 18: Effect of stem rust Ug99 on protein content (%) of barley 43

Table 19: Effect of stem rust Ug99 on zeleny content (%) of barley 44

Table 20: Effect Of Stem Rust Ug99 On Gluten Content Of Barley 45

Table 21: Phenotypic correlation coefficient (r) among yield component, growth parameters, quality aspects, final disease and AUDPC for barley 47

Table 22: Factor patterns of variables 49

LIST OF FIGURES

Figure 1. Life cycle of stem rust pathogen (Kurt and Szobo, 2005).....7

LIST OF PLATES

Plate 1: Stem rust symptoms on stem and leaf.....14

Plate 2: Infection types (ITs) of *Puccinia graminis* f. sp. *tritici*. as by Leonard and Szabo 2005.....15

LIST OF APPENDICES

Appendix A. Map of barley growing locations in Kenya.....64

Appendix B. Crop production areas in Kenya.....65

Appendix C. Kenya yearly barley production.....66

Appendix D. Barley production in African country in 1000 MT.....66

Appendix E. Monthly total rainfall (mm), minimum and maximum temperature at the experimental sites in the year 2008 / 09.....67

Appendix F. Chemical characteristics of soils at the experimental site.....68

Appendix G. Field design utilized in both season.....69

Appendix H. Number of rows of the 20 barley genotypes.....70

Appendix I. ANOVA of stem rust on barley season 1.....70

Appendix J. ANOVA of stem rust on barley season 2.....73

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	-Analysis of Variance
APS	-American Phytopathological Society
AUDPC	-Area under Disease Progress Curve
CAN	-Calcium Ammonium Nitrates
CIMMYT	-International Centre for Maize and Wheat Improvement
DAP	-Di-Ammonium Phosphate
EABL	-East Africa Breweries Limited
FAO	-Food and Agriculture Organization
FDS	-Final Disease Score
GOK	-Government of Kenya
KARI	-Kenya Agriculture Research Institute
KML	-Kenya Malting Limited
LSD	-Least Significant Difference
MT	-Metric Tones
NCPB	-National Cereal and Produce Board
Pgt	*-Puccinia graminis tritici*
Rpg	- stem rust resistance gene in barley
SAS	-Statistical Analysis System
Sr	- Stem rust resistance gene in wheat
USDA	- United States Department of Agriculture

DEFINITION OF TERMS

Avirulence	The property of a pathogen by which it cannot infect a host plant because of the effectiveness of one or more major genes for resistance
Differential set	A set of lines or cultivars by which races of a natural enemy can be characterized or distinguished
Forma specialis (f.sp)	Unit within the pathogen that is distinguished by its host range
Host	A plant that is invaded by a parasite
Horizontal resistance	Race-non-specific resistance
Inoculum	The pathogen or part of it that can cause infection
Isolate	A sample of a pathogen that is stored alive or maintained in isolation on plant or in natural media
Pathogen	An entity that can incite a disease
Race	A group of genotypes within a pathogen species that is distinguished by its virulence
Resistant	Possessing qualities that hinder the development of a given pathogen
Susceptible	Lacking the inherent ability to resist attack by the pathogen
Tolerance	Ability of host to avoid or minimize loss in productivity although it has been infected by a pathogen
Virulence	The degree of pathogenicity of a given pathogen
Vertical resistance	Race specific resistance

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Barley (*Hordeum vulgare* L.), one of the most important cereal crops, was introduced in Kenya in 1920 by the colonial government as animal feed (EABL, 2005). Later, in 1929, the crop was commercialized for beer making. Barley does well in high and medium altitude areas (1500 – 3000masl) with mean annual rainfall of at least 635 mm. In Kenya barley is grown in Timau, Moiben, Nakuru, Molo and Mau Narok. The cultivars of barley recommended for production in Kenya include 'Sabini', 'Nguzo' and 'Bima' (KML, 2007). Currently about 30,000 ha of land in Kenya is under barley production but 55,000 ha are unutilized (EABL, 2005). In 2007 about 75,000MT, against the expected potential of 120000MT was harvested (NCPB, 2007). In the last one decade there was a reduction in barley production by more than 15 MT in the country (USDA, 2008).

Rust fungi, responsible for diseases like stem rust, yellow rust and leaf rust are major contributors to the sub optimal crop yields. Breeding for resistance has previously been used as the main method of protection against the fungi. The rust fungi can however overcome host resistance genes and spread new strains through wind dispersal of spores (Hovmøller and Justesen, 2007; Njau *et al.,* 2010). Stem rust caused by *Puccinia graminis* f.sp *tritici*, Eriks. and E. Henn. is one of the most important plant diseases whose devastating epidemics often result in major grain losses (CIMMYT, 2005). Between 1996 and 2003 severe infection of stem rust was recorded on many commercial wheat cultivars in Mau-Narok, Molo, Njoro and Lanet areas of Kenya (Wanyera, 2008). The problem of wheat stem rust was previously contained through the use of genetic resistance resulting from genes such as Sr31 (CIMMYT, 2005). In 1999 and 2001 a new race Ug99 or TTKS with virulence to stem rust resistant cultivars, was detected in Uganda and Kenya respectively (Wanyera *et al.*, 2006; Pretorius *et al.,* 2000). Preliminary work indicates that race TTKS is virulent on barley cultivars carrying the durable stem rust resistance gene *Rpg1* (Steffenson and Jin, 2006). More recently stem rust races Ug99 variants like PTKST, TTKSF, TTKSK, TTKSP, TTKST and TTTSK, have been detected (Jin *et al.*, 2009; Jin *et al.*, 2008b; Jin *et al.*, 2008, and Jin *et al.*, 2007). The TTKST variant caused severe epidemics and rendered about half of the previously known Ug99-resistant global wheat materials susceptible in some regions of Kenya (Singh *et al.,* 2008). The new variants of Ug99 PTKST, TTKSF, TTKST and TTTSK with virulence to genes Sr21, *Sr24,* and Sr36 imply that the race Ug99 is

evolving. It is further clear that continued monitoring of the Ug99 race cluster is necessary to keep abreast with the distribution patterns and new variants (Pretorius and Park, 2008).

Stem rust race Ug99 is responsible for up to 100% yield loss of wheat. The race also affects barley where control using fungicides has been unsuccessful. Much of the work including genetic manipulation of wheat to control the disease has had little success. Barley is another crop that is affected by the Ug99 race. Little work has however been done to evaluate the barley cultivars for resistance to the new race of stem rust.

1.2 Statement of the Problem

Stem rust, caused by *Puccinia graminis* f.sp *tritici,* is one of the most destructive diseases affecting barley and wheat. The disease was previously controlled by the use of host plant resistance which the pathogen has lately overcome. In 1999, a new race of stem rust designated as race TTKSK or Ug99 appeared in Uganda and has since spread to Kenya. The race is responsible for more than 70% yield loss of wheat annually. The conventional control of the disease using fungicides is ineffective and an environmental hazard. The pathogens' polycyclic life cycle further negates the continued use of fungicides. Most wheat genotypes are susceptible hence the need to search for resistance in other genotypes such as barley. While it is generally known that barley is affected by the fungus, little work has been done to quantify the yield and quality reduction associated with the disease.

1.3 Objectives

1.3.1 General objective

To increase barley yield and quality through stem rust management.

1.3.2 Specific objectives

1. To determine the infection response of seedling and adult barley genotypes to stem rust Ug99 under greenhouse conditions.
2. To determine the infection response and disease severity of barley genotypes to stem rust Ug99under field conditions.
3. To determine yield and quality losses associated with stem rust Ug99 disease in barley.

1.4 Hypotheses

1. The infection response of adult barley plant does not differ from that of seedling barley under greenhouse conditions.
2. Barley genotypes have no resistance to the current physiological race of stem rust Ug99 under field conditions.
3. Stem rust infection has no effect on yield and quality of barley.

1.5 Justification

Barley is a principal raw material used to process malt, a vital ingredient for beer brewing. The industry is one of the highest corporate taxpayers with annual turnover of Kshs. 28.9 billion and employs more than 1600 people across the region (EABL, 2005). The area suitable for barley production in Kenya is estimated to be 85,000 ha (EABL, 2005). Over the next 20 years, the world demand for barley is likely to increase by 50%, because of the rapidly growing animal feed use and meat consumption (Borlaug, 2007). Diseases however reduce the yield and quality of barley hence results in lower returns to the grower (Chelkowski *et al.,* 2003; Williams, 2003). Stem rust is one of the major diseases of barley that contributes to about $5 billion loss annually (FAO, 2004). Use of fungicides for stem rust control affects the environment and are expensive hence not recommended. The search for and deployment of resistant cultivars therefore continues to be just important as its selection for quality attributes in malting barley improvement. Most of the Kenyan wheat germplasm are susceptible or partially susceptible to the new virulent African stem rust race Ug99 and there is need to screen for new sources of resistance (Njau *et al.,* 2009). Barley being one of the crops affected by stem rust need to be screened for resistance to the local stem rust physiological races including Ug99, so that breeding programmes can be effectively sustained both in barley and wheat.

CHAPTER TWO

LITERATURE REVIEW

2.1 Barley

Barley (*Hordeum vulgare*), is a member of the *Poaceae* family just like wheat, *Triticum* spp. and rye, *Secale cereale* (Bothmer and Jacobsen 1985). The genus, *Hordeum*, has a worldwide distribution with three species constituting the barley under cultivation (Allard, 1988). Barley is an annual grass which may be either winter or spring annual used for malting, as human food and as livestock feed (Nevo, 1992). For malting, the grains are steeped in aerated water for 45 to 65 hours, and then transferred to germinating compartments for 5 to 7 days at temperatures of 20-24^{0}C. During this treatment root sprouts, the malt is then dried in hot air kilns. For making beer the malt is crushed between rollers, mixed in proper proportions with slightly warm water, and held under controlled temperatures (EABL, 2005). During this stage starch is converted into maltose and dextrins, while the proteins are broken down by enzyme action. The protein content of malting barley is affected by many factors, especially the growing conditions. Fertilizer application may also impact the final protein level Barley can also be used directly for human food, where, the abrasive disks are used to grind the hulls and bran off the kernels (Beaven, 1947). When the hull and most bran are removed, the remaining part of kernel is used in soups and dressings, breakfast cereals, and in baking. The smooth-awned or awnless varieties are used as a hay crop in some areas.

In Kenya barley is ranked fourth after maize, wheat and rice in production per unit area (NCPB, 2007). About 85,000 ha of cultivated land are suitable for barley production in Kenya, yet only 30,000 ha are used (EABL, 2005). The average yield of 2.6 ton/ha out of the expected potential of 4.0ton/ha put Kenya, in the leading in East Africa; 8th in Africa and 42nd in the world production (USDA, 2008). Analysis show that barley yields was 90 MT (3ton/ha) from 1995 to 1998 but declined immensely from 1999 to 2.6ton/ha (NCPB, 2007). The main cause of this sub-optimal yield from 1999 is the resurgent of stem rust virulent race Ug99 (TTKSK) that is virulent to the previously resistant genes rpg1 among other factors. More yield losses are expected due to the increased variation in the stem rust Ug99 race (Pretorius and Park, 2008).

2.2 Stem rust of barley

Stem rust also known as 'black rust' or 'summer rust' because of the abundant production of shiny black teliospores that form in the uredinium at the end of the season or with unfavorable conditions is caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn (Anikster, 1984). The stem rust fungus, *Puccinia graminis*, is functionally an obligate biotroph (Bushnell and Bosacker, 1982).

2.3 Disease distribution and taxonomy

The stem rust fungus almost co-exists with barley and wheat throughout the world (Walker, 1957; Agrios, 2005). In Kenya barley and wheat are produced in different places and seasons hence its host availability throughout the year. In wheat the disease contributes to 70% reduction in yield compared to approximately 30% in barley (Jin *et al.,* 2007). *Puccinia graminis* is a rust fungus in the division Basidiomycota, class Basidiomycetes, order Uredinales, and family Pucciniaceae which contains 17 genera and about 4121 species, of which the majority are in the genus *Puccinia* (Kirk *et al.*, 2001). Wheat stem rust, *P. graminis* f. sp. *tritici* , naturally infect 28 species belonging to eight genera while other formae speciales of *P. graminis* have narrower host ranges (Kurt *et al.,* 2005).

2.3.1 Distribution of race Ug99

Ug99 was first identified in Uganda in 1998 (Pretorius *et al.,* 2000) although some reports indicate that the race may have been present in Kenya as early as 1993 (Sing*h et al,* 2008). Since identification in Uganda the disease has spread rapidly. In 2006 and 2007 the race was reported to be in Eastern Sudan and Iran respectively (Nazari *et al.,* 2009). The observed expansion into new areas is in-line with previous predictions on the likely movement of Ug99 (Singh *et al.,* 2006) and fits the step-wise dispersal model following prevailing winds.

2.4 Etiology

Stem rust is caused by one of the true heteroecious fungi, *Puccinia graminis* (Kurt *et al.,* 2005). The fungus is macrocyclic i.e produces all five [basidiospores, pycniospores (spermatia), aeciospores, urediniospores (uredospores), and teliospores] spore stages (Leonard, 2001a). The fungus produces aecia and spermagonia on Barberry while it produces telia and uredia on barley (Agrios, 2005). Several grasses including goat grass are also affected and serve as source of primary inoculum (Wiese, 1977). The fungus undergoes full/ complete life cycle when the alternate host (Barberry) is present but in the absence it undergoes asexual life cycle (De Millano, 1983). As a result of the wide variations in cropping seasons in Kenya, rust inoculum is present throughout the year (Harder *et al.*, 1972).

The disease cycle starts with the exposure of the new host crop to stem rust inoculum (Agrios, 2005). In regions with warm climates, the volunteer plants carry the spores over summer and urediniospores from the infected volunteer wheat are the source of initial inoculum for wheat planted in the next fall to start a disease cycle (Kurt *et al.,* 2005). In the regions with a cold winter, aeciospores are the main source of initial inoculum for wheat stem rust that is produced on barberry, the most common alternate host (Kurt *et al.,* 2005). On barberry, *P. graminis* starts its life cycle by producing black thick walled, diploid teliospores that keep *P. graminis* dormant over winter. In spring, each teliospore germinates to produce two identical thin walled haploid basidiospores (Kurt *et al.,* 2005). Mature basidiospores are carried by air currents to re-infect barberry on which they germinate and form a haploid mycelium growing on the leaf surface. From the mycelium, pycnia are produced on the upper leaf surface of barberry to produce receptive hyphae that serve as female gametes and pycniospores as male gametes. Pycniospores are produced in honeydew that is attractive to insects that helps in dispersing together with rain splashing (Agrios, 2005). When pycniospores are paired with receptive hyphae, cross-fertilization occurs successfully and dikaryotic mycelium forms. Then a cup-shaped dikaryotic aecium forms to release aeciospores. Normally aeciospores stalked in chains produced on barberry are transported by wind to start a new disease cycle in susceptible wheat cultivars (Kurt *et al.,* 2005).

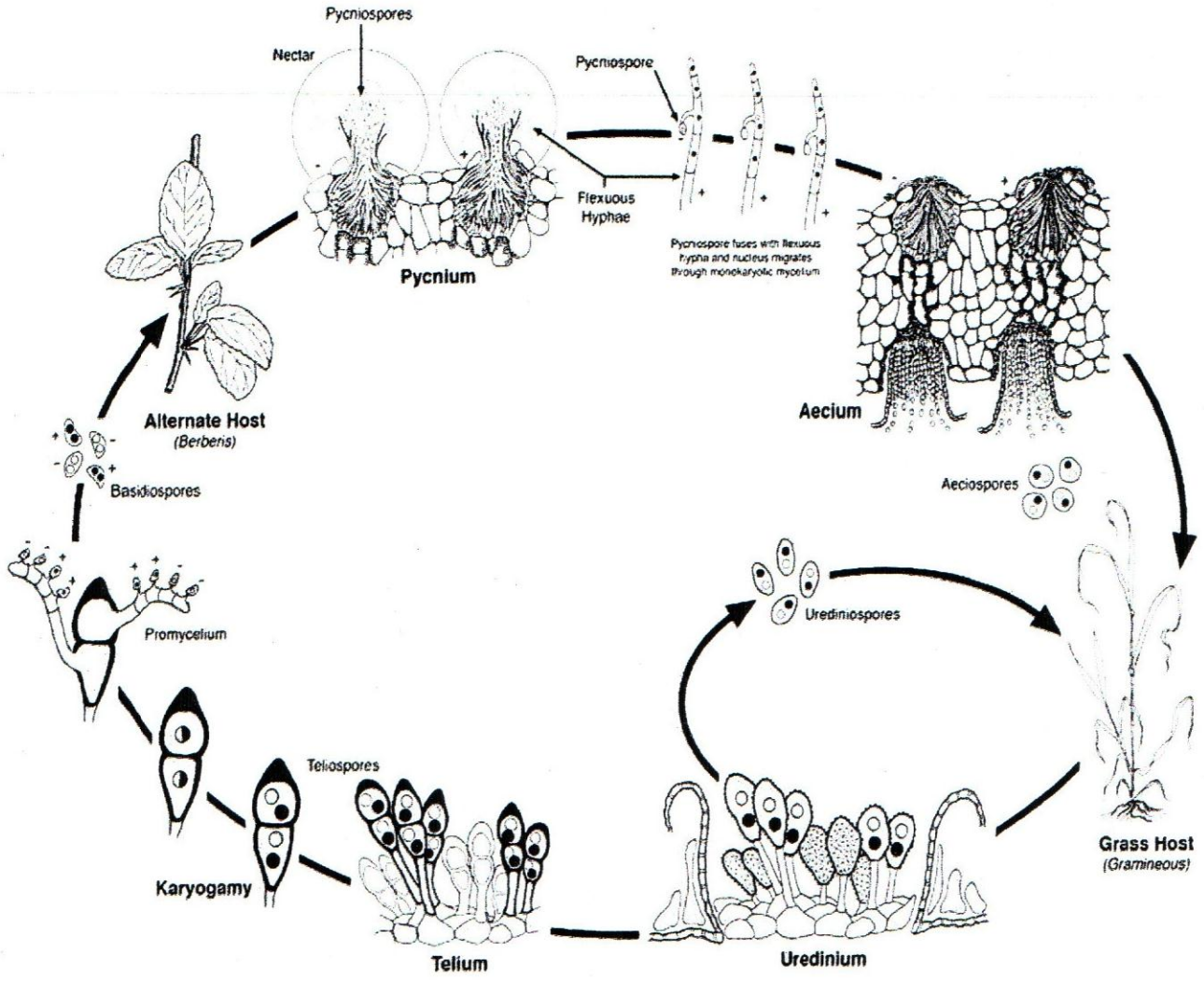


Figure 1. Life Cycle of Stem Rust Pathogen (Kurt and Szobo, 2005)

2.4.1 Pathogen races and race identification

Physiological races are biotypes of a pathogen that vary in their ability to infect different varieties of the same host species. Walker (1957) reported 200 distinct races stem rust, while Agrios (2005) reported several hundreds with new races appearing every year. In fungi, production of new physiological races is achieved through parasexual recombination, mutation, sexual reproduction and heterokaryosis (Agrios, 2005).

Stem rust pathogen can be divided into many different races according to their reactions to different resistance genes in wheat and barley. The first report on stem rust race (races 1 and 2) was in 1916. Previously classification of races of *P.* g. f. sp. *tritici* was based on the gene concept, and the

differential host genotypes of single resistance genes (Roelfs and Martens, 1988). A new international system of nomenclature that uses, differential lines that carry single genes Sr 5, 6, 7b, 8a, 9a, 9e, and 15 in Chinese spring background and Sr 9d, 10, 13, 15 and 36 in the background of W2691, a highly susceptible line (Sr9e) combination VII (SR13AND SR17) and Triumph (SrTmp) are now days employed as testers (Roelfs and Martens, 1988; Kurt *et al.*, 2005).

The testers are divided into one set of genes e.g. Sr 5, 7b, 9d, and 9e. Another set of genes classified for resistance or susceptibility are coded from B to T, omitting the vowels (Table 1). The pathogenicity 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. Jin e*t al.* (2008b) revised the North America nomenclature by adding four more genes (Sr24, Sr31, Sr38 and Sr*McN*).

Table 1: The coding system used in the U.S to designate stem rust races and indicate their pathogenicity

	Wheat lines carrying genes			
Set 1	Sr5	Sr21	Sr9e	Sr7b
Set 2	Sr11	Sr6	Sr8a	Sr9g
Set 3	Sr36	Sr9b	Sr30	Sr13+17
Set 4	Sr9a	Sr9d	Sr10	Sr*Tmp*
Set 5	Sr24	Sr31	Sr38	Sr*McN*
Code		Host	reaction	
B	R†	R	R	R
C	R	R	R	S
D	R	R	S	S
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	R
S	S	S	S	R
T	S	S	S	S

The first letter indicates the reaction of the lines in set 1; the second letter set 2 etc. †R= Resistant S= Susceptible (Roelfs and Martens, 1988)

2.4.2 Races recorded in Kenya

Since 1927, several stem rust physiological races have been identified (Table 2)

Table 2: Races of stem rust recorded in Kenya from 1928 to 2008

Race Kenya number	International number	Year Identified
K1	21	1928
K2	17	1928
K3	14	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	TTKSK	2003
	TTKST	2006
	TTTSK	2007
	TTSSK	2008

2.4.3 Evolution and dispersal of new races

Occurrence of new races in a geographic/epidemiologic region can be attributed to migration from an outside the region, mutation and selection for a particular virulence, and recombination; both sexual (on barberry) and asexual (Jin *et al.,* 2008b).

Migration

Urediniospores can move several hundreds or thousands of kilometers (Luig, 1985). Long distance spread is influenced by latitude and by local and global wind patterns. The occurrence of barley yellow

rust in South America and its further spread, to North America and the gradual spread of the *Yr9*-virulent wheat stripe rust from eastern Africa to the South Asia can be attributed to the global wind patterns (Dubin and Stubbs, 1986). Increased transcontinental travel and human errors have also contributed to the movement of races from one area to another. New strains of diseases and pests can move to and establish at any location on earth if they have a selective advantage. If the new region is occupied by large area of cultivars susceptible to incoming inoculum, then major epidemics and heavy crop losses may result (McIntosh, 1988).

Mutations followed by selection

Mutation is one of the most common mechanisms that lead to the development of new virulence in stem rust fungi. Deployment of a resistance gene on a large scale leads to the selection and increase in the frequency of those mutants that have acquired virulence (McIntosh, 1988). The increase of such races leads to rust epidemics. With only a few exceptions, the life of a newly deployed, race-specific resistance gene is 3-5 years (Roelfs *et al.,* 1992). As in the case of Sr31, the longer a resistance gene remains effective the greater the breeding community depends upon it for continued protection. This leads to complacency and excessive use arising from the assumption that the original problem had been solved, ultimately resulting in genetic vulnerability (McIntosh, 1988).

Recombination

Recombination occurs primarily during the sexual reproduction of fungi whenever two haploid (1N) nuclei, containing genetic material that may differ in many loci, unite to form a diploid (2N) nucleus called a zygote (Agrios, 2005). The zygote, sooner or later, may divide meiotically and produce new haploid cell (gametes, spores, and mycelium). Recombination of genetic factors (different genes or alleles of the same genes) occurs during the meiotic division of the zygote as a result of genetic crossovers in which part of the chromatids (and the genes they carry) of one chromosome of a pair are exchanged with the part of the chromatids of other chromosome of the pair. Recombination of the genes of two parental nuclei takes place in the zygote, and the eventual haploid nuclei or gametes resulting after meiosis are different both from the gametes that produced the zygote and from one another (Agrios, 2005). Recombination can also occur during the mitotic cell division in the course of growth of an individual and it is thought to account for a significant amount of genetic exchange in fungi (Nelson *et al.,* 1955).

2.5 Epidemics

Stem rust needs green plant tissue to be able to infect. It is favored by hot humid days of 25 to 35°C mild nights of 15 to 20°C and frequent dews (Kurt *et al.,* 2005). Both aeciospores and urediniospores require free water for germination as do the other spore stages (Roelfs *et al.,* 1992). The urediniospores, the asexual infective spores for cereal and grass hosts, are spread over long distances by wind and deposited by rain (USDA, 2008). They can also be moved as contaminants on human clothing (Singh *et al.,* 2006). The level of disease injury/ epidemic in a given area and season is a function of the crop and the environment. The crop affects the level of epidemic through its susceptibility or resistance to the pathogen which determines the amount of inoculum. The environment affects disease development through levels of moisture, temperatures and prevailing winds. As a result of the diverse cropping seasons and continuous availability of inoculum in Kenya, the pathogen is available throughout to cause infection (Harder *et al.*, 1972).

2.5.1 Infection process

Urediniospores are produced in large numbers and can be blown to considerable distances by wind (Kurts *et al.,* 2005). However, most urediniospores are deposited close to their source under the influence of gravity. Urediniospores are relatively long lived and can survive in the field away from the host for periods of several weeks. They can also withstand freezing if the moisture content is lowered to 20% to 30% viability rapidly decreases at moisture content of more than 50% (Roelfs *et al.,* 1992). Infection of cereal or grass plants by germinating urediniospores follows an intricate series of stages and structures, which apparently are essential for the establishment of a successful parasitic relationship of the stem rust fungus with its host (Staples and Macko, 1984; Wiethölter *et al.*, 2003b). Urediniospore germination occurs if the spore is in contact with water on the stem or leaf surface within 6 to 8 hours. The germinating urediniospore 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 (Heath, 1997). Proper orientation, which requires that the germ tube be appressed to the waxy cuticle on the epidermis, maximizes the chances that the germ tube will encounter a stoma through which penetration of the host may occur (Roelfs *et al.,* 1992). At the stoma the germ tube stops elongating and forms an appressorium over the stomatal opening (kurt *et al.,* 2005). The two nuclei, which migrated from the urediniospore into the tip of the germ tube, enter the appressorium and undergo mitosis after they are cut off from the now empty germ tube by a septum (Mendgen, 1981). Germination and appressorium formation usually occur at night when dew is present on the plant

surface. Development in *P. graminis* typically stops after formation of the appressorium until dawn when fungal growth resumes (Agrios, 2005). This resumption of 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 *et al.*, 2005).

Under conducive 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 (Yirgou and Caldwell, 1968). There it produces an elongate sub-stomatal vesicle in which a second round of synchronous mitoses occurs. From each end of the vesicle an infection hypha may be produced, and a pair of nuclei migrates into the developing infection hypha. When the tip of the infection hypha contacts a host cell, it differentiates a haustorial mother cell that is separated from the hypha by a septum. At this stage, the haustorial mother cell typically contains 2–4 nuclei. The haustorial mother cell produces a narrow peg that penetrates the host cell wall, apparently by enzymatic dissolution as well as pressure. The haustorial mother cell contains a specialized cell wall that includes two additional layers not found in the intercellular hyhae (Chong *et al.*, 1985). Upon penetrating the plant cell wall, a specialized fungal hypha expands to form an enlarged haustorium in the periplasmic space of the host cell. While the haustorium is forming, the infection hypha may produce a new branch just proximal to the first haustorial mother cell, resume growth and form a new haustorial mother cell and haustorium when it contacts another host cell (Jager and Reisener, 1969). The process may be repeated to produce a third haustorium in yet another host cell, but by this time the nutrient reserves of the original urediniospore have been exhausted and further development depends upon the success of the haustoria in extracting nutrients from the host without inducing a resistant response (Staples and Macko, 1984; Wiethölter *et al.*, 2003a). 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 (Mendgen, 1981).

2.6 Disease symptoms

Infections in cereals or grasses occur mainly on stems and leaf sheaths and occasionally on the leaf blades and glumes. Plants do not usually show obvious disease symptoms until 7 to 15 days after infection when the oval pustules (uredinia) of powdery, brick-red urediniospores break through the

epidermis (Agrios, 2005). 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 millimetres long and a few millimetres wide is formed by rupture of the host epidermis from pressure of a mass of brick-red urediniospores produced in the infection. These uredinial pustules are generally linear or diamond shaped and may enlarge up to 10 mm long (Kurt *et al.,* 2005). The powdery masses of urediniospores appear similar to rust spots on a weathered iron surface (Roelfs *et al.*, 1992). With age, the infection ceases production of brick-red urediniospores and produces a layer of black teliospores in their place, causing the stems of heavily infected plants to appear blackened late in the season (Kurt *et al.,* 2005).



Plate 1: Stem Rust Symptoms on Stem and Leaf

2.6.1 Rating of seedling infection

Infection types for uredinia on seedling leaves are described by Stakman *et al.* (1962) giving a scale ranging from 0-4. This gives infection types as 0 1, 2, 3 and 4 with extra class designated as X, for heterogenous or mesothetic infection. Infection types 0, 0; 1, 2 and X are rated as resistance while 3 and 4 are rated as susceptible.

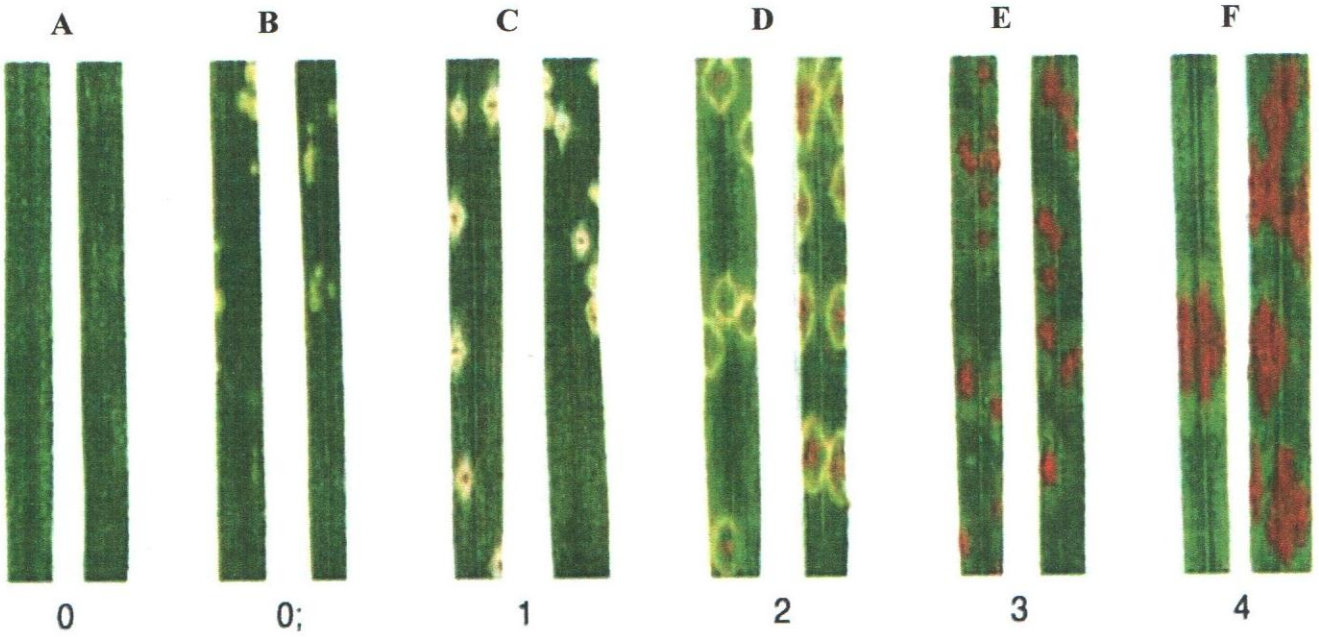


Plate 2: Infection types (ITs) of *Puccinia graminis* f. sp. *tritici*. at a 0 - 4 scale. A) IT=0 (no uredia); B) IT=0; (fleck); C) IT=1 (small uredia); D) IT=2 (small to medium uredia); E) IT=3 (medium uredia without chlorosis or necrosis) F) IT=4 (large uredia without chlorosis or necrosis).

2.6.2 Relation between disease symptoms and amount of the pathogen

i. Inoculum density

Inoculum density is the total available pathogen that can cause infection in a given specified area; this may obscure real differences in quantitative resistance (Agrios, 2005). In order to prevent escape of genotypes from infection, there is a tendency to apply high inoculum densities. Complete resistance in such cases is easily detectable, but small differences in susceptibility tend to disappear. The optimal inoculum density is the density whereby escapes are largely prevented while only the most susceptible cultivars are strongly affected (Roelfs *et al.*, 1992).

ii. Earliness

Where hosts differ in earliness the periods of exposure to the pathogen also vary (Singh *et al.,* 1998). Resistance to head blight caused by *Fusarium* in wheat is considerably overestimated in late cultivars due to this aspect (Parlevliet, 1993).

iii. Plant habitat

The environment in which the plant is will determine the increase in the pathogen density (Roelfs *et al.*, 1992). In dense crops and short plants the amount of tissue affected tends to increase. In loose crops and tall plants it tends to decrease. This is probably due to micro-climatic effects. Short wheat cultivars are more affected than tall cultivars by *Septoria* leaf and glume blotch (Agrios, 2005).

2.7 Crop losses due to stem rust of barley

Stem rust typically causes losses of 10%–50 %, but this could increase to up to 90% when it occurs in early spring (Ciara *et al.,* 2006). In the mid of 20th century, yield losses reached 20% - 30% in eastern and central Europe (Zadoks, 1963) and many other countries, including Australia, China and India (Rees, 1972; Joshi and Palmer, 1973; Roelfs, 1977). In the United States, from 1920 to 1960, stem rust epidemics in Minnesota, North Dakota and South Dakota caused average yield losses of over 20% and up to 50% in 1935 (Leonard, 2001b). In 1994 in Ethiopia wheat stem rust reduced the yield of Enkoy; a wheat variety which was widely grown in the highlands, by 60 to 100% while in Kenya yield losses up to 80% were recorded (KARI, 2005b). A crop that appears healthy 3 weeks before harvest can be devastated by explosive buildup of stem rust if sufficient inoculum arrives from a heavily infected crop in some distant region (Kurts *et al.,* 2005). Severe infection of stems interrupts nutrient flow to the developing heads, resulting in shriveled grain. In addition, stems weakened by rust infection are prone to lodging and further loss of grain (Roelfs *et al.*, 1992; Agrios, 2005).

Stem rust causes cereal yield losses in several ways. The fungus absorbs nutrients from the plant tissues that would be used for grain development in a healthy plant (Williams and Allan, 1984). As pustules break through the epidermal tissue, it becomes difficult for the plant to control transpiration, so its metabolism becomes less efficient (Agrios, 2005). Desiccation or infection by other fungi and bacteria also can occur. Interference with the vascular tissues results in shriveled grains. Stem rust also can weaken wheat stems, so plants lodge, or fall over, in heavy winds and rain. Where severe lodging occurs, crops cannot be mechanically harvested. In extreme cases, infected plants may die (Kurts *et*

al., 2005). Infected plants may also show increased water loss because they transpire more due to the loss of water through ruptured tissues (Agrios, 2005). Photosynthetic area is also reduced leading to reduced photosynthesis. Seed filling is affected through induction of early maturity; affecting seed size, quality and number (Agrios, 2005).

2.8 Disease management

2.8.1 Cultural practice

No single practice is effective under all conditions, but using a series of cultural practices greatly enhances the existing resistance (Roelfs *et al.*, 1992). Since moisture on leaves and excessive foliar nitrogen favor rust fungi infections appropriate spacing, row orientation, and fertilizer schedules should therefore be used (Agrios, 2005). In some areas, summer wheat crops are irrigated, which may increase the survival of infected volunteer plants Use of earlier-maturing barley varieties in the central Great Plains of the U.S. has helped reduce the threat of stem rust epidemics by limiting the length of time for stem rust epidemics to develop and the numbers of urediniospores that can contribute to epidemics (Roelfs *et al.*, 1992). Zadoks and Bouwman (1985) emphasized the importance of the green bridge in carrying the disease from one crop to the other. This can be lengthened by some farmers planting early and others late. Removing the green bridge by tillage or herbicides is an effective control measure for epidemics that would result from dangerous inoculum. In some places volunteer plants must be controlled several times during the season when the wheat is grown (Roelfs *et al.*, 1992).

Alternative host such as Barberry are an important early source of inoculum. A single barberry plant can produce as many as 64 billion aeciospores (Agrios, 2005). Removal of alternate host eliminates the sexual cycle, leaving only asexual urediniospores to maintain the fungus. This reduces the genetic variation in the fungal population and hence the number of races of stem rust against which breeders must seek resistance (Roelfs *et al.*, 1992). Finally, epidemics are delayed by several weeks in many of the major barley and wheat producing areas of the U.S. and Canada because aeciospores were released before the first arrival of urediniospores from the south . Barberry eradication increases the durability of resistance genes, can delay disease onset, initial disease severities and may reduce need for chemical or cultural measures. Barberry eradication is often not economically feasible and not our problem in the tropics (Roelfs *et al.*, 1992). Cultural practice can reduce environmental pollution,

enhance effectiveness of chemicals when used, enhance effectiveness of resistance, and delay disease onset and thereby severity (Roelfs *et al.,* 1992).

2.8.3 Chemical control

Where barley stem rust occurs in susceptible varieties, economic control can be achieved with fungicide applied at early disease onset (Wanyera *et al.*, 2009). Fungicides that inhibit the synthesis of sterols [Sterol Biosynthesis Inhibitors (SBIs) or Demethylation Inhibitors (DMIs)] are effective, but the cost of application is prohibitive. In the developing world, treatment with fungicide when epidemics occur would not be feasible for many farmers and countries given the constraints to mounting well-coordinated mobilization campaigns (Marasas *et al.,* 2004). Since most farmers are not able to identify the rust, it is recommended to apply two sprays, at 60 days and 75-80 days respectively, after planting (Wanyera *et al.*, 2009). Thereafter, the farmers can start scouting for the disease on the stems on a weekly basis. A third spray will be required if the disease is observed on the crop. Farmers in Kenya can effectively reduce/suppress stem rust by the application of foliar fungicides and still benefit (Wanyera, 2008). Continual use of fungicides however, can lead to development of resistance (Roelfs *et a.l.,* 1992).

2.8.4. Genetic resistance/ tolerance

Resistance is expressed as a reduction in the number of lesion, a reduction in the size of the sporulation area, an increase in the length of the latent period (Roelfs *et al.,* 1992). Some resistances function throughout the life span of the host, others only at certain growth stages and in certain tissues, and still others only under certain environmental conditions (Mclntosh, 1988). Genetic resistance is the most commonly used and the most effective means to control stem rust (Hiebert *et al.,* 2010). In wheat the adult plant resistance gene Sr2 result in an absence of uredinia pustules in the internode tissues, while Sr13 gene provides a reduction in pustule size (Roelfs *et al.*, 1992).

In barley, 7 stem rust resistance genes (rpg1–5, rpgBH, and rpg6) have been described (Sun and Steffenson, 2005). The rpg4 locus confers resistance to the *P. graminis* f. sp. *tritici* pathotype, Pgt-QCC and the Rpg5 locus confers resistance to the rye stem rust pathogen *Puccinia graminis* f. sp. *secalis,* isolate 92-MN-90. Rpg5 is unique among plant disease resistance genes in that it encodes the NBS-LRR and the protein kinase domains all in one gene (Kleinhofs *et al.*, 2009). The barley stem rust resistance gene Rpg1 confers resistance to many pathotypes of the stem rust fungus *Puccinia graminis* f. sp. *tritici* (Rostoksa *et al.,* 2004). Since the introduction of barley cultivars with this -gene in 1938, gene Rpg1 has provided resistance to stem rust (Steffenson, 1992). Race TTKSK is however

not only virulent to wheat cultivars with Sr31, but also barley cultivars with the widely used gene Rpg1 (Steffenson *et al.,* 2007). Resistance to race TTKSK identified in cultivated, Swiss landrace, and wild barley Q2861 is conferred by a single gene that co segregates with QCC and rye stem rust resistance in bin 13 of chromosome 7(5H). Research is underway to ascertain whether TTKSK resistance is conferred by Rpg5/Rpg4 or another nearby R gene candidate (Steffenson *et al.,* 2010; Steffenson *et al.,* 2007).

The currently used resistance genes can not however remain effective as new races of the fungus begin to appear. The fungus is capable of overcoming resistance genes, primarily through mutation (Mundt, 1991). Breeders use a combination of vertical resistance genes against specific races of *P. graminis* and horizontal resistance genes that slow the development of the epidemic by offering some resistance to all pathogen races (Mclntosh *et al.*, 2003). Generally resistance to infection in host plants: quantitative (representing the combined effect of many minor genes), or qualitative resistance, (controlled by major genes, single genes with large effects).

When qualitative host resistance exist, the associated pathogens generally have corresponding major genes that determine virulence, such systems are typically referred to as 'gene-for-gene' interactions (Flor, 1956). The central assumption is that each resistance (R) gene in the host interacts specifically with a corresponding avirulence (Av) gene in the parasite, with resistance being dominant to susceptibility and avirulence dominant to virulence (Flor, 1971). Plant race-specific disease resistance genes function by recognizing the avirulence gene product of a pathogen and, presumably, by activating signaling cascades leading to resistance (Hammond - Kosack and Jones, 1997; Hulbert *et al.*, 2001).

In barley leaf rust, *P. hordei* the quantitative resistance (QR) to stem rust pathogen is inherited polygenically (Parlevliet, 1978). Most cultivars, though, carry considerable levels of QR, preventing the barley leaf rust from becoming a major pathogen (Parlevliet, 1989). All cultivars selected for their major gene resistance carry moderate to fair levels of QR hidden behind that major gene (Hulbert *et al.,* 2001). McIntosh (1997) noted that the ideal sources of resistance are those present in closely related, commercial genotypes, and any effort to transfer resistance from related species and genera should be considered long term. Resistance is considered durable if it remains effective when used for many years over a substantial area (Johnson, 1981). Much of the resistance used by breeders has not broken down; multiple major gene resistance and all QR based on some to several genes appeared to

be durable (Parlevliet, 1997). The Rpg1 gene was durable, but did not last forever. And in the evolutionary sense, no resistance will last forever (Prabhu, 2008).

Resistance to pathogens with a wide host range (generalists) are usually of a quantitative nature and mostly durable (Parlevliet, 1993). But, there are exceptions, such as, the major resistance genes Mi in tomato and Rk in cow pea against the root knot nematode, *M. incognita* (Roberts, 1995). Monogenic resistance against specialists of a non-hypersensitive nature is often durable. The non-hypersensitive resistance genes Rpg2 (Sr-2*)* and Rpr34 (Lr-34) of wheat/barley to stem rust and leaf rust respectively and the mlo-gene of barley to powdery mildew have already lasted for a considerable time.

2.8.5 Crop resistance to diseases

i. Acquired resistance

Some susceptible plants become systemically resistant in response to localized infections, a phenomenon known as acquired resistance. Plants with acquired resistance have high levels of pathogenesis - related proteins (Scheffer, 1997). Induced resistance may be localized at the site of the inducing treatment or it may be systemic. The latter is commonly called SAR (Systemic acquired or activated resistance) and is effective in some, all or newly emerging plant parts distant from the site of induction (Schonbeck and Steiner, 1997).

ii. Resistance produced through transformation

Breeders have generally been transferring major resistance genes across the species barrier by crossing them with related species. Several *Triticum* species and species of at least six other genera from the family *Triticeae* have been used successfully as donors of resistance genes. Transformation of plants with a gene of a viral genome often gives rise to plants resistant to the virus from which the gene was derived; this is known as pathogen-derived resistance (Lomonossoff, 1995). This pathogen-derived resistance (PDR) varies greatly from protecting against very high levels to very low levels of inoculum.

2.9 Evaluation of barley genotypes for stem rust resistance

Improved grain yield, grain quality and resistances to various biotic and a biotic stresses are the most important breeding objectives in most crop improvement programs (Singh *et al.*, 1998). The agronomic performance of crop varieties and advanced lines could be evaluated by multi-environment

trial, genotype environment interaction analysis, yield with its component analysis, agronomic trait analysis, correlation together path analysis, additive main effects and multiplicative interaction (Ozgen, 1993; Truberg and Huhn, 2002; Wamatu and Thomas, 2002; Voltas *et al.*, 2005).

Effective genetic control of rust disease requires a coordinated effort, including race monitoring, collection and characterization of sources of resistance and resistance breeding (McIntosh *et al.*, 1995). This ensures continued genetic diversity within cultivar arrays to buffer the extent of loss when relevant pathogenic changes occur. In wheat seedlings are often used to screen sources of resistance and to study the genetics of resistance (Lui and Harder, 1996). This method is also applicable for barley. Adult plant resistance assessment and the timely detection of stem rust races with virulence are important to producers aiming at maximum yields (McIntosh *et al.*, 1995). The mode of inheritance of resistance to physiological races of stem rust appears to involve one or two major genes (Singh, 2004). Most of which are rapidly being broken down, putting multiple race-specific genes in a single genotype minimizes the probability that a single mutation in the pathogen can overcome all the resistance genes (Mundt, 1991; Huang *et al.*, 1997; McIntosh and Brown, 1997). A substantial effort on the identification and characterization of rust resistance genes occurred in the second half of the last century (McIntosh *et al.*, 2003).

A case in point is TTKSK (i.e. isolate Ug99), a new race of *P. g.* f. sp. *tritici*, which was first discovered in Uganda in 1999 (Pretorius *et al.*, 2000) and has now spread in east Africa (Wanyera *et al.*, 2006). Race TTKSK is virulent on many wheat and barley cultivars (Pretorius *et al.*, 2000; Jin and Singh, 2006; Singh *et al.*, 2006; Steffenson and Jin, 2006). Breeding for multigene resistance to Ug99 involves crossing disease-resistant lines with barley varieties adapted to local conditions in the world's barley-growing countries. Seed multiplication then follows to produce sufficient planting material for areas at risk from Ug99 (Anon, 2008).

Wild barley grows over a remarkably wide range of ecological habitats; hence it exhibits a high level of genetic diversity for both a biotic and biotic stresses (von Bothmer *et al.*, 2003). The long co-evolution of this subspecies with various pathogens in its natural range makes it one of the most important sources of disease resistance genes for cultivated barley (Fetch *et al.* 2003). In particular, wild barley is considered a rich source of resistance genes to leaf rust, stem rust, and stripe rust (Moseman *et al.*, 1990; Fetch *et al.*, 2003). There is however a great concern about the lack of durability of disease resistance (Johnson, 1992). Several strategies can be adopted to prolong the durability of the stem rust resistance genes, such as gene pyramiding, diversification and application of cultivar mixtures (McDonald and Linde, 2002). Another possibility would be to introduce resistance

EGERTON UNIVERSITY LIBRARY

types that are intrinsically durable like partial resistance, which is characterized by a slow epidemic build-up despite a high infection type (IT) (non hypersensitive type of resistance) (Parlevliet and Van Ommeren, 1975).

2.9.1 Rust resistance genes

Genetic resistance to wheat stem rust (*Puccinia graminis* f.sp. *tritici*) has largely been based on two types of resistance; seedling and adult plant resistance (MacIntosh, 1988). Seedling resistance genes (which also work at the adult plant stage), also referred to as major genes usually confer strong resistance response but not to all rust races (Roelfs *et al.,* 1992). *Sr31* is a good example of adult plant resistance (APR) or post-seedling resistance often provide a partial resistance response and tend to be race non-specific (MacIntosh, 1997). Some of the APR genes have also been described as "slowrusting" due to the longer latency period from infection to the full development of rust epidemic or sporulation. Of the 46 catalogued stem rust resistance genes in wheat only one, *Sr2*, falls into the APR category. A few more APR genes have been described for resistance to wheat leaf and stripe rust caused by *Puccinia striiformis* and *Puccinia triticina.* Only a single plant stem rust resistance gene has been cloned to date, which is the protein kinase encoding barley *Rpg1* gene (Steffenson and Jin, 2006). A few seedling leaf rust resistance genes have been cloned in wheat and other cereals and are predominantly from the nucleotide binding site-leucine rich repeat (NB-LRR) class of major resistance (R) genes extensively studied in the flax rust system(Ellis *et al.*, 2007; Ayliffe *et al.,* 2008). The nature of Adult plant resistance (APR) genes is quite different from the seedling resistance proteins and therefore augers well for exploiting different mechanisms within the innate immune system of wheat in efforts at developing more durable rust resistance (Prabhu, 2008). While a number of seedling resistance genes effective against Ug99 stem rust have been identified, the search for other additional APR genes to complement Sr2, are going to be pivotal in strategies for ensuring durable stem rust resistance (Singh *et al.,* 2006)

CHAPTER THREE

MATERIALS AND METHODS

3.1 General methods

3.1.1 Site description

The experiment was carried out at the Kenya Agricultural Research Institute (KARI) –Njoro situated along the Njoro- Mau Narok road in Nakuru County, located at 0^0 20'S 35^0 56'E and at 2185 meters above sea level. It receives an average rainfall of 939 mm per annum, with a mean temperature range of 9.5^0C to 24^0C and falls within the agro-ecological zone III (Jaetzold and Schmidts, 1983). The soils are predominantly *mollic Andosols.*

3.1.2 Barley germplasm

Twenty barley germplasm that included three Kenyan commercial cultivars (Nguzo, Sabini and Karne), two (1512-5 and 1385-13) new introductions from the Kenya Malting company and 15 introductions from ICARDA were used (Table 3).

3.1.3 Stem rust isolate preparation

The spores used for inoculation were collected from experimental plots in KARI-Njoro. The stem rust spore (urediniospore) samples consisted of bulk isolate collected using a portable powered suction pump (Hoover Model, 2944B) ® from wheat cultivar Chozi. The inoculum was pre-tested on differential hosts in the greenhouse according to Roelfs *et al.* (1989) and confirmed to be those of PgtUg99. To generate large quantities of urediniospores, the isolate was inoculated onto seedlings of the susceptible wheat cultivar chozi having gene Sr31. The Urediniospores were suspended in distilled water where light mineral of oil- (tween 20 solution) was added and the suspension sprayed onto the plants using a small bowler- atomizer (11422) ®. The plants were then kept in a dark humidity chamber overnight before taking them to clear plastic chambers in the greenhouse to prevent contamination resulting from the movement of urediniospores. Urediniospores were harvested from sporulating pustules at 14 and 21 days after inoculation using a cyclone spore collector (Groove 568) ®. The spores were then dried for 2 to 3 days over silica gel in the laboratory.

Table 3: Type and source of barley genotypes used in the experiment

Serial No	Accession	Source
1.	CBSS99MOO39IT-H-IM-IY-IM-IY-OM	ICARDA
2.	CBSS99MOO317T-AH-2M-IY-IM-IY-OM	ICARDA
3.	CBSS00YOO113T-A-OY-OM-2Y-OM	ICARDA
4.	CBSS99MOO429T-L-IM-IY-OM	ICARDA
5.	CBSW99WMOOO95T-B-IM-IY-IM-OM	ICARDA
6.	CBSS00YOOO48S-OY-OM-2Y-OM	ICARDA
7.	CBSSOOYOO475T-O-OY-OM-2Y-OM	ICARDA
8.	CBSSOOYOO236T-E-0Y-OM-2Y-OM	ICARDA
9.	CBSS99MOO349T-F-3M-IY-IM-OM	ICARDA
10.	CBSS99MOO468T-H-IM-IY-OM	ICARDA
11.	CBSSOOYOO479T-D-OY-OM-IM-OM	ICARDA
12.	CBSW98WOOO54S-BY-2M-IY-2M-IY-OM	ICARDA
13.	CBSS99MOO315T-F-IM-F-IM-IY-IM-IY-OM	ICARDA
14.	CBSSOOYOO225T-C-OY-OM-2Y-IM-OM	ICARDA
15.	CBSSOOYOO278D-G-OY-OM-2Y-OM	ICARDA
16.	1385-13	KENYA
17.	NGUZO	KENYA
18.	1512-5	KENYA
19.	KARNE	KENYA
20.	SABINI	KENYA

The table above will be used throughout the thesis where the serial number will be used to represent the accession that will **NOT** be repeated. It will be represented as genotype serial number.

3.2 EXPERIMENT I: RESPONSE OF BARLEY GERMPLASM TO STEM RUST UNDER GREENHOUSE CONDITIONS

3.2.1 The infection type at seedling stage

The greenhouse experiment was arranged in a completely randomized design (CRD) with three replicates done by sowing the 20 barley germplasm in 15 t diameter plastic pots filled with forest soil that had been autoclaved at 121^{0}C and 15 pounds pressure for one hour (Welty *et al.,* 1992). The urediniospore suspension (1mg of urediniospore per 100ml of distilled water) was mixed with 4 drops of tween 20, surfactant using a small bowler- atomizer and inoculated on the seedlings. The inoculation involved uniform spraying of the upper and lower leaf surfaces of individual plants 10 days after germination. Immediately after inoculation the seedlings were incubated for 24 hours in a dark dew chamber kept moist by frequently spraying with water to maintain humidity of 80-100%, and temperatures between 16^{0}C and 22^{0}C. The seedlings were transferred to the greenhouse after incubation. For the first two hours, the leaves were sprayed with water for 15 minutes at an interval of 30 minutes using an atomizer. Temperature of 22-30^{0}C was there after maintained in the green house, following the procedure by McIntosh *et al.*, (1995). Fourteen days after inoculation the seedlings were assessed for disease severity according to Stakman *et al.* (1962) scale for stem rust (Table 4).

Table 4: Infection codes used in classifying the reaction to stem rust on seedling barley leaves

Host response	Infection type	Disease symptoms
Immune	0	No sign of infection to the naked eye but minute flacks may be visible under low magnification
Very resistant	;	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
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

(Adapted from Stakman et al., 1962)

3.2.2 The reaction type to stem rust at adult plant stage

Twenty barley germplasm were sown in 20cm diameter plastic plots filled with sterile forest soil. Diammonium Phosphate (18%N and 46% P_2O_5) at the rate of 50Kg/ha that translates to125mg per pot was thoroughly mixed with the soil where 10 seeds of each cultivar were sown in one pot. Two weeks after emergence plants were thinned to eight. The pots were placed on trays with gravel and watering done every other day for 30 minutes. At flag leaf stage plants were inoculated with urediniospores suspension (1mg urediniospores/ 100 ml of distilled water plus 5 drops of tween 20 surfactant) using a syringe and a hypodermic needle. The urediniospore suspension was injected into elongating stems, where each plant received 0.5ml of the suspension (Welty *et al.*, 1992), and then in a dark dew chamber over night. The plants were then moved to the greenhouse and placed on a bench where a spray of water was applied on the leaves every 15 minutes for 2 hours to maintain temperature between 22-30°C.

The experiment was laid out as a completely randomized design (CRD) with three replicates. The disease reaction was rated based on the size of the pustules and associated necrosis or chlorosis 14 days after inoculation and weekly thereafter as described by Roelfs *et al.* (1992).

Table 5: Host infection response used in classifying stem rust reaction at adult stage

Host response	Infection response	Description of symptom
Resistant	R	Small uredia surrounded by necrosis
Moderately resistant	MR	Small to mediam uredinia surrounded by chlorosis or necrosis
Moderately susceptible	MS	Medium sized uredia, usually surrounded by a light green chlorosis
Susceptible	S	Large uredia with limited amount of chlorosis; may be diamond shaped

(Adapted from Roelfs et al., 1992)

3.3 EXPERIMENT II: PERFORMANCE OF THE DIFFERENT BARLEY ACCESSION IN A STEM RUST PRESSURE IN THE FIELD

Twenty barley cultivars were screened for their level of resistance to stem rust disease in the field. The experiments were planted for two seasons at KARI Njoro during the 2008/09 crop seasons. Each cultivar was sown in plots of 3m length at spacing of 20 cm, at seed rate of 125 kg/ha with six rows per plot. The same set was sown as control under fungicides application of tebuconazole (folicur) at 0.75l/ha, 3 times in the season starting at stem elongation stage (Zadoks *et al.,* 1974) at an interval of 21 days. The experimental design was randomized complete block design (RCBD) in split plot arrangement with the main plots as the 20 barley cultivars while the subplots consisted of fungicide (protected and unprotected), replicated three times. Susceptible wheat cultivars, chozi was planted perpendicular to test plots in the middle of the 1-m pathways on both sides of experimental plots to serve as spreader. The furrows were cut by machine and planting done by hand. A basal dose of DAP fertilizer (18%N and 46% P_2O_5) at a rate of 50Kg/ha was applied at planting and followed with a top dress of CAN (23%N) at 25Kg/ha a month later. Weeds were controlled using Stomp 500E, a pre-emergency herbicide at a rate of 3.0L/ha. Buctril MC (bromoxyil and iso-octyl esters) a post emergent herbicide was applied at the rate of 1.25L/ha to control broad-leaved weeds (KARI, 2005a). Metasystox insecticide at the rate of 0.75 L/ha was used to control Russian wheat aphid (RWA) and other insect pests. The spreader rows were inoculated with *P. g. tritici* pathotype TTKSK at the early jointing stage by injecting an aqueous suspension of urediniospores into the hollow culm of plants at 0.75-m intervals along each spreader. The infection type responses as described by Roelfs *et al.* (1992), McIntosh *et al.* (1995) and disease severities 0-100%, Peterson *et al.* (1948) were recorded in both trials starting from when 50% of the plants reached heading stage at an interval of 14 days until hardening stage.

3.3.1 Barley quality analysis

Germination Percentage was determined as described by ASBC Barley 3C, IOB, and EBC procedure. An Infratec 1241 Grain Analyzer was used to determine protein, starch, zeleny and glutan.

3.4 Parameter measured

In the field infection type responses as described by Roelfs *et al.* (1992); McIntosh *et al.* (1995) and disease severities 0-100%, Peterson *et al.* (1948) were recorded in both trials starting from heading stage at an interval of 14 days until hardening stage. Final disease severity (FDS) score was taken at hardening. The disease was assessed as the incidence of plant infected with stem rust in a cultivar, and

the percent of the disease severity (modified cobb scale) was used to calculate the area under disease progress curve (AUDPC) of each cultivar as described by Shaner and Finney (1977) and Mwandemele (1993).

Chlorophyll content was taken after every two weeks on the flag leaf of three plants in the plot selected at random using a chlorophyll meter and the average was calculated. The tillering capacity was determined by counting the number of spikes per meter squared. Harvesting was done after physiological maturity to ensure all the spikes were sufficiently dry for harvesting. A half meter square quadrant was thrown in the middle of each plot and a fifty tillers harvested by cutting them at the ground level using a sickle and put into a labeled paper bag for subsequent drying in an oven at 65^0C (48h) until constant weight and weighted at 13% moisture content. The spikes from each plot were fed into a portable thresher and winnowed. The 1000 kernel weight was determined after counting the grain with an electric seed counter (Model: PFEUFFER GmbH FlugplatzstraBe70D-97318 Kitzingen). The yield of the whole plot was determined by harvesting the whole plot and threshing them. The grain yield loss for each cultivar was calculated by subtracting the yield of untreated plot from the treated and then divided by the yield of treated plot multiplied by 100. The grain shriveling was determined by sampling grains from each plot and screening them through 2.5mm sieve.

3.5 Data analysis

Resistance was deduced from the final disease severity (FDS), from the area Under Disease Progress Curve (AUDPC), infection response determined during the adult plant growth stage and from the infection types (ITs) and reaction evaluated at seedling growth stage in the greenhouse. Data from the field was analysed using SAS (SAS Institute, 1999). Mean comparisons were made using DMRT.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Disease severity under varying conditions

4.1.1 Infection levels of stem rust in the greenhouse

The genotypes showed varying levels of resistance to stem rust (Table 6). At seedling stage, the infection levels ranged from 0 to 2, except in 9 and 12 which showed infection types 3 and 3,4 respectively. In adult plants reaction 60% of the population were susceptible, 20% moderately susceptible, 10% moderately susceptible to moderately resistant and 10% moderately resistant. Genotypes 1 and 14 were moderately resistant (MR) while, 17 was resistant to moderately resistant (R -MR). 19 was however moderately resistant to moderately susceptible reaction (MR - MS).

Table 6: Seedling infection type and adult plant reaction of barley to PgtUg99 in the greenhouse

Genotypes serial No.	**Seedling infection type**[a]	**Adult plant reaction**[b]
1.	;	MR
2.	0	S
3.	0;	S
4.	0	S-MS
5.	1	S
6.	1	MS
7.	1	MS-S
8.	1	S
9.	3	S
10.	0	S-MS
11.	; 1	S
12.	4, 3	S
13.	; 0	S
14.	2	MR
15.	2	S
16.	1	S
17.	0	R-MR
18.	0, 2	S
19.	2	MS-MR
20.	1	S

[a] *Seedling infection type as described by Stakman et al. (1962).* [b] *Adult plant reaction based on modified cobb scale (Roelfs et al.,1992).*

4.1.2 Stem rust severity under field conditions

Disease pressure was higher in season 1 compared to season 2 probably because of differences in relative humidity and temperature. Among the 20 genotypes that were evaluated for adult plant resistance, 1, 17 and 19 were moderately resistant while the rest were susceptible or moderately susceptible (Table 7). Genotype 18 showed the highest severity of (93%) in season 1, while 20 had the highest severity of 30% in the second season. 17 had the lowest disease severity of 16% and 5% in season 1 and 2 respectively. Jin *et al.* (1994) similarly noted an interaction between host genotypes and rust pathotypes which they attributed to variation in environmental temperature. Calpouzos *et al.* (1976); Dill – Macky *et al.* (1990) noted that terminal disease severities were a reliable indicator of resistance to stem rust in wheat and barley. Barley germplasm that showed the highest final disease percent were considered very susceptible.

4.1.3 Stem rust development and expression of resistance in the field

Fast rusting genotypes are generally associated with high disease progress and final disease rating. The mean final disease severity was higher in season 1 compared to season 2 (Table 7). This may be attributed to the higher relative humidity and cooler temperatures in season 1. The area under disease progress curve (AUDPC) varied with season and genotypes. The highest final disease was noted in 20, 18, 16 and 11. Genotype 11 had the highest AUDPC and final disease severity (FDS) in season 1. The lowest AUDPC and final disease severity were recorded in 1 in season 2. The variation in disease progress may be attributed to genotype and phenotype differences of plant types.

Similarly, Lal *et al.* (2004) found a positive relationship in values for final diseases and AUDPC in slow rusting and susceptible cultivar among barley genotypes. The slow rusting ability is due to the effect of a combination of genes working together against the disease. According to Kleinhofs *et al.* (2009) the rpg4 and rpg5 genes confer resistance to several wheat stem rust races, including TTKSK also called Ug99 that is threatening the world wheat and barley. Steffenson *et al.* (2010) confirmed that lines with gene combinations of rpg1/rpg3 and rpg1/rpg4 provided a highest level of protection to stem rust epidemic than those with rpg1/rpgU combination.

Table 7: The final disease severity (%) and area under disease progress curve of barley genotypes

Genotypes serial No.	% FDS [X]		AUDPC[Y]		
	Season1	**Season2**	**Season1**	**Season2**	**Season Difference**
1.	23*MR**	7MR	800.3 ± 171..36^{fgh}	51.7 ± 25.85 ^{d}	**
2.	28S	6S	825.3 ± 247.08 ^{fgh}	162.7 ± 89.25^{bcd}	**
3.	56S	18S	1828.3 ± 134.89^{cde}	479.0 ± 210.32^{abc}	**
4.	46S-MS	13MS	1713.7 ± 337.20^{cde}	323.3 ± 119.66^{abcd}	**
5.	41S	13S	1732.0 ± 549.10^{cde}	280.0 ± 67.69^{abcd}	**
6.	23MS	9MS	608.3 ± 143.73^{h}	179.7 ± 105.28^{bcd}	**
7.	40MS-S	16MS	1573.3 ± 120.58^{cdef}	325.0 ± 105.01^{abcd}	**
8.	45S	16MS	1676.0 ± 145.10^{cde}	401.67 ± 100.29^{abcd}	**
9.	53S	15S	1696.0 ± 82.97^{cde}	410.0 ± 212.20^{abcd}	**
10.	46MS	15MS	1541.0 ± 295.10^{def}	310.0 ± 101.15^{abcd}	**
11.	73S	13S	2358.3 ± 106.27^{abc}	400.3 ± 152.43^{abcd}	**
12.	50S	15S	1494.3 ± 286.01^{defg}	360.3 ± 49.21^{abcd}	**
13.	63S	21S	2186.0 ± 70.71^{bcd}	559.3 ± 126.54^{ab}	**
14.	20MS	6MS	654.7 ± 157.31^{h}	128.0 ± 75.97^{cd}	**
15.	36S	16S	1551.7 ± 12.26^{def}	436.7 ± 92.90^{abcd}	**
16.	86S	16S	2992.7 ± 54.36^{a}	345.0 ± 139.54^{abcd}	**
17.	16R-MR	5MR	755.3 ± 220.72^{gh}	84.0 ± 77.09^{cd}	**
18.	93S	21S	2756.7 ± 206.82^{ab}	559.3 ± 130.18^{ab}	**
19.	46R-MR	13MR	1174.7 ± 417.31^{efgh}	186.0 ± 86.02^{bcd}	**
20.	63S	30S	2067.7 ± 538.30^{cd}	616.0 ± 105.55^{a}	**

*[X] Final disease severity (FDS %) last rating following the modified Cobb scale (Peterson et al., 1948). [Y] Area under disease progress curve (AUDPC).Any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$)*

*** or NS** *means the genotype was significantly and not significantly different between season*

4.2 Effect of stem rust Ug99 on growth and development of barley

4.2.1 Dry matter

A mean dry matter loss of 20.7 and 23.0 % attributed to stem rust was observed in season 1 and 2 respectively. The highest dry matter loss of 41.1% and 43.1 was observed in 7 and 9 in season 1 and 2 respectively. Genotype 5 had the lowest (9.0%) in season 2 (Table 8). Harder and Legge (2000) similarly found dry matter reduction of up to 25.5 % under natural infection of stem rust. In addition to the effect on dry matter, the rust pathogen also affects the translocation patterns of the photosynthates, which are preferentially directed toward the infected zones where they are used up by the rust pathogen at the expense of the host growth and development (Bushnell and Roelfs, 1984). Durbin (1967) reported that the rusted host had higher respiration rates and depressed photosynthesis that led to reduction in growth and accumulation of dry matter.

Table 8: Effect of stem rust infection on Dry matter (Kgs) of barley genotypes

	Season1	**Season 2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	25.6 ± 13.35^{abc}	26.9 ± 11.91^{abc}	**NS**
2.	22.3 ± 1.03^{abc}	16.3 ± 2.42^{abc}	**
3.	14.7 ± 10.83^{bc}	16.7 ± 5.09^{abc}	**NS**
4.	26.7 ± 13.50^{abc}	25.6 ± 15.18^{abc}	**NS**
5.	17.7 ± 3.77^{bc}	9.0 ± 4.13^{c}	**
6.	31.5 ± 8.04^{bc}	34.5 ± 5.12^{abc}	**NS**
7.	41.1 ± 9.06^{a}	41.3 ± 10.21^{3a}	**NS**
8.	18.7 ± 5.82^{abc}	13.0 ± 4.21^{bc}	**
9.	16.7 ± 9.55^{bc}	43.1 ± 18.90^{a}	**
10.	24.8 ± 3.88^{abc}	32.3 ± 7.05^{abc}	**
11.	9.2 ± 6.65^{c}	10.3 ± 6.62^{bc}	**
12.	21.7 ± 10.39^{bc}	22.4 ± 11.90^{abc}	**NS**
13.	34.8 ± 3.21^{ab}	38.0 ± 2.97^{ab}	**
14.	23.4 ± 4.02^{abc}	$20.9 \pm 6.03a^{bc}$	**NS**
15.	11.4 ± 5.94^{bc}	10.9 ± 8.25^{bc}	**NS**
16.	27.3 ± 15.10^{abc}	27.9 ± 15.91^{abc}	**NS**
17.	8.3 ± 2.40^{c}	25.5 ± 15.33^{abc}	**NS**
18.	10.8 ± 4.33^{bc}	7.8 ± 2.19^{c}	**NS**
19.	18.4 ± 6.84^{abc}	20.9 ± 7.98^{abc}	**
20.	28.1 ± 3.34^{abc}	33.2 ± 14.82^{abc}	**NS**

*Any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

4.2.2 Leaf area

The leaf area was higher in treated plots than in untreated plots in both seasons (Table 9). In season 1 the highest and lowest loss of 67.3% and 17.8% was recorded on 20 and 16 respectively. In season two 8 and 14 had the highest (35.1%) and lowest (8.9%) amount of losses respectively. The reduction in the photosynthetic leaf area was attributed to flaking (Bushnell and Roelfs, 1984). Extensive pustule cover on the stems and leaves, compiled with reduced leaf surface area hence light interception and photosynthesis, could have affected plant performance.

Table 9: Effect of stem rust Ug99 on leaf area (cm) of different barley genotypes

	Season 1	**Season 2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	42.0 ± 5.53^{abc}	30.5 ± 4.32^{a}	**
2.	42.3 ± 8.33^{abc}	20.8 ± 6.96^{ab}	**
3.	44.6 ± 15.55^{abc}	10.9 ± 3.66^{ab}	**
4.	46.7 ± 20.17^{abc}	30.8 ± 16.73^{a}	**
5.	21.4 ± 10.20^{bc}	31.9 ± 2.48^{a}	NS
6.	31.4 ± 14.62^{bc}	16.1 ± 4.94^{ab}	NS
7.	34.6 ± 9.85^{bc}	19.8 ± 12.41^{ab}	**
8.	47.2 ± 8.08^{ab}	35.1 ± 10.55^{a}	**
9.	42.6 ± 5.91^{abc}	19.1 ± 7.12^{ab}	**
10.	26.5 ± 8.01^{bc}	18.7 ± 6.86^{ab}	**
11.	31.1 ± 4.80^{bc}	9.8 ± 2.85^{c}	**
12.	29.4 ± 12.36^{bc}	23.3 ± 5.45^{ab}	NS
13.	40.6 ± 11.88^{abc}	25.1 ± 6.51^{ab}	**
14.	24.9 ± 11.01^{bc}	8.9 ± 3.79^{c}	**
15.	38.2 ± 10.83^{abc}	19.5 ± 5.05^{ab}	**
16.	17.8 ± 1.02^{c}	27.1 ± 6.87^{ab}	NS
17.	24.0 ± 4.25^{bc}	28.0 ± 9.96^{ab}	NS
18.	32.9 ± 0.73^{bc}	11.6 ± 4.28^{ab}	**
19.	30.4 ± 15.13^{bc}	18.8 ± 10.49^{ab}	**
20.	67.3 ± 5.84^{a}	13.6 ± 8.24^{ab}	**

*Any two means in a column whose SE values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

4.2.3 Chlorophyll index

Irrespective of season, higher chlorophyll index was noted in treated plots than in untreated plots (Table 10). The highest chlorophyll losses of 56.2 in 17 and 54.9% in 18 were noted in season 1 and 2 respectively. Herrera- Foessel *et al.* (2006) attributed the reduction in chlorophyll content to the hypersensitive reaction on the leaves sheaths. Owere *et al.* (1981) found the loss of chlorophyll to be the principal limiting factor of photosynthesis in barley affected by leaf rust. Increase in ethylene and abscisic acid content of stem rust infected wheat could promote senescence and premature yellowing of healthy leaves (Gordon and Duniway, 1982). Tani *et al.* (1973) reported a reduction in amount of Ribosomal RNA in an oat infected with crown rust suggesting that synthesis of chloroplast protein may be generally depressed by rust infections.

Table 10: Effect of stem rust Ug99 on the chlorophyll index of barley genotypes

	Season1	**Season2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	18.2 ± 5.29^{cd}	22.4 ± 2.16^{ab}	**NS**
2.	37.7 ± 7.42^{abcd}	24.6 ± 16.16^{ab}	**NS**
3.	21.0 ± 9.13^{bcd}	13.3 ± 6.41^{b}	**
4.	20.8 ± 12.49^{bcd}	27.3 ± 10.46^{ab}	**
5.	29.4 ± 7.41^{abcd}	26.4 ± 11.76^{ab}	**NS**
6.	17.6 ± 7.46^{cd}	39.3 ± 16.17^{ab}	**
7.	33.0 ± 15.35^{abcd}	17.6 ± 9.66^{b}	**
8.	14.3 ± 4.96^{d}	24.9 ± 7.38^{ab}	**
9.	38.7 ± 7.23^{abcd}	24.9 ± 19.70^{ab}	**NS**
10.	20.2 ± 9.18^{bcd}	12.6 ± 5.72^{b}	**
11.	45.0 ± 9.01^{ab}	28.5 ± 5.91^{ab}	**
12.	24.0 ± 2.05^{bcd}	23.9 ± 17.30^{ab}	**NS**
13.	37.6 ± 14.49^{abcd}	1.7 ± 28.43^{b}	**
14.	26.4 ± 3.54^{bcd}	6.7 ± 3.22^{b}	**
15.	38.8 ± 14.32^{abcd}	31.0 ± 19.25^{ab}	**NS**
16.	17.0 ± 5.23^{cd}	37.6 ± 13.55^{ab}	**
17.	56.2 ± 8.24^{a}	43.2 ± 16.18^{ab}	**NS**
18.	29.6 ± 17.61^{abcd}	54.9 ± 26.51^{a}	**
19.	41.4 ± 4.27^{abc}	40.5 ± 14.05^{ab}	**NS**
20.	35.3 ± 13.06^{abcd}	48.6 ± 26.55^{ab}	**NS**

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$)*

*** or NS means the genotype was significantly and not significantly different between season*

4.2.4 Tillering capacity

The numbers of effective tillers in protected plots were higher than those in unprotected plots across the seasons (Table 11). Germplasm 14 and 13 had the highest (45.8%) and lowest (8.7%) reduction in number of effective tillers in season 1. In season 2, 16 had a loss of 24% and 15 with 2.7%. Plant processes are generally intimately related. Changes in photosynthate distribution induced by localized infections of rust fungi will have physiological and metabolic repercussion in other part of the plant and at later times in its development (Agrios, 2005). Bushnell and Rowell (1968) found that in severely rusted wheat shoot desiccation and death led to a drastic decline in the roots capacity to water absorption.

Table 11: Effect of stem rust Ug99 on the number of effective tillers (m^{-2}) of barley genotypes

	Season 1	**Season2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	15.4 ± 5.74^{bcd}	11.2 ± 4.71^{ab}	**
2.	19.4 ± 6.58^{bcd}	9.2 ± 6.77^{ab}	**
3.	26.6 ± 10.61^{abcd}	11.6 ± 3.90^{ab}	**
4.	22.7 ± 5.86^{abcd}	21.2 ± 6.14^{a}	**
5.	36.1 ± 15.61^{abc}	9.1 ± 3.34^{ab}	**
6.	39.9 ± 10.40^{ab}	22.8 ± 2.82^{a}	**
7.	20.4 ± 5.54^{bcd}	23.3 ± 11.56^{a}	**NS**
8.	30.9 ± 18.71^{abcd}	20.6 ± 11.42^{a}	**NS**
9.	27.2 ± 13.62^{abcd}	15.1 ± 6.30^{ab}	**NS**
10.	30.1 ± 9.57^{abcd}	10.0 ± 4.68^{ab}	**
11.	11.0 ± 8.79^{cd}	16.1 ± 5.88^{ab}	**NS**
12.	14.9 ± 6.08^{bcd}	12.9 ± 5.03^{ab}	**NS**
13.	8.7 ± 0.66^{d}	1.7 ± 5.65^{b}	**NS**
14.	45.8 ± 3.46^{a}	13.8 ± 6.58^{ab}	**
15.	39.4 ± 11.46^{abc}	2.7 ± 0.10^{b}	**
16.	12.0 ± 1.89^{cd}	24.0 ± 7.84^{a}	**
17.	15.7 ± 6.53^{bcd}	10.5 ± 5.05^{ab}	**
18.	22.9 ± 7.10^{abcd}	8.2 ± 3.17^{ab}	**
19.	30.5 ± 7.58^{abcd}	19.8 ± 6.25^{ab}	**
20.	41.9 ± 9.61^{ab}	18.6 ± 7.07^{ab}	**

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*
*** or NS means the genotype was significantly and not significantly different between season*

4.3.5 Plant height

Plant height is an important proxy of growth, variation of plant height is a function of genotype and season. In season 1, a significant loss in plant height in 4, 7, 8, 15 and 17 was noted. In season two, 7, 16 and 17 had the highest loss in height (Table 12). Harder and Legge (2000) however found no reduction in plant height caused by stem rust in barley. The difference could be as a result of factors like the stage of plant development at the time of infection, location of infection on the plant, and the environmental condition can modify the magnitude of the effect.

Table 12: Effect of stem rust Ug99 on plant height (cm) of barley genotypes

	Season1	**Season2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	7.8 ± 4.42^{abcd}	4.7 ± 3.59^{b}	**
2.	3.3 ± 1.38^{d}	1.1 ± 1.45^{b}	**
3.	9.7 ± 3.77^{abcd}	7.9 ± 4.57^{ab}	**
4.	12.6 ± 6.10^{abc}	1.2 ± 1.29^{b}	**
5.	8.1 ± 2.65^{abcd}	2.0 ± 1.88^{b}	**
6.	8.9 ± 1.47^{abcd}	1.9 ± 1.76^{b}	**
7.	13.7 ± 5.31^{a}	13.2 ± 3.51^{a}	NS
8.	13.3 ± 2.68^{ab}	0.2 ± 1.03^{b}	**
9.	4.6 ± 2.73^{bcd}	4.2 ± 4.19^{b}	NS
10.	3.5 ± 0.84^{cd}	5.0 ± 4.20^{b}	NS
11.	11.0 ± 0.97^{abcd}	1.9 ± 2.46^{b}	**
12.	9.1 ± 1.51^{abcd}	3.8 ± 1.28^{b}	**
13.	9.5 ± 1.92^{abcd}	6.0 ± 3.66^{ab}	**
14.	6.8 ± 4.69^{abcd}	1.0 ± 1.47^{b}	NS
15.	14.1 ± 4.68^{a}	1.0 ± 2.19^{b}	**
16.	5.2 ± 2.38^{abcd}	9.9 ± 4.15^{ab}	**
17.	12.6 ± 1.16^{abc}	4.4 ± 7.19^{b}	NS
18.	5.5 ± 1.50^{abcd}	1.0 ± 2.35^{b}	**
19.	10.3 ± 4.47^{abcd}	9.1 ± 4.26^{ab}	**
20.	6.8 ± 2.72^{abcd}	2.3 ± 1.72^{b}	**

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT*Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

4.3 Effect of stem rust Ug99 epidemics on yield and yield components of barley

4.3.1 Total grain yield

In untreated plots a high yield of 3.2t/ha was obtained in 1 and 20, while the highest loss of 32.1% was obtained in 16 a Kenyan line (Table 13). The new race Ug99 of stem rust caused severe loss on the susceptible genotypes. The yield loss in untreated plots was highest in 10, 13 and 20 across the seasons (Table 13). This means that when environmental conditions are ideal for proliferation and rapid progression of stem rust, reduction in crop yields would be expected. Dill-Macky *et al.* (1990) in Australia similarly reported a reduction in yield ranging from 20 – 60 % caused by stem rust.

Some genotypes showed resistance to Ug99 stem rust race at seedling and adult plant stage but still suffered yield loss in untreated conditions. Loughman *et al.* (2005) reported that stem rust can cause yield losses irrespective of the level of resistance. Generally plants respond to infection using energy – demanding physiological processes, such as defense reaction hence the yield declines (Smedegaard – Petersen and Tolstrup, 1985). The fungicides increase the yields of the treated cultivars through protection against the disease or and phytotonic effect. Ransom and McMullen. (2008) found that there was an increase in yield of hard winter wheat cultivars. This explains the increase in yield in protected plots across the season especially in the first season. Mayfield (1985) found a clear relationship between grain yield and disease severity and demonstrated that increasing stem rust severity by about 1% increased grain loss by 2%. Most of the barley germplasm did not show significant reduction in kernel weight. The grain yield loss in susceptible cultivars was attributed to interruption in movement of photosynthates, water and minerals caused by wheat stem rust (Agrios, 2005).

Table 13: Effect of stem rust on yield (t/ha) of barley in 2008 and 2009

Genotypes serial No.	Season1	Season2	Season Difference
	Mean %Loss	**Mean %Loss**	
1.	24.7 ± 2.51^{bcd}	13.9 ± 2.64^{abc}	**
2.	17.5 ± 1.55^{bcd}	7.2 ± 1.72^{c}	**
3.	27.9 ± 14.82^{abcd}	24.1 ± 13.57^{abc}	**
4.	17.1 ± 9.57^{bcd}	16.6 ± 2.23^{abc}	NS
5.	14.4 ± 2.62^{bcd}	8.2 ± 4.13^{bc}	**
6.	21.9 ± 8.23^{bcd}	20.9 ± 6.69^{abc}	NS
7.	19.0 ± 3.94^{bcd}	22.0 ± 6.32^{abc}	NS
8.	53.8 ± 17.50^{a}	8.1 ± 1.77^{bc}	**
9.	15.2 ± 8.64^{bcd}	14.3 ± 4.21^{abc}	NS
10.	22.6 ± 11.97^{bcd}	27.6 ± 5.89^{ab}	NS
11.	$24..2 \pm 5.64^{bcd}$	25.7 ± 11.43^{abc}	NS
12.	6.9 ± 1.98^{d}	14.6 ± 7.93^{abc}	NS
13.	15.3 ± 2.64^{bcd}	20.6 ± 14.54^{abc}	NS
14.	16.5 ± 3.84^{bcd}	9.0 ± 6.05^{bc}	**
15.	29.8 ± 11.21^{abcd}	15.4 ± 7.72^{abc}	**
16.	14.0 ± 7.46^{cd}	32.3 ± 7.38^{a}	**
17.	42.1 ± 8.24^{ab}	5.3 ± 3.88^{c}	**
18.	40.6 ± 15.18^{ab}	9.6 ± 5.34^{bc}	**
19.	27.3 ± 8.66^{bcd}	21.3 ± 12.53^{abc}	NS
20.	15.8 ± 6.71^{bcd}	13.6 ± 3.53^{abc}	NS

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

4.3.2 Thousand kernel weight

A thousand kernel weights were higher in treated plots than in untreated plots in both seasons. The highest weight of 4.6gm was recorded in 16, 19 and 18 compared to 3.4gm the lowest in 6 and 3. In untreated plots 4.3gm was recorded in 16 as the highest while 3.2gm in 6 was the lowest. The highest loss (14.8%) was associated with the disease on genotype 20 in season 1 (Table 14). Harder and Legge (2000) similarly reported a loss ranging from 7 – 43 % in kernel weight in barley for resistant and susceptible varieties respectively. Gaunt (1981) described tolerance as the ability of the plant to maintain desirable yield, quality and performance in the presence of the disease symptom or if the plant appears susceptible to the disease. The 1000 kernel weight is not likely to describe disease effects on the yield than the yield measurement itself, because they were not consistent across the seasons.

Table 14: Effect of stem rust on the 1000 kernel weight (gm) of barley

	Season 1	**Season2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	2.5 ± 1.61^{cd}	1.6 ± 0.80^{a}	**NS**
2.	2.7 ± 1.73^{cd}	3.8 ± 0.69^{a}	**NS**
3.	10.8 ± 5.71^{abcd}	6.5 ± 5.17^{a}	**
4.	9.2 ± 4.50^{abcd}	2.4 ± 1.38^{a}	**
5.	10.2 ± 4.13^{abcd}	1.8 ± 3.05^{a}	**
6.	7.8 ± 2.66^{abcd}	1.9 ± 1.80^{a}	**
7.	8.0 ± 2.93^{abcd}	1.7 ± 1.71^{a}	**
8.	1.7 ± 1.91^{d}	5.3 ± 1.65^{a}	**
9.	5.8 ± 1.88^{bcd}	0.7 ± 1.20^{a}	**
10.	7.3 ± 1.51^{abcd}	2.5 ± 2.44^{a}	**
11.	5.2 ± 0.17^{bcd}	3.4 ± 2.15^{a}	**NS**
12.	2.4 ± 1.55^{cd}	2.2 ± 0.08^{a}	**NS**
13.	10.7 ± 5.39^{abcd}	5.7 ± 3.06^{a}	**
14.	5.5 ± 2.97^{bcd}	4.6 ± 3.26^{a}	**
15.	7.2 ± 3.01^{abcd}	6.0 ± 3.19^{a}	**
16.	11.5 ± 2.59^{abc}	6.6 ± 3.69^{a}	**
17.	9.4 ± 3.00^{abcd}	2.9 ± 0.72^{a}	**
18.	14.5 ± 5.24^{ab}	5.2 ± 1.70^{a}	**
19.	10.6 ± 1.76^{abcd}	2.3 ± 1.28^{a}	**
20.	14.8 ± 3.78^{a}	1.6 ± 0.80^{a}	**

Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).

***** or NS** means the genotype was significantly and not significantly different between season*

4.3.3 Grain size

Grain size is an important parameter of yield and quality of any crop. Grains from untreated plots passed through the 2.5mm sieve than from the treated plots (Table 15). The highest loss of 69% in 18 and 49.5% in 6 was recorded in the two seasons respectively. The genotype 18 had the highest loss, final disease and second last area under disease progress curve in season 1 that was not the case for 6 in season 2. The reduction in the grain size can be greatly attributated to the negative effect of stem rust on grain filling process. Calpouzos *et al.* (1976) and Johnson and Wilcoxson (1979) attributed reduction in grain size and weight had been associated with stem rust of wheat and barley leaf rust caused by *Puccinia hordei*. The disease affects the photosynthetes partitioning to the grain, reducing the size and adversely affecting the quality of barley. Barley grains of different sizes vary in malting behavior and produce malts with different properties (Burger and La Berge, 1985). The small grains from rusted plants were generally unsuitable for the production of malt. When infection occur during critical periods such as anthesis or grain filling, productivity can be drastically curtailed because the maturation period is reduced hence the grain size (Bushnell and Roelfs, 1984).

Table 15: Effect of stem rust on grain size of barley

	Season 1	**Season 2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	3.0 ± 2.17^{e}	31.8 ± 4.62^{ab}	**
2.	2.6 ± 1.37^{e}	11.2 ± 5.47^{b}	**
3.	14.1 ± 7.36^{de}	24.1 ± 8.21^{ab}	**
4.	21.4 ± 6.67^{cde}	34.2 ± 14.98^{ab}	NS
5.	34.6 ± 6.21^{abcde}	36.4 ± 10.77^{ab}	NS
6.	21.2 ± 14.89^{cde}	49.5 ± 4.99^{a}	**
7.	29.0 ± 14.95^{bcde}	20.4 ± 7.28^{b}	NS
8.	29.3 ± 16.76^{bcde}	30.0 ± 3.54^{ab}	NS
9.	34.5 ± 16.16^{abcde}	13.6 ± 7.19^{b}	**
10.	35.9 ± 17.25^{abcde}	29.9 ± 12.64^{ab}	NS
11.	29.3 ± 21.47^{bcde}	37.9 ± 7.91^{ab}	NS
12.	45.3 ± 15.97^{abcd}	17.9 ± 8.14^{b}	**
13.	42.9 ± 19.13^{abcd}	16.6 ± 9.91^{b}	**
14.	30.2 ± 24.21^{bcde}	25.8 ± 4.47^{ab}	NS
15.	36.3 ± 22.79^{abcde}	26.0 ± 14.03^{ab}	NS
16.	56.4 ± 12.68^{abc}	22.8 ± 8.53^{ab}	**
17.	29.1 ± 26.66^{bcde}	29.0 ± 5.61^{ab}	NS
18.	69.3 ± 7.82^{a}	12.4 ± 2.90^{b}	**
19.	58.5 ± 17.57^{ab}	26.5 ± 11.40^{ab}	**
20.	41.7 ± 21.64^{abcd}	32.5 ± 1.72^{ab}	NS

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

4.4 Effect of stem rust Ug99 epidemic on the quality of barley

4.4.1 Germination percent

Percentage germination varied with genotype and treatment, high percent germination was observed in treated plots compared to the untreated ones. The disease significantly affected the germination percent of barley. In season 1, the highest reduction in percent germination (54.1% and 38.3%) was recorded in 16 and 10 in season 1and 2 respectively (Table 16). The reduction in germination percent could be attributed to low level of stored starch. Malting barley is one of the few grains where the seed is required to germinate for product development, i.e. production of malt (Briggs and Woods, 1993). The failure of barlely to germinate at an acceptable level (>95 %), could introduce problems during malting process. Benech- Arnold (2000) noted that the most significant and relevant aspects of dormancy or pre –harvest sprouting are hormones and enzymes.

Table 16: Effect of stem rust disease on germination percent of barley genotypes

	Season1	**Season2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	20.4 ± 5.58^{c}	19.6 ± 7.18^{abcde}	**NS**
2.	23.5 ± 9.93^{c}	19.1 ± 8.32^{abcde}	**
3.	17.6 ± 3.80^{c}	3.8 ± 1.99^{e}	**
4.	50.6 ± 8.94^{ab}	33.1 ± 7.70^{ab}	**
5.	21.1 ± 4.07^{c}	15.9 ± 6.91^{bcde}	**NS**
6.	16.5 ± 2.63^{c}	7.5 ± 4.61^{de}	**
7.	38.9 ± 12.42^{abc}	16.1 ± 1.10^{bcde}	**
8.	19.5 ± 10.16^{c}	8.4 ± 5.51^{de}	**
9.	25.4 ± 8.17^{c}	10.6 ± 6.40^{cde}	**
10.	35.7 ± 13.48^{abc}	38.3 ± 5.09^{a}	**NS**
11.	23.8 ± 7.88^{c}	8.7 ± 3.13^{de}	**
12.	15.6 ± 8.08^{c}	12.1 ± 9.43^{cde}	**
13.	19.5 ± 7.92^{c}	25.6 ± 3.40^{abc}	**NS**
14.	21.2 ± 5.55^{c}	2.6 ± 1.24^{e}	**
15.	29.5 ± 11.16^{c}	2.7 ± 2.64^{e}	**
16.	54.1 ± 9.71^{a}	17.1 ± 4.98^{bcde}	**
17.	34.5 ± 5.53^{abc}	17.1 ± 4.72^{bcde}	**
18.	24.9 ± 7.29^{c}	5.4 ± 1.80^{e}	**
19.	18.5 ± 14.67^{c}	28.8 ± 18.49^{abc}	**
20.	$51.5 \pm 7.82^{ab*}$	29.8 ± 3.82^{abc}	**

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

4.4.2 Starch content

The stem rust Ug99 epidemics significantly reduced the starch content of barley (Table 17). Irrespective of season, the disease had the greatest effect on 5, 3and 17. Since the rust fungus is an obligate parasite, it derives the requirement from the host plant. Pfeiffer *et al.* (1969) observed that glucose fed to rusted wheat leaves was utilized by the fungus. The ratio of carbon in respired Co_2 contributed by C_6 and C_1 from hexose substrates (the C_6: C_1 ratio) declines from about 0.5 in healthy tissue to 0.3 at sporulation in rusted tissue (Antonelli and Daly, 1966). The fungus utilizes available glucose, with reduced photosynthesis and increased respiration hence the total glucose available for storage as starch is reduced.

Table 17: Effect of stem rust Ug99 on starch content of barley grains

	Season 1	**Season 2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	0.4 ± 0.32^{b}	0.4 ± 0.05^{b}	**NS**
2.	0.8 ± 0.21^{ab}	0.5 ± 0.34^{b}	**
3.	1.7 ± 0.29^{ab}	1.7 ± 0.44^{a}	**NS**
4.	1.0 ± 0.47^{ab}	1.0 ± 0.83^{ab}	**NS**
5.	1.5 ± 0.51^{ab}	1.5 ± 0.38^{ab}	**NS**
6.	0.9 ± 0.06^{ab}	0.6 ± 0.46^{ab}	**NS**
7.	0.5 ± 0.24^{b}	0.6 ± 0.27^{ab}	**
8.	0.4 ± 0.45^{b}	0.7 ± 0.19^{ab}	**NS**
9.	0.6 ± 0.15^{b}	0.7 ± 0.26^{ab}	**NS**
10.	0.2 ± 0.16^{b}	0.5 ± 0.38^{ab}	**NS**
11.	0.7 ± 0.21^{ab}	0.7 ± 0.42^{ab}	**NS**
12.	0.8 ± 0.36^{ab}	0.8 ± 0.11^{ab}	**NS**
13.	0.8 ± 0.21^{ab}	0.3 ± 0.39^{b}	**
14.	0.5 ± 0.10^{b}	0.4 ± 0.28^{b}	**NS**
15.	1.1 ± 0.46^{ab}	1.1 ± 0.24^{ab}	**NS**
16.	1.1 ± 0.10^{ab}	1.0 ± 0.35^{ab}	**NS**
17.	2.4 ± 2.02^{a}	2.4 ± 0.41^{a}	**NS**
18.	0.5 ± 0.47^{b}	1.1 ± 0.28^{ab}	**
19.	0.8 ± 0.41^{ab}	0.8 ± 0.73^{ab}	**NS**
20.	0.9 ± 0.69^{ab}	0.9 ± 0.51^{ab}	**NS**

Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ *by DMRT.* *Means followed by the same letter in column are not significantly different at* ($p \leq 0.05$)

** ***or NS*** *means the genotype was significantly and not significantly different between season*

4.4.3 The protein, zeleny and gluten content

The highest loss (9.00 %) in protein content was observed in 20 in season 1 (Table 18). A mean loss of 4.0 and 16.3% in zeleny content was noted in season 1 and 2 respectively (Table 19). A mean of 17.1 and 53.2% in genotype 17 was noted in season 1 and 2 respectively. Table 20 shows the effect of stem rust Ug99 on gluten content. Genotype20 and 17 gave gluten loss of 8.1 and 16.3% in season 1 and 2 respectively. The fungus being an obligate parasite it utilizes the protein in the host for its structural growth and development. More frequently, the total protein of the host and parasite remain constant (Johnson *et al.,* 1968). Much of the total protein can be assumed to be in the developing fungus, especially at sporulation and thereafter. Probably little protein is left in the host cell and subsequently little in the grain storage.

Table 18: Effect of stem rust Ug99 on protein content (%) of barley

	Season1	**Season2**	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	1.8 ± 0.14^{b}	2.1 ± 0.39^{a}	**NS**
2.	3.7 ± 0.83^{ab}	2.9 ± 0.50^{a}	**
3.	6.0 ± 3.10^{ab}	3.3 ± 2.40^{a}	**
4.	5.0 ± 0.24^{ab}	7.1 ± 3.09^{a}	**NS**
5.	3.6 ± 2.10^{ab}	2.0 ± 1.32^{a}	**
6.	5.4 ± 0.91^{ab}	1.6 ± 0.79^{a}	**
7.	4.7 ± 3.65^{b}	4.8 ± 1.10^{a}	**NS**
8.	4.1 ± 1.86^{ab}	3.9 ± 2.12^{a}	**NS**
9.	0.8 ± 0.72^{b}	3.4 ± 1.24^{a}	**
10.	4.1 ± 1.10^{ab}	3.8 ± 1.14^{a}	**
11.	2.6 ± 0.88^{ab}	4.3 ± 2.41^{a}	**NS**
12.	3.4 ± 1.91^{ab}	3.6 ± 3.32^{a}	**NS**
13.	0.8 ± 0.45^{b}	4.2 ± 0.77^{a}	**
14.	2.1 ± 0.67^{b}	4.7 ± 2.97^{a}	**NS**
15.	4.0 ± 2.40^{ab}	2.1 ± 0.77^{a}	**NS**
16.	3.9 ± 0.69^{ab}	2.2 ± 1.47^{a}	**
17.	2.5 ± 0.45^{b}	1.8 ± 0.49^{a}	**
18.	4.5 ± 1.50^{ab}	3.2 ± 0.86^{a}	**
19.	3.4 ± 2.27^{ab}	3.2 ± 2.86^{a}	**NS**
20.	9.0 ± 3.77^{a}	4.0 ± 2.24^{a}	**

*Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*
***** or NS** means the genotype was significantly and not significantly different between season*

Table 19: Effect of stem rust Ug99 on Zeleny content (%) of barley

	Season1	Season2	
Genotypes serial No.	**Mean %Loss**	**Mean %Loss**	**Season Difference**
1.	3.8 ± 0.73	9.4 ± 0.15^{abc}	**
2.	2.2 ± 1.66^{b}	6.5 ± 2.06^{bc}	**
3.	6.6 ± 2.62^{ab}	19.4 ± 4.82^{abc}	**
4.	2.8 ± 0.91^{b}	32.0 ± 8.00^{ab}	**
5.	1.7 ± 0.94^{b}	7.9 ± 3.89^{abc}	**
6.	3.0 ± 1.58^{b}	4.2 ± 2.02^{c}	**
7.	1.7 ± 1.35^{b}	16.4 ± 1.00^{abc}	**
8.	6.5 ± 4.98^{ab}	9.8 ± 3.03^{abc}	NS
9.	1.5 ± 0.58^{b}	14.6 ± 1.88^{abc}	**
10.	2.7 ± 0.27^{b}	4.8 ± 1.73^{c}	NS
11.	2.6 ± 0.92^{b}	18.6 ± 5.18^{abc}	**
12.	4.3 ± 1.28^{b}	6.4 ± 3.18^{bc}	NS
13.	2.0 ± 1.30^{b}	41.9 ± 14.79^{a}	**
14.	2.1 ± 0.60^{b}	29.2 ± 11.90^{ab}	**
15.	2.2 ± 1.58^{b}	6.5 ± 1.57^{bc}	**
16.	5.3 ± 0.99^{b}	5.0 ± 3.15^{bc}	NS
17.	17.1 ± 15.18^{a}	53.1 ± 16.71^{a}	**
18.	3.2 ± 0.88^{b}	16.2 ± 5.79^{abc}	**
19.	5.1 ± 2.64^{b}	7.9 ± 5.73^{abc}	NS
20.	8.0 ± 2.54^{ab}	16.1 ± 5.98^{abc}	**

** Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. *Means followed by the same letter in column are not significantly different at ($p \leq 0.05$).*

***** or NS** means the genotype was significantly and not significantly different between season*

Table 20: Effect of stem rust Ug99 on gluten content of barley

Genotypes serial No.	Season1 Mean %Loss	Season 2 Mean %Loss	Season Difference
1.	2.4 ± 0.90^{bc}	3.7 ± 1.39^{b}	**
2.	8.7 ± 5.05^{ab}	5.2 ± 2.14^{b}	NS
3.	5.4 ± 0.58^{abc}	3.0 ± 0.94^{b}	**
4.	5.7 ± 1.86^{abc}	8.9 ± 2.80^{ab}	**
5.	3.4 ± 1.34^{bc}	2.2 ± 0.33^{b}	NS
6.	5.8 ± 0.40^{abc}	2.2 ± 1.24^{b}	**
7.	4.3 ± 1.29^{abc}	1.8 ± 3.97^{b}	NS
8.	4.8 ± 1.70^{abc}	4.5 ± 1.31^{b}	NS
9.	1.4 ± 0.76^{c}	4.0 ± 1.90^{b}	**
10.	5.5 ± 1.31^{abc}	5.6 ± 2.33^{b}	NS
11.	3.2 ± 1.14^{abc}	4.6 ± 1.91^{b}	NS
12.	3.5 ± 1.58^{abc}	3.8 ± 1.80^{b}	NS
13.	1.5 ± 0.42^{c}	3.2 ± 1.70^{b}	NS
14.	2.0 ± 0.73^{c}	6.5 ± 3.45^{b}	NS
15.	1.2 ± 0.51^{c}	2.0 ± 1.14^{b}	NS
16.	3.8 ± 0.90^{abc}	5.3 ± 0.51^{b}	**
17.	9.8 ± 7.17^{a}	3.6 ± 2.25^{b}	NS
18.	4.0 ± 0.97^{abc}	5.4 ± 2.73^{b}	NS
19.	0.8 ± 1.45^{c}	5.0 ± 3.69^{b}	NS
20.	8.1 ± 2.62^{ab}	16.3 ± 7.7^{a}	NS

** Any two means in a column whose S.E. values overlap are not different at $\alpha = 0.05$ by DMRT. * Means followed by the same letter in column are not significantly different at ($p \leq 0.05$)*

***** or NS** means the genotype was significantly and not significantly different between season*

4.5 Correlation and factor analysis

4.5.1 Correlations

The phenotypic correlation coefficient (r) among final disease, area under disease progress curve, yield components, growth parameters and quality aspects are shown in Table 21. The correlation coefficient depicted both negative and positive values that ranged from 0 to 0.97. The 1000 kernel weight had a positive correlation with grain size. It was however negatively correlated with starch, protein, leaf area, zeleny and gluten. As expected dry matter was positively correlated to plant height, yield and leaf area. Grain size was significantly correlated to leaf area, protein, starch, yield, chlorophyll, Zeleny, gluten, final disease and area under disease progress curve. Yield had a positive correlation with plant height.

Protein had low and moderate correlations with Zeleny, tillers and gluten respectively. Starch had a significant negative correlation with chlorophyll, moderate significant correlation with plant height and high positive correlations with final disease, area under disease progress curve, zeleny and gluten respectively. Leaf area had positive significant correlation with germination, Zeleny, gluten, final disease and area under disease progress curve. Tillering capacity had a low positive significant association with germination. Chlorophyll was negatively and significantly correlated to height, Zeleny, gluten and area under disease progress curve and positively to germination percentage. Germination has a negative low significant correlation with zeleny and gluten. Plant height had a positive moderate significant correlation with Zeleny, gluten, final disease and area under disease progress curve. Zeleny had a high positive significant correlation with gluten, final disease and area under disease progress curve. Gluten had a high positive significant relation with final disease and area under disease progress curve. Final disease was highly positively and significant correlated with area under disease progress curve.

Table 21: Phenotypic correlation coefficient (r) among yield component, growth parameters, quality aspects, final disease and AUDPC

Variable	1000 Kernel	Dry matter	Grain size	Yield	Protein	Starch	Leaf area	Tillers	Chloro phyll	Germina tion	Height	Zeleny	Gluten	Final disease	AUI
1000 Kernel	1.00														
Dry matter	-0.05	1.00													
Grain size	0.64**	-0.14	1.00												
Yield	028	0.33*	-0.28*	1.00											
Protein	-0.23*	0.04	0.32*	-0.01	1.00										
Starch	-0.26*	0.01	0.64**	-0.08	0.20	1.00									
Leaf area	-0.22*	0.24*	0.44*	0.16	0.38	0.63**	1.00								
Tillers	0.19	-0.06	-0.21	0.23	0.21*	-0.12	0.11	1.00							
Chlorophyll	0.22	0.18	-0.31*	0.04	0.04	-0.38*	-0.11	0.29*	1.00						
Germination	0.16	0.22	-0.28*	0.16	-0.10	-0.27	-0.02	0.18	0.29*	1.00					
Height	-0.11	0.30*	0.21	0.49*	0.36	0.41*	0.57*	0.16	-0.03*	-0.02	1.00				
Zeleny	-0.37*	0.04	0.72**	-0.01	0.39*	0.89*	0.70**	-0.09	-0.33*	-0.32*	0.51*	1.00			
Gluten	-0.37*	0.04	0.73**	-0.03	0.50*	0.87*	0.71**	-0.06	-0.33*	-0.28*	0.51*	0.97**	1.00		
F disease	-0.03	-0.08	0.44*	-0.06	0.09	0.72*	0.53*	0.06	0.31*	-0.26	0.43*	0.68**	0.65**	1.00	
AUDPC	-0.11	-0.07	0.49*	-0.01	0.09	0.76*	0.55*	-0.04	-0.38*	-0.24	0.40*	0.71**	0.67**	0.96**	1.00

**, **- Significant at $P \leq 0.05$ and 0.01, respectively, AUDPC – area under disease progress curve*

4.5.2 Factor analysis

Factor analysis is important because it helps to summarize the data and removes autocorrelations. Seven out of seventeen principal components accounted for 71% of the total variation (Table 22). Factor 1, which can be described as disease consisted of; final disease, area under disease progress curve and season and accounted for 23% of the variation. The second factor consisted of germination percent, Protein, starch, zeleny and gluten that accounted for 12% of variation and they were summarized as quality. The third factor that accounted for 9% of the variation was yield and yield components and they included; 1000 kernel weight, Yield, Dry matter, Grain size, and variety. The fourth factor was plant height and chlorophyll that accounted for 8% of the variation. Leaf area, number of tillers represented factor 5 and 6 each accounting for 7 and 6% respectively. Finally the number of tiller per meter square was the seventh factor that accounted for 6% of the variation. Generally the fourth, fifth sixth and seventh factors were put in the same category that was described as the growth component factor.

Table 22: Factor Patterns of Variables

Variable / statistic	Factor loading						
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
1000 Kernel	0.13	0.08	**0.59**	- 0.26	0.11	0.19	- 0.05
Dry matter	-0.06	0.08	**-0.66**	0.41	0.37	0.08	0.01
Grain size	0.34	- 0.02	**0.53**	0.01	0.37	- 0.03	0.41
Yield	0.27	- 0.27	**0.59**	- 0.49	- 0.27	0.30	0.04
Protein	0.16	**- 0.71**	0.23	- 0.25	0.01	0.01	0.09
Starch	-0.08	**0.68**	- 0.03	0.16	0.23	- 0.11	0.32
Leaf area	0.40	0.04	-0.10	-0.07	**-0.51**	0.20	-0.36
Tillers	0.35	-0.05	-0.27	-0.12	-0.12	**0.63**	**0.51**
Chlorophyll	0.09	0.19	0.30	**0.57**	-0.33	0.35	-0.40
Germination	0.18	**0.51**	-0.15	0.34	0.37	0.04	0.06
Height	0.36	0.14	-0.10	**-0.52**	-0.30	-0.20	0.39
Zeleny	-0.43	**0.64**	0.16	-0.12	0.09	-0.18	-0.11
Gluten	-0.06	**0.71**	-0.13	-0.08	0.20	0.17	0.01
F disease	**0.87**	0.01	0.19	-0.17	0.14	-0.23	-0.12
AUDPC	**0.88**	-0.01	0.11	-0.17	0.07	-0.25	-0.13
Season	**-0.85**	0.07	0.15	0.06	0.10	0.07	-0.05
Variety	0.21	0.24	**0.67**	0.16	0.10	0.38	-0.09
Eigen value	3.85	2.12	1.52	1.31	1.17	1.07	1.04
Proportion of variance explained	0.23	0.12	0.09	0.08	0.07	0.06	0.06
Cumulative variance explained	0.23	0.35	0.44	0.52	0.59	0.65	0.71

Figures in bold represent significant contribution to variable ($p \leq 0.05$), Factor loading 1 can be described as disease, factor 2 as quality, factor 3 as yield and yield component and factor 4, 5, 6 and 7 as growth component.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Stem rust Ug99 race caused yield loss of up to 53.80% in susceptible germplasm CBSSOOYOO236T-E-0Y-OM-2Y-OM. Most of the germplasm were susceptible at adult plant stage and showed resistance at the seedling stage. There were generally high losses of quality and yield parameter caused by stem rust Ug99 epidemic than reduction in growth parameters. Most of the Kenyan grown cultivars are susceptible to the new race of stem rust PgtUg99.

Resistance due to 'slow rusting' genes appears to be useful especially under high disease pressure with some cultivars posting very high resistance to PgtUg99. Stem rust severity measured towards the end of the epidemic can be used to select for slow rusting cultivars, if the resources are limiting and several disease reading are not available to complete AUDPC, this is based on the high correlation between final disease (FD) and AUDPC. The study has demonstrated the pathogenicity of PgtUg99 to barley despite the fact that it poses a great threat to wheat production in the world. The information in this study is beneficial to both researchers, barley and wheat producers in confronting a pathogen that is devastating with feasible chemical control though expensive and unfriendly to the environment.

Disease pressures as realized in this studies are not common in commercial fields; therefore, the level of slow rusting resistance in some of the germplasms could be sufficient to protect the crop in large scale production where inoculum must be produced on the slow rusting genotypes for the disease to progress. Under the conditions of our study this measure did not discriminate the resistant, moderately resistant, and moderately susceptible lines; however, it is possible that under less severe stem rust conditions these lines may have shown more significant differences because susceptible lines showed the greatest acceleration to maturity. The very early senescence of these lines due to stem rust would be an important factor in limiting potential yield and in reducing quality.

5.2 Recommendations

1. Apart from adopting the slow rusting lines used in this study for commercial production and breeding against the disease, there is need to screen more cultivars to identify new sources of resistance to the new race of stem rust Ug99.

2. Studies should be done to understand the genes that are responsible for slow rusting to the new race in barley so that they can be incorporated in the Kenyan commercial cultivars.

3. Kenya government should advise farmers to grow varieties that are resistant and use fungicides to control the disease to reduce the inoculum load in the country and also not to grow barley and wheat in a rotation or in adjacent field without controlling stem rust in barley.

4. The genetic basis of the slow rusting germplasm should be studied so as to understand the mechanism by which they reduce the disease and identify similar genes in other sources of resistance.

REFERENCES

Agrios, G.N. (2005). Plant pathology 5th edition. Academic press, San Diego U.S.A. pp 565-571.

Allard, R. W. (1988). Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. Journal of Heredity 79:225-238.

Anikster, Y. (1984). The formae speciales. In The Cereal Rusts, Vol. 1 (Bushnell,W.R. and Roelfs, A.P., eds). Orlando, FL: Academic Press, pp 115-130.

Anon, (2008). Wheat crops at risk killer fungus spells disaster. Red Orbit News. 12,March2008.http://www.redorbit.com/news/science/1293009/ wheat crops at Risk killer fungus spellsdisaster/ index.html? Source= r science. Accessed on 9th September 2008.

Antonelli, E. and Daly, J. M. (1966). Decarboxylation of indolecsetic acid by near isogenic lines of wheat resistant or susceptible to *Puccinia graminis* f. sp. *tritici*. Phytopathology 56: 610-618.

Ayliffe, R., Michael, L., Singh, R., and Lagudah. E. (2008). Durable resistance to wheat stem rust needed. Current Opinion in Plant Biology 11: 187-192.

Beaven, E.S. (1947). Barley: 50 years of observation and experimentation. London: Duck worth. Pp394.

Benech-Arnold, R. (2000). Bases of pre-harvest sprouting resistance in barley: physiology, molecular biology and environment control of dormancy in the barley grain. In "Barley science: recent advances from molecular biology to agronomy of yield and quality". (Eds G. A. Slafer, J.L. Molina-Cano R. Savin, J.L. Arausi, I. Romagos) (Food products press New York)

Borlaug, N. E. (2007). Sixty-two years of fighting hunger: personal recollections. Euphytica 157:287-297

Bothmer, R. v. and Jacobsen, N. (1995). Origin, taxonomy, and related species. Pp. 19-56 *in* D. C. Rasmusson, ed. Barley. American Society of Agronomists, Madison, Wis.

Burgeon, J., Cadena, A.R., Crossa, J., Banzinger, M., Gilmour, A.R. and Cullis, B.R. (2000). User's Guide for Spatial Analysis of Field Variety Trial using ASREML, CIMMYT, Mexico. D.F.

Briggs,D.E. and Woods, J.L. (1993). Dormancy in malting barley: Studies on drying, storage biochemistry and physiology. HGCA Project report No. 84, pp. 146.

Burger, W.C., and La Burge, D.E. (1985). Malting and brewing quality. In 'Barley'.(Ed.D.C. Rasmusson.) Agronomy Series 26 : 367 – 401.

Bushnell W.R., and Roelfs, A.P. (1984). The cereal rusts. Vol 1. Origins, specificity, structure, and physiology. Academic press. Orlando, pp. 333 – 373.

Bushnell, W. R., and Bosacker, P. L., (1982). Nuclear volume and number in long-term in vitro cultures of *Puccinia graminis*. Canadian Journal of Botany 60: 1827-1836.

Bushnell, W. R., and Rowell, J.B. (1968). Premature death of adult rusted wheat plant in relation to carbon dioxide evolution by root systems. Phytopathology 58: 651 - 258.

Calpouzos, L., Roelfs, A.P., Madson, Martin, F.B., Welsh, J.R., and Wilcoxson, R.D. (1976). Anew model to measure yield losses caused by stem rust in spring wheat. Trends of British Mycological Society 55: 467 – 479.

Chelkowski, J., Tyrka, M., and Sobkiewiez, A. (2003). Resistance genes in barley and the identification with molecular markers. Journal of Applied Genetics 44: 291-309

Chong, J., Harder, D.E. and Rohringer, R. (1985) Cytological studies on *Puccinia graminis* f. sp. *tritici* in a compatible wheat host. I. Walls of intercellular hyphal cells and haustorial mother cells. Canadian Journal of Botany 63: 1713-1724.

Ciara, B., Kith, J., Geoff T., and Robert, L. (2006). Managing stem rust of wheat. Plant Pathology, Department of Agriculture, Western Australia, Pp. 123.

CIMMYT (2005). Sounding the Alarm on Global Stem Rust. An Assessment of Ug99 in Kenya and Ethiopia and Potential for Impact in neighboring Regions and beyond. 29 May 2005. pp 26.

De Millano W.A.J., (1983). Improvement of Wheat in Zambia using incomplete resistance against rusts. Lusaka, Zambia, Pp. 113.

Dill – Macky, R. Rees, R. G., and Platz, G. J., (1990). Stem rust epidemics and their effects on grain yield and quality in australian barley cultivars. Australian journel of agricultural research 41: 1057 – 1063.

Dubin, H.J., and Stubbs, R.W. (1986). Epidemic spread of barley stripe rust in South America. Plant Disease 70: 141-144.

Durbin, R. D. (1967). Obligate parasite: effect on the movement of solutes and water. In "The dynamic role of molecular constituents in plant - parasite interactions" C.J. Mirocha and I. Uritani, edititors , pp.80 – 99.

East African Breweries Ltd (2005). The Kenyan Beer Industry. Nairobi, Kenya,Pp. 543

Ellis, J. G., Dodds, P. N., and Lawrence, G. J. (2007). Flax rust resistance gene specificity is based on direct resistance-avirulence protein interactions. Annual Review of Phytopathology 45: 289-306.

FAO. (2004). Meeting of the Technical Subgroup of the Expert Group on International Economic and Social Classifications. United Nations Department of Economic and Social Affairs Statistics Division. New York, U.S.A Pp. 643.

Fetch, T. G., Steffenson, B. J., Nevo, E. (2003). Diversity and sources of multiple disease resistance in *Hordeum spontaneum*. Plant Disease 87: 1439-1448.

Flor, H.H. (1956). The complementary genic systems in flax and flax rust. Advanced Genetics 8: 29-54.

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

Gaunt, R.E. (1981). Disease tolerance – an indicator of threshold? Phytopathology 71: 915 – 916.

Gordon, T.R. and Duniway, J.M. (1982). Effects of Powdery Mildew Infection on The Effects of Co2 Fixition and Light Utilization On Sugar Beet Leaves. Plant Physiology 69: 139 – 142.

Harder, G.E, Mathenge, G.R and Mwaura, H.D. (1972). Physiological specialization and epidemiology of Wheat stem Rust in East Africa. Nairobi, Kenya Pp. 45.

Harder, D.E., and Legge, W.G. (2000). Effectiveness of different sources of stem rust resistance in barley. Crop science 40: 826 – 833.

Hammond-Kosack, K.E., and Jones, J.D.G. (1997). Plant disease resistance genes. Annual Review of Plant Physiology and Plant Molecular Biology 48: 575-607.

Heath, M.C. (1997). Signaling between pathogenic rust fungi and resistant or susceptible host plants. Annals of Botany 80: 713-720.

Herrera-Foessel, S.A., Singh, R.P., Huerta-Espino, J., and Djurle,A. (2006). Effect of Leaf Rust on Grain Yield and Yield Traits of Durum Wheats with Race-Specific and Slow-Rusting Resistance to Leaf Rust. Plant Disease 90:1065-1072.

Hiebert,W.C., Fetch, G.T., Zegeye, T., Thomas, B.J., Somers,D.J., Humphreys, G.D., McCallum,B.D., Cloutier,S., Singh, D. and Knott, R.D. (2010) Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars 'Peace' and 'AC Cadillac'.Theoretical and Applied Genetics in press.

Hovmøller M.S. and Justesen A.F, (2007). Rates of evolution of avirulence phenotypes and DNA markers in a North-West European population of *Puccinia striiformis* f.sp. *tritici*. Molecular Ecology 16: 4637-4647.

Huang, N., Angeles,E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaradivel, N., Bennett, J., and Khush, G.S. (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theories of Applied Genetics 95: 313-320.

Hulbert, S.H., Webb, C.A., Smith,S.M., and Sun, Q. (2001). Resistance gene complexes: evolution and utilization. Annual Review of Phytopathology 39,285-312.

Jäger, K. and Reisener, H.J. (1969) Host-parasite metabolic relationship between *Puccinia graminis* var. *tritici* and *Triticum aestivum* (wheat) I. Uptake of amino acids from the host. Planta, 85: 57-72.

Jaetzold, R., and Schmidt, H. (1983). Farm management handbook of Kenya. Vol. II Natural condition and farm management information. Ministry of Agriculture, Kenya in cooperation with Germany Agricultural team (GAT) of the Germany Agency for technical cooperation (GTZ) Nairobi Kenya Pp. 345.

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

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

Jin, Y., Steffenson, B.J., and Miller, J.D. (1994). Inheritance of Resistance to Pathotypes QCC and MCC of *Puccinia graminis* f. sp. *tritici*. In barley lines Q21861 and Temperature Effect on the Expression of Resistance. Phytopathology 84: 452 – 455.

Jin Y, Pretorius Z.A, Singh, R.P., (2007). New virulence within race TTKS (Ug99) of the stem rust pathogen and effective resistance genes. Phytopathology 97: 137 - 143.

Jin, Y., Szabo, L., and Pretorius, Z.A. (2008b). Virulence variation within the Ug99 lineage. In: Proceedings of the 11th International Wheat Genetics Symposium, Brisbane, Australia Pp. 342.

Jin, Y., Singh, R. (2006). Resistance to recent eastern African stem rust isolates with virulence to *Sr31* in US wheat. Plant Disease 90: 476-480.

Joshi, L.M., and Palmer, L.T. (1973). Epidemiology of stem, leaf and stripe rusts of wheat in Northern India. Plant Disease Report. 57:8-12.

Johnson, R. (1992). Reflections of a plant pathologist on breeding for disease resistance, with emphasis on yellow rust and eyespot of wheat. Plant Pathology 41: 239-254.

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

Johnson , D.A., and Wilcoxson, R.D. (1979). Yield losses of fast and slow rusting barley infected with *Puccinia hordei*. Plant Disease Report 63: 764 – 768.

Johnson, L.B., Brannaman, B.L., and Zscheile, F. B., Jr. (1968). Protein and enzyme changes in wheat leaves following infection with *Puccinia recondite*. Phytopathology 58 : 578 – 583.

Kenya Agriculture Institute (KARI), (2005a). Crop production hand book for wheat, oil crops and horticulture. Kinyua,M.G. and Ochieng, J. A. W. (Eds.). G. Gansdil printers & stationary , Nairobi, Kenya. Pp 1 – 19.

Kenya Agricultural Research Institute(KARI), (2005b). Effect of a new race on wheat production/use of fungicides and its cost in large vs. small scale farmers, situation of current cultivars. KARI, Njoro.Pp 1- 102.

Kenya Maltings Ltd. (2007) Annual Reports. Nairobi, Kenya Pp 342.

Kirk, P.M., Cannon, P.F., David, J.V. and Stalpers, J.A. (2001) Ainsworthand Bisby's Dictionary of the Fungi, ninth edition, pp. 569, 610, 624. Wallingford, UK: CAB International.

Kleinhofs, K., Brueggeman, R., Nirmala, J., Zhang, L., Mirlohi, A., Druka, A., Rostocks, N., and Steffenso B.J.(2009). Barley stem rust resistance genes: Structure and function. The plant genome 2 (2): 109-120.

Kurt, J., Leonard, G and Szabo, J. (2005). Stem rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6, (2): 99-111.

Leonard, K.J. (2001a). Black stem rust biology and threat to wheat growers. The Central Plant Board Meeting. February 5-8, Lexington, KY.Pp. 234.

Leonard, K.J. (2001b). Stem rust-future enemy? In Stem Rust of Wheat: from Ancient Enemy to Modern Foe (Peterson, P.D., ed.):pp. 119-146.

Liu, J.Q., and Harder, D.E. (1996). Component of Resistance in Barley to Stem Rust: receptive, urediospore production, and latent period and infection response in adult plants. Canadian Journal of Plant Science 74: 1298-1304.

Lomonossoff, G.P. (1995). Pathogen-derived resistance to plant viruses. Annual Review of Phytopathology 33: 323-343.

Loughman, R., Jayasena, K., and Majewski.J. (2005). Yield Loss and Fungicide Control of Stem Rust of Wheat. Australian Journal of Agricultural Research 56:323 – 327.

Luig, N.H. (1985). Epidemiology in Australia and New Zealand. In A.P. Roelfs & W.R. Bushnell, eds.Cereal Rusts Vol. II; Diseases, Distribution, Epidemiology, and Control, p. 301-328. AcademicPress, Orlando.Pp. 345.

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

Mayfield, A.H. (1985). Efficacies of fungicides for control of stem rust of wheat. Australia Journal of Agriculture 25:440 – 443.

McIntosh, R.A., and Brown, G.N. (1997). Anticipatory breeding for resistance to rust diseases in wheat. Annual Review of Phytopathology 35: 311-326.

McIntosh, R.A. (1997). Breeding wheat for resistance to biotic stress. In: Braun, H.J., Altay, F., Kronstad, W.E., Beniwal, S.P.S. & McNab, A. (Eds.). Wheat: prospects for global improvement. Proc. 5th Int. Wheat Conf. 1996, Ankara, Turkey. Kluwer Acad. Publ. Dordrecht. The Netherlands. pp 71-86.

McIntosh, R.A. (1988). The role of specific genes in breeding durabe resistance in wheat and triticales. Pp1-9 in N.W. Simmonds and S.Rajaram eds. Breeding strategies for resistance to the rusts of wheat. CIMMYT: Mexico, D.F.

McIntosh, R.A., Wellings, C.R., and Park, R.F. (1995). Wheat Rusts. An Atlas of Resistance genes. (CSIRO Publications, East Melbourne, Australia)

McIntosh, R.A., Yamazaki, Y., Devos, K.M., Dubcovsky, J., Rogers, W.J., and Appels, R. (2003). Catalogue of gene symbols for wheat. In 'Proceedings of the 10th International Wheat Genetics Symposium'. Paestum, Italy. (Eds NE Pogna, M Romano, A Pogna, G Galterio).

Mendgen, K. (1981). Nutrient uptake in rust fungi. Phytopathology 71: 983-989.

Moseman, J.G., Nevo, E., and El-Morshidy, M.A. (1990). Reactions of *Hordeum spontaneum* to infectionwith two cultures of *Puccinia hordei* from Israel and United States. Euphytica 49: 169-175.

Mundt, C.C (1991) Probability of mutation to multiple virulence and durability of resistance gene pyramids: further comments. Phytopathology *81*: 240-242

Mwandemele, O.D. (1993).Hand book of plant breeding, 2nd Ed, EMC, Egerton university, Njoro, Kenya. Pp 90-110

National Cereals and Produce Board, (2007). Control and regulation of cereals production, marketing, storage, distribution and standard measures. A report on barley. Nairobi, Kenya.

Nazari, K., Mafi, M., Yahyaoui, A., Singh, R. P., and Park R. F. (2009). Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici) race* TTKSK (Ug99) in Iran. Plant Disease 93(3): 317.

Nevo, E. (1992). Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum,* in the Fertile Crescent. Pp. 19-43 *in* P. R. Shewry, ed. Barley: genetics, biochemistry, molecular biology and biotechnology. C.A.B. International, The Alden Press, Oxford Pp. 543.

Njau, P.N., Jin,Y., Huerta-Espino, J., Keller, B., and Singh, P.R. (2010). Identification and Evaluation of Sources of Resistance to Stem Rust Race Ug99 in Wheat. Plant Disease 94: (4) 413-419

Njau P.N., Wanyera R., Macharia G.K., Macharia J., Singh R., and Keller B (2009). Resistance In Kenyan Bread Wheat to Recent Eastern African Isolate of Stem Rust, *Puccinia graminis* f.sp. *tritici,* Ug99. Journal of Plant Breeding and Crop Science 1(2): 022-027.

Owera, S.A.P., Farrar,J.F., and Whitbread, R. (1981). Growth and photosynthesis in barley infected with brown rust. Physiology of plant pathology 18: 79 – 90.

Ozgen, M. (1993). Environmental adaptation and stability relationships between grain yield and some agronomic traits in winter oat. Journal of Agronomy and Crop Science 170: 128-135.

Parlevliet, J.E. (1997.) Durable resistance. In: H. Hartleb, H., Heitefuss, R. and Hoppe, H.H. (Eds.). Resistance of crop plants against fungi. Gustav Fisher, Jena, Germany. pp. 238-253.

Parlevliet, J.E. (1978). Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica 27: 369-379.

Parlevliet, J.E., (1989). Identification and evaluation of quantitative resistance. In: Leonard, K.J. & Fry, W.E. (Eds.). Plant disease epidemiology - Genetics, Resistance and Management. Vol. 2 McGraw - Hill Publishing Company, New York. pp. 215-248.

Parlevliet, J.E. (1993). What is durable resistance, a general outline. In: Jacobs, Th. & Parlevliet, J.E. (Eds.) Durability of disease resistance. Kluwe Academic Publisher pp. 23-39.

Parlevliet, J.E., and Van Ommeren, A. (1975). Partial resistance of barley to leaf rust, *Puccinia hordei* II. Relationship between field trials, micro plot tests and latent period. Euphytica 24: 293-303

Peterson, R.F., Campbell, A.B., Hannah, A.E. (1948). A diagramic scale for estimating rust intensity of leaves and stems cereals. Canadian Journal of Research 26: 496-600.

Pretorius, Z. A., Singh, R.P., Wagoire, W.W., and Payne, T.S. (2000). Detection of virulence to wheat stem rust resistance genes *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Disease 84: 203.

Pretorius, Z. A., and Park, R. F. (2008). Origin, evolution, distribution and virulence of Ug99 and the global stem rust monitoring system. In: International Conference on Wheat Stem Rust Ug 99- A Threat to Food Security. November 6th -8th , 2008. NewDelhi, India.

Prabhu, K. V. (2008). Marker assisted shuttle breeding and gene pyramiding strategies for bringing durable resistance. In: International Conference on Wheat Stem Rust Ug 99- A Threat to Food Security. November 6th -8th, 2008. NewDelhi, India.

Ransom, J.K., and McMullen, M.V. (2008). Yield and Disease Control on Hard Winter Wheat Cultivar with Folior Fungicides. Agronomy Journal 100: 1130 – 1137.

Rees, R.G. (1972). Uredospore movement and observations on the epidemiology of wheat rusts in North-eastern Australia. Australian Journal of Agricultural Research 57:215-223.

Roberts, P.A. (1985). Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. Annual Review Phytopathology 33:199-221.

Roelfs, A.P. (1977). Foliar fungal diseases of wheat in the People's Republic of China. Plant Disease

Report 46: 836-841.

Roelfs, A.P., Singh, R.P., and Saari, E.E. (1992). Rust Diseases of Wheat: Concepts and Methods of Disease Management. Mexico, DF: CIMMYT.

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

Rostoksa, N., Steffenson, B.J., and Kleinhofs, A. (2004). Structure and Expression of the Barley Stem Rust Resistance Gene Rpg1 Messenger RNA. Physiological and Molecular Plant Pathology 64: 91-101.

Scheffer, R.P. (1997). The nature of Disease in Plants. Cambridge University Press.Pp. 56.

Schonbeck, F., and Steiner, U. (1997). Induced resistance. Pages In: Hartleb, H., Heitefuss, R. and Hoppe, H.H. (Eds.) Resistance of Crop plant against fungi. Gustav Fischer, Jena, Stuttgart. pp. 272-297.

Shaner, G., and Finney, R.E. (1977). The effect of nitrogen fertilizer on the expression of slow-mildew resistance in Knox wheat. Phytopathology 67:1051 -56.

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y. Njau, P., Wanyera, R ., Herrera-Foessel, .S.A. Bhavani, S., Singh, D., and Singh, P.K. (2008). Global Status of Ug99 Spread and Efforts to Mitigate the Threat. In: International Conference on Wheat Stem Rust Ug 99-A Threat to Food Security. November 6th -8th ,2008. NewDelhi, India. Pp. 15 - 20

Singh, R.P. (2004). Breeding for resistance in CIMMYT wheat germplasm: Progress and challenges. In: 12th proceedings of regional wheat workshop East, Central and South Africa. Nakuru, Kenya.Pp. 130 – 200.

Singh, R.P., Hodson, D.P., Jin, Y., Huerta-Espino, J., Kinyua, M.G., Wanyera, R., Njau, P., and Ward, R.W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 1 (No. 054), 13 pp.

Singh, R.P., Rajaram, S., Miranda A., Huerta-Espino, J., and Autrique, E. (1998). Comparison of two crossing and four selection schemes for yield, yield traits, and slow rusting resistance to leaf rust in wheat. Euphytica 100: 35-43.

Smedegaard – Peterson, V., and Tolstrup, K. (1985). The Limiting Effects of Disease Resistance on Yield. Annual Review of Phytopathology 23:475 – 490.

Stakman, E.C. Stewart, D.M., and Loegering, W.Q. (1962). Identification of physiological races of *Puccinia graminis* var *tritici.* U.S Department of Agriculture Research Service, U.S.A.

Staples, R.C., and Macko, V. (1984). Germination of urediospores and differentiation of infection structures. In The Cereal Rusts, Vol. 1 (Roelfs, A.P., and Bushnell, W.R., eds). Orlando, FL: Academic Press, pp. 255-289.

Steel, R.G.D., and Torrie, J.M. (1980). Principles and Procedures of Statistics. A Biometrician Approach. 2nd Edition, McGraw-Hill Book Company, New York .Pp. 23

Steffenson, B.J. (1992). Analysis of durable resistance to stem rust in barley. Euphytica 63:153-167.

Steffenson, B.J., and Jin, Y. (2006). Resistance to race TTKS of *Puccinia graminis* f. sp. *tritici* in barley. Phytopathology 96: 110 -115.

Steffenson, B. J. , Jin, Y., Brueggeman, R.S., Kleinhofs, A. and Sun, Y.(2010). Resistance to Stem Rust Race TTKSK Maps to the rpg4/Rpg5 Complex of Chromosome 5H of Barley. Phytopathology 99: (10) 1135-1141.

Steffenson,B.J., Olivera, P., Roy, J.K., Jin, Y., Smith, P.K. and Muehlbauer, G.J. (2007). A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Australian Journal of Agricultural Research, 58: 532-544

Sun, Y., and Steffenson, B.J. (2005). Reaction of barley seedlings with different stem rust resistance genes to *Puccinia graminis* f. sp. *tritici* and *Puccinia graminis* f. sp. *secalis*. Canadian Journal of Plant Pathology 27: 80-89.

Truberg, B., and Huhn, M. (2002). Contributions to the analysis of genotype1· environment interactions: experimental results of the application and comparisonof clustering techniques for the stratification of field test sites. Journal Agronomy and Crop Science. 188, 113-122.

USDA,(2008). Wheat stem rust. <http://www.ars.usda.gov/Main/docs.htm?docid=9910>. Accessed on 16th September 2008.

Voltas, J., Lo´ pez-Co, H.,´ rcoles, and Borra´ s, G. (2005). Use of biplot analysis and factorial regression for the investigation of superior genotypes in multi-environment trials. European Journal Of Agronomy 22: 309-324.

Von Bothmer, R., Sato K., Komatsuda, T., Yasuda S., and Fishbeck, G. (2003). The domestication of cultivated barley. In 'Diversity in barley (*Hordeum vulgare*)'. (Eds R von Bothmer, T van Hintum, H Knupffer, K Sato) pp. 9-27. (Elsevier: New York).

Walker, J.C. (1957). Plant pathology.3rd edition McGraw-Hill book Company New York pp 425-525.

Wamatu, J. N., and Thomas, E. (2002). The influence of genotype-environment interaction on the grain yields of 10 pigeon pea cultivars grown in Kenya. Journal of Agronomy and Crop Science 188: 25-33.

Wanyera, R., Kinyua, M.G., Jin, Y., and Singh R.P. (2006). The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Disease. 90: 113 - 117.

Wanyera, R. (2008). Chemical and Cultural Management of TTKSK (Ug99) Of Wheat Stem Rust Pathogen In Kenya. In: International Conference on Wheat Stem Rust Ug 99- A Threat to Food Security. November 6^{th} -8^{th}, 2008. New Delhi, India.

Wanyera, R., Macharia, G.K., Kilonzo, S.M., and Kamundia J.W (2009). Foliar Fungicides In the Control of Wheat Stem Rust, Race TTKS (Ug99), In Kenya. Plant Disease 93: 9 - 113.

Wiese, M.V. (1977). Compendium of Wheat Diseases. The American Phytopathology society St Paul Minnesota pp35-40.

Wiethölter, N., Graessner, B., Mierau, M., Mort, A.J. and Moerschbacher, B.M. (2003a). Differences in the methyl ester distribution of homogalacturonans from near-isogenic wheat lines resistant and susceptible to the wheat stem rust fungus. Molecular Plant-Microbe Interaction 16: 945-952.

Wiethölter, N., Horn, S., Reisige, K., Beike, U. and Moerschbacher, B.M. (2003b). In vitro differentiation of haustorial mother cells of th wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*, triggered by synergistic action of chemical and physical signals. Fungal Genetic Biology 38: 320-326.

Williams, K.J. (2003). The molecular genetics of disease resistance in barley. Australia Journal of Agriculture Research 54: 1065-1079.

William, R.B., and Allan, R. (1984). The cereal Rusts, Academic press INC. Orlando, FL. PP. 200.

Welty, R. E. and Barker, R.E. (1992). Evaluation of resistance to stem rust in perennial ryegrass grown in controlled and field condition. Plant Disease 76:637 – 641.

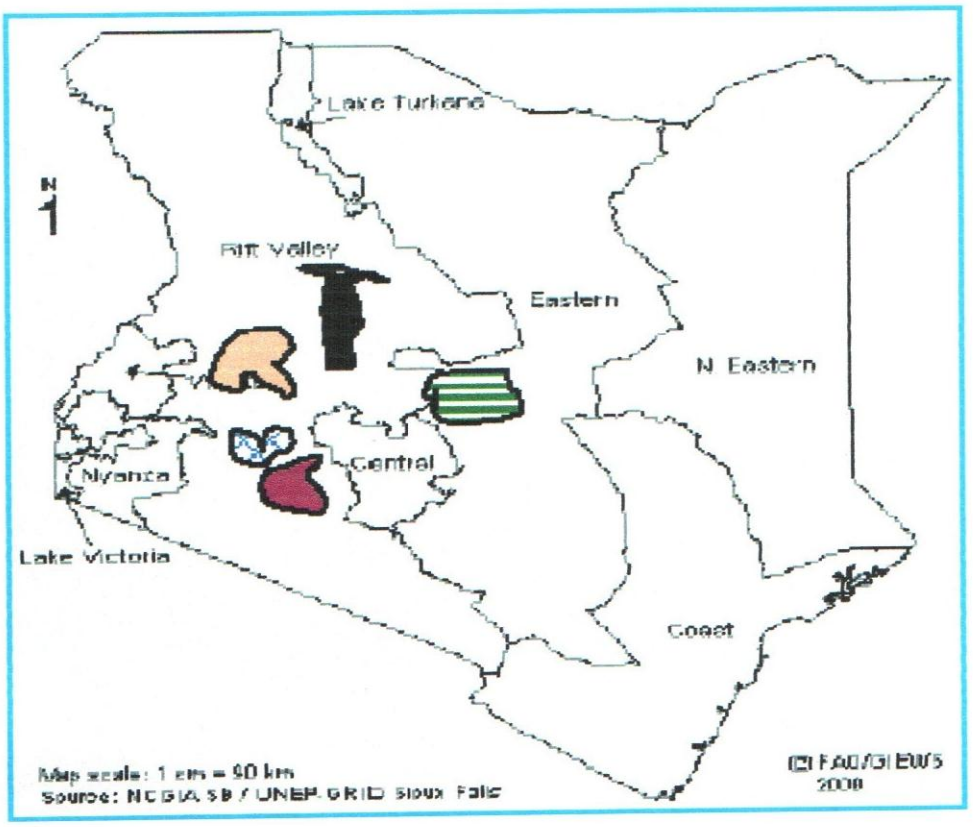
Yirgou, D., and Caldwell, R. (1968). Stomatal penetration of wheat seedlings by stem and leaf rusts in relation to effects of carbon dioxide, light, and stomatal aperture. Phytopathology 58: 500-507.

Zadoks, J.C. (1963). Epidemiology of wheat rust in Europe. FAO Plant Protection Bulleting 13:97-108.

Zadoks, J.C., and Bouwman, J.J. (1985). Epidemiology in Europe. In The Cereal Rusts, Vol. 2 (Roelfs, A.P. and Bushnell, W.R., eds). Orlando, FL: Academic Press, pp. pp. 329-369.

Zadoks, J.C., Chang, T.T. and Konazak, C.F. (1974). A decimal code for growth stages of cereals. Weed research 14:415 – 421.

Appendix A. Map of barley growing locations in Kenya

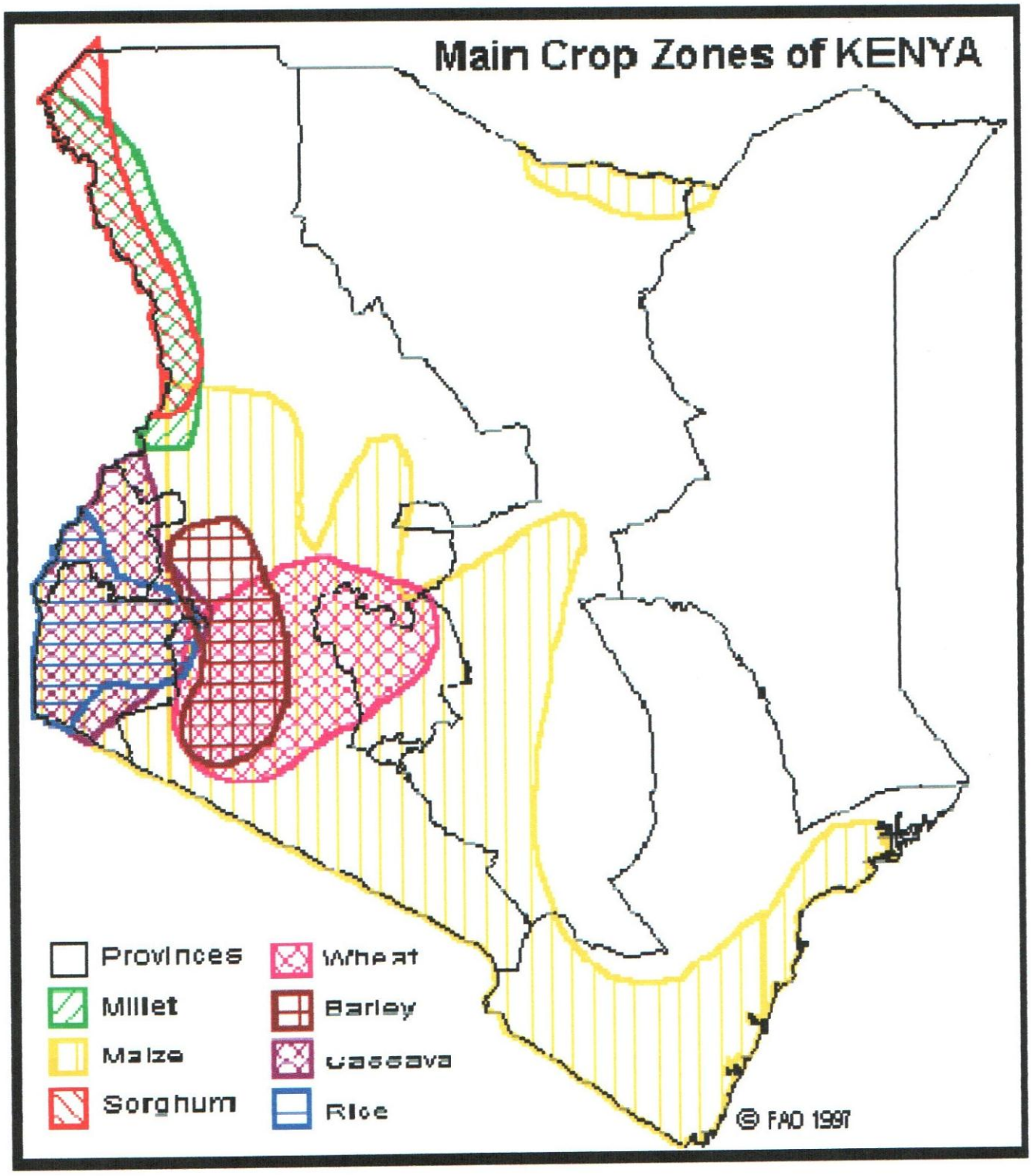


Key

- Timau near Mt Kenya
- Moiben near Eldoret town
- Nakuru*
- On the wetter escarpment of Samburu district near Maralal town
- Molo near Nakuru town

**Although Nakuru has potential, the soil has high nitrogen content, which is not suitable for growing barley for beer brewing*

Appendix B. Crop production areas in Kenya



Appendix C. Kenya Yearly Barley Production (1000 MT)

Market Year	Production	change
1995	90	0.00 %
1996	90	0.00 %
1997	90	0.00 %
1998	90	0.00 %
1999	60	-33.33 %
2000	75	25.00 %
2001	75	0.00 %
2002	75	0.00 %
2003	75	0.00 %
2004	75	0.00 %
2005	75	0.00 %
2006	75	0.00 %
2007	75	0.00 %

Year of Estimate: 2007, **Source:** United States Department of Agriculture

Appendix D. Barley Production in African Country in 1000 MT

	Country	Production (1000 MT)
1	Ethiopia	2,500.00
2	Morocco	763.00
3	Algeria	700.00
4	Tunisia	520.00
5	South Africa	205.00
6	Egypt	160.00
7	Libyan Arab Jamahiriya	85.00
8	Kenya	75.00

Year of Estimate: 2007, **Source:** United States Department of Agriculture

Appendix E. Monthly total rainfall (mm), minimum and maximum temperature at the experimental sites in the year 2008 / 09

Month	Rainfall (mm)	Minimum temps(^{0}C)	Maximum temps(^{0}C)
January (2008)	20.6	7.65	23.74
February	4.2	8.66	24.38
March	70.6	8.90	24.16
April	112.7	8.9	21.7
May	62.3	7.77	22.97
June	49.8	9.43	22.87
July	89.8	9.1	22.20
August	86.4	8.48	22.10
September	83.2	9.1	23.43
October	155.1	9.87	21.22
November	94.0	9.7	22.17
December	3.4	8.26	24.26
January (2009)	21.7	8.77	25.19
February	5.7	7.75	26..71
March	24.8	8.80	28.10
April	173.3	9.40	25.60
May	62.7	9.42	23.77
June	13.6	7.57	23.70
July	42.2	7.10	23.30
August	56.3	9.13	24.80

Appendix F. Chemical characteristics of soils at the experimental site

Parameters	
pH	6.2
Organic carbon (%)	1.8
Total nitrogen (%)	0.33
Phosphorous (ppm)	321
Sodium (ppm)	50
Potassium (ppm)	676
Calcium (ppm)	2796
Magnesium (ppm)	151
Copper (ppm)	11
Zinc (ppm)	27
Manganese (ppm)	525
Iron (ppm)	32

Appendix G. Field design utilized in both season

	RIPLICATION I		RIPLICATION II		RIPLICATIONII		
	B O R		D E R		R O W S		
	UNPROTECT	PROTECT	PROTECT	UNPROTECT	UNPROTECT	PROTECT	
B	VAR3	VAR3	VAR18	VAR18	VAR13	VAR13	B
	VAR7	VAR7	VAR14	VAR14	VAR3	VAR3	
O	VAR10	VAR10	VAR4	VAR4	VAR11	VAR11	O
	VAR14	VAR14	VAR11	VAR11	VAR18	VAR18	
R	VAR17	VAR17	VAR6	VAR6	VAR8	VAR8	R
	VAR19	VAR19	VAR20	VAR20	VAR2	VAR2	
D	VAR13	VAR13	VAR10	VAR10	VAR9	VAR9	D
	VAR9	VAR9	VAR2	VAR2	VAR17	VAR17	
E	VAR4	VAR4	VAR17	VAR17	VAR6	VAR6	E
	VAR1	VAR1	VAR5	VAR5	VAR4	VAR4	
R	VAR6	VAR6	VAR13	VAR13	VAR20	VAR20	R
	VAR11	VAR11	VAR8	VAR8	VAR14	VAR14	
	VAR15	VAR15	VAR7	VAR7	VAR12	VAR12	
R	VAR20	VAR20	VAR3	VAR3	VAR16	VAR16	R
	VAR18	VAR18	VAR15	VAR15	VAR7	VAR7	
O	VAR16	VAR16	VAR1	VAR1	VAR19	VAR19	O
	VAR12	VAR12	VAR16	VAR16	VAR5	VAR5	
W	VAR8	VAR8	VAR1	VAR1	VAR1	VAR1	W
	VAR5	VAR5	VAR12	VAR12	VAR15	VAR15	
S	VAR2	VAR2	VAR19	VAR19	VAR10	VAR10	S
	B O R		D E R		R O W S		

Appendix H. Number of rows of the 20 barley genotypes

Serial No	Genotypes	No. of rows
1.	CBSS99MOO39IT-H-IM-IY-IM-IY-OM	6
2.	CBSS99MOO317T-AH-2M-IY-IM-IY-OM	2
3.	CBSS00YOO113T-A-OY-OM-2Y-OM	6
4.	CBSS99MOO429T-L-IM-IY-OM	6
5.	CBSW99WMOOO95T-B-IM-IY-IM-OM	6
6.	CBSS00YOOO48S-OY-OM-2Y-OM	6
7.	CBSSOOYOO475T-O-OY-OM-2Y-OM	6
8.	CBSSOOYOO236T-E-0Y-OM-2Y-OM	6
9.	CBSS99MOO349T-F-3M-IY-IM-OM	6
10.	CBSS99MOO468T-H-IM-IY-OM	6
11.	CBSSOOYOO479T-D-OY-OM-IM-OM	6
12.	CBSW98WOOO54S-BY-2M-IY-2M-IY-OM	6
13.	CBSS99MOO315T-F-IM-F-IM-IY-IM-IY-OM	6
14.	CBSSOOYOO225T-C-OY-OM-2Y-IM-OM	2
15.	CBSSOOYOO278D-G-OY-OM-2Y-OM	6
16.	1385-13	2
17.	NGUZO	2
18.	1512-5	2
19.	KARNE	6
20.	SABINI	2

Appendix 1. ANOVA of stem rust on barley season 1

Appendix I. 1: ANOVA of stem rust on 1000kernel weight season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	53.6270456	26.8135228	0.84	0.5392
VARIETY	19	828.3095502	43.5952395	1.37	0.0015*
Error	38	1211.813988	31.889842		
Corrected Total	59	2093.750584			

CV = 4.40%, *- Significant at 5% (0.05) level of probability

Appendix I. 2: ANOVA of stem rust on 50 tiller dry matter season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	262.291512	131.145756	0.66	0.5225
VARIETY	19	4370.426862	230.022466	1.16	0.0395*
Error	38	7545.94544	198.57751		
Corrected Total	59	12178.66382			

CV = 12.70%, *- Significant at 5% (0.05) level of probability

Appendix I. 3: ANOVA of stem rust on grain screening through 2.5mm sieve season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	19231.75229	9615.87615	28.92	0.7001
VARIETY	19	16189.09127	852.05744	2.56	0.0067*
Error	38	12636.97714	332.55203		
Corrected Total	59	48057.82070			

CV = 14.70%, *- Significant at 5% (0.05) level of probability

Appendix I. 4: ANOVA of stem rust on grain yield season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	198.391768	99.195884	0.40	0.6703
VARIETY	19	6475.121999	340.795895	1.39	0.1904*
Error	38	9325.67533	245.41251		
Corrected Total	59	15999.18910			

CV = 16.60%, *- Significant at 5% (0.05) level of probability

Appendix I. 5: ANOVA of stem rust on protein content season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	8.3870512	4.1935256	0.40	0.6708
VARIETY	19	183.1558964	9.6397840	0.93	0.0567*
Error	38	394.9487544	10.3933883		
Corrected Total	59	586.4917020			

CV = 2.40%, *- Significant at 5% (0.05) level of probability

Appendix I. 6: ANOVA of stem rust on starch content season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	14.43536958	0.75975629	0.73	0.7621
VARIETY	19	59.14053093	1.57026547	1.52	0.2324
Error	38	39.34081681	1.03528465		
Corrected Total	59	56.91671732			

CV = 0.60%, *- Significant at 5% (0.05) level of probability

Appendix I. 7: ANOVA of stem rust on leaf area season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	1820.776613	910.388307	3.10	0.5640
VARIETY	19	7085.200450	372.905287	1.27	0.2575*
Error	38	11143.44233	293.24848		
Corrected Total	59	20049.41939			

CV = 21.86 %, *- Significant at 5% (0.05) level of probability

Appendix I. 8: ANOVA of stem rust on tillering capacity season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	687.620549	343.810275	1.35	0.7252
VARIETY	19	6111.567525	321.661449	1.26	0.0658*
Error	38	9708.93287	255.49823		
Corrected Total	59	16508.12095			

CV = 17.18 %, *- Significant at 5% (0.05) level of probability

Appendix I. 9: ANOVA of stem rust on chlorophyll index season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	2177.372116	1088.686058	4.39	0.6192
VARIETY	19	7044.945149	370.786587	1.50	0.0026*
Error	38	9417.78981	247.83657		
Corrected Total	59	18640.10708			

CV =17.78 %, *- Significant at 5% (0.05) level of probability

Appendix I. 10: ANOVA of stem rust on germination percentage season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	251.240415	125.620208	0.53	0.5943
VARIETY	19	8082.841601	425.412716	1.79	0.0630*
Error	38	9047.52100	238.09266		
Corrected Total	59	17381.60301			

CV = 13.62 %, *- Significant at 5% (0.05) level of probability

Appendix I. 11: ANOVA of stem rust on plant height season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	89.6928334	44.8464167	1.44	0.8491
VARIETY	19	670.5519423	35.2922075	1.13	0.0087*
Error	38	1181.732731	31.098230		
Corrected Total	59	1941.977507			

CV =4.22 %, *- Significant at 5% (0.05) level of probability

Appendix I. 12: ANOVA of stem rust on zeleny content season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	80.10487883	40.0591833	0.95	0.5345
VARIETY	19	1482.124483	78.0243942	1.85	0.1715*
Error	38	1604.551263	42.225033		
Corrected Total	59	2521.724535			

CV =5.13 %, *- Significant at 5% (0.05) level of probability

Appendix I. 13: ANOVA of stem rust on gluten content season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	26.4437585	13.2218793	0.83	0.5447
VARIETY	19	369.0042624	19.4212770	1.22	0.0057*
Error	38	606.933781	15.971942		
Corrected Total	59	1002.381802			

CV =3.02%, *- Significant at 5% (0.05) level of probability

Appendix I. 14: ANOVA of stem rust final disease severity season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	863.33	431.67	5.26	0.6496
VARIETY	19	25341.25	1333.75	16.24	<.0001*
Error	38	3120.00	82.11		
Corrected Total	59	29324.58			

CV = 19.04%, *- Significant at 5% (0.05) level of probability

Appendix I. 15: ANOVA of stem rust area under disease progress curve season 1

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	912227.03	456113.52	2.66	0.0826
VARIETY	19	25622206.98	1348537.21	7.88	<.0001*
Error	38	6505668.97	171201.81		
Corrected Total	59	33040102.98			

CV = 25.87%, *- Significant at 5% (0.05) level of probability

Appendix J. ANOVA of stem rust on barley season 2

Appendix J. 1: ANOVA of stem rust on 1000 kernel weight season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	3.2046388	1.6023194	0.09	0.9110
VARIETY	19	170.3174345	8.9640755	0.52	0.004*
Error	38	651.8683866	17.1544312		
Corrected Total	59	825.3904599			

CV =2.99%, *- Significant at 5% (0.05) level of probability

Appendix J. 2: ANOVA of stem rust on 50 tiller dry matter season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	821.147859	410.573930	1.36	0.7684
VARIETY	19	6043.343386	318.070705	1.06	0.0292*
Error	38	11455.64907	301.46445		
Corrected Total	59	18320.14031			

CV =16.70%, *- Significant at 5% (0.05) level of probability

Appendix J. 3: ANOVA of stem rust on grain screening through 2.5 mm sieve season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	210.969732	105.484866	0.47	0.6267
VARIETY	19	4675.459168	246.076798	1.10	0.0853*
Error	38	8473.02498	222.97434		
Corrected Total	59	13359.45388			

CV=20.78%, *- Significant at 5% (0.05) level of probability

Appendix J. 4: ANOVA of stem rust on grain yield season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	1229.030920	614.515460	4.23	0.8220
VARIETY	19	3089.792003	162.620632	1.12	0.0372*
Error	38	5521.862468	145.312170		
Corrected Total	59	9840.685392			

CV =11.80%, *- Significant at 5% (0.05) level of probability

Appendix J. 5: ANOVA of stem rust on protein content season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	8.13786158	4.37567693	0.45	0.9678
VARIETY	19	126.731393	6.67069660	0.68	0.5106
Error	38	370.5088442	9.7502327		
Corrected Total	59	466.9880990			

CV =2.44%, *- Significant at 5% (0.05) level of probability

Appendix J. 6: ANOVA of stem rust on starch content season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	2.71386036	1.35693018	3.00	0.6180
VARIETY	19	9.17827832	0.48306728	1.07	0.0176*
Error	38	17.19377163	0.45246767		
Corrected Total	59	29.08591030			

CV = 0.76%, *- Significant at 5% (0.05) level of probability

Appendix J. 7: ANOVA of stem rust on leaf area season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	68.941670	34.470835	0.18	0.8347
VARIETY	19	3085.201703	162.379037	0.86	0.0336*
Error	38	7213.19791	189.82100		
Corrected Total	59	10367.34128			

CV = 17.09%, *- Significant at 5% (0.05) level of probability

Appendix J. 8: ANOVA of stem rust on tillering capacity season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	610.645248	305.322624	2.83	0.7150
VARIETY	19	2044.957674	107.629351	1.00	0.0845*
Error	38	4098.855445	107.864617		
Corrected Total	59	6754.458367			

CV =10.79%, *- Significant at 5% (0.05) level of probability

Appendix J. 9: ANOVA of stem rust on chlorophyll content season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	936.951380	465.102704	0.79	0.7076
VARIETY	19	68478.0230	3604.111511	6.09	0.0051*
Error	38	22476.18376	591.47852		
Corrected Total	59	38521.35816			

CV = 27.24%, *- Significant at 5% (0.05) level of probability

Appendix J. 10: ANOVA of stem rust on germination season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	277.384648	138.692324	1.06	0.556
VARIETY	19	6170.164840	324.745518	2.48	0.0084*
Error	38	4972.95063	130.86712		
Corrected Total	59	11420.50012			

CV = 8.92%, *- Significant at 5% (0.05) level of probability

Appendix J. 11: ANOVA of stem rust on plant height season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	394.4783586	197.2391793	8.32	0.6510
VARIETY	19	578.5732891	30.4512257	1.28	0.0092*
Error	38	900.705186	23.702768		
Corrected Total	59	1873.756834			

CV = 5.35%, *- Significant at 5% (0.05) level of probability

Appendix J. 12: ANOVA of stem rust on zeleny content season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	66.091686	33.045843	0.23	0.7947
VARIETY	19	2637.023349	138.790703	0.97	0.0113*
Error	38	5430.469153	142.907083		
Corrected Total	59	8133.584187			

CV = 14.41%, *- Significant at 5% (0.05) level of probability

Appendix J. 13: ANOVA of stem rust on gluten content season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	16.1062824	8.0531412	0.37	0.6959
VARIETY	19	454.1401705	23.9021142	1.09	0.4006
Error	38	836.073464	22.001933		
Corrected Total	59	1306.319917			

CV =4.65%, *- Significant at 5% (0.05) level of probability

Appendix J. 14: ANOVA of stem rust final disease season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	8.03	4.02	0.09	0.7865
VARIETY	19	2020.73	106.35	2.32	0.0035*
Error	38	1743.97	45.89		
Corrected Total	59	3772.73			

CV = 26.51%, *- Significant at 5% (0.05) level of probability

Appendix J. 15: ANOVA of stem rust area under disease progress curve season 2

Source of variation	DF	SS	Mean Square	F Value	Pr > F
REP	2	67824.30	33912.15	0.81	0.9872
VARIETY	19	1473856.73	77571.41	1.86	0.0015*
Error	38	1586994.37	41763.01		
Corrected Total	59	3128675.40			

CV = 31.95%, *- Significant at 5% (0.05) level of probability