- DIVERSITY OF ELITE KENYAN BREAD WHEAT MUTANT LINES IN STEM RUST (*Puccinia graminis* f. sp. *tritici)* RESISTANCE

BY

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without prior written permission of the author and/or University of Eldoret

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Declaration by the Supervisors

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

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ABSTRACT

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is a major constraint to wheat production in Kenya. The re-emergence of a new fungal race Ug99 and its variants have caused devastating losses to wheat production since the disease overcome many resistant genes that were used against stem rust and most commercial wheat varieties grown in Kenya are now susceptible to stem rust. Breeding for rust resistance is the best mechanism to manage this disease as it's economical, durable and environmentally friendly. The objective of this study was to determine the diversity in stem rust resistance and yield potential of selected elite bread wheat mutant lines. The study was divided into three experiments. The first experiment was to determine yield potential of the selected elite mutant lines in comparison to their parent varieties. The study found that two mutant lines SP-26 of Kwale (SP-K) and SP-21 of Njoro II (SP-N) gave significantly ($P \leq 0.05$) higher grain yield of 4.04 t/ha and 4.34 t/ha respectively. Their parent varieties SP-K and SP-N gave 1.91 t/ha and 2.92 t/ha respectively. The other mutant lines gave grain yield of between 0.89 t/ha – 2.32 t/ha. The commercial checks gave grain yield of between 0.69 t/ha – 2.27 t/ha. The second experiment was to determine the genetic diversity of the wheat genotypes using morphological characteristics and molecular markers. The study found out that wheat genotypes showed variations for morphological characteristics, being separated mostly by grain yield per spike, days to maturity, 1000 seed weight and number of tillers per plant. The SSR markers used in this study grouped the genotypes into two major clusters with four sub clusters with mutant lines clustering with their respective parents. The third experiment was to evaluate the diversity of stem rust resistance in the elite mutant lines in comparison with their parent varieties and commercial checks in the green house and field conditions. Ten polymorphic SSR markers were used and the genotypes exhibited different reactions to stem rust. Significant ($P \leq 0.05$) genotypic, location and seasonal effects were recorded in the field. The genotypes evaluated had high genetic diversity regarding their response to stem rust. From the genotypes evaluated, the most resistant genotypes were SP-21 and SP-26. The study added to the pool of knowledge through identifications of high yielding stem rust resistant genotypes that can be recommended to farmers for growing as new varieties.

DEDICATION

I dedicate this thesis with all my love to my father Clement Chemwok and mother Maria Chemwok, and to my entire family members Mary Chebichii Kebenei and children Patricia, Patrick, Precious, Caren, Michelle, Keren, Marion, Shalom, Ben, Dan, Angel and Jewel, my brothers, sisters, nieces, nephews, in-laws, Pastors and all my friends and colleagues whom together, regardless of whatever situation they found themselves, provided a conducive environment for my study. May God abundantly bless you.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
CIMMYT	International Centre for Maize and Wheat Improvement
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FDS	Final Disease Severity
GS	Growth Stage
GENSTAT	Generation Statistics
Gy	Gray
IAEA	International Atomic Energy Agency
IT	Infection Type
IR	Infection Response
KALRO	Kenya Agricultural Livestock Research Organization
Kwt	Thousand kernel weight (Test weight)
MVD	Mutant Variety Database
Pgt	*Puccinia graminis* f.sp *tritici*
r	Pearson coefficient of correlation (coefficient of correlation)
Sr	Stem rust resistance gene
SP	Single Plant
SSR	Simple sequence repeat
TTKSK	Stem rust race virulent to Sr 31
UPGMA	Unweighted pair group arithmetic mean

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Wheat (*Triticum aestivum.*L) is an important cereal crop worldwide (722.4 million tons per year) produced for domestic and commercial baking (FAO, 2017). Wheat contributes to food security in Kenya and his ranked second important cereal crop after maize. The area under wheat production in Kenya on average is 170,000 ha with an average annual production of 450,000 MT. Annual wheat consumption in Kenya is estimate at 1,200,000 MT and therefore, Kenya is a net importer of wheat, annually importing on average about 60% of its total wheat consumption requirements (FAO, 2017). But wheat production in Kenya is low compared to increasing demand. Wheat production in Kenya is constrained by unstable weather conditions, continued use of recycled seed, prevalence of stem rust race (Ug99) and sub-division of family-owned farms into smaller units (KALRO, 2016). With population growth, increased urbanization, gradual increase in incomes per head and changing diets, the demand for wheat products in Kenya will continued to exceed production and Kenya will continue to import its wheat requirements to meet domestic consumption (USDA, 2017). Importation of close to two-thirds of its wheat requirements for domestic use is a worrying trend to Kenya with fertile soils and where agriculture contributes nearly two thirds or 63 percent of the gross domestic products (GDP) (KIPPRA, 2013).

The climatic requirement for wheat production in Kenya is met at altitudes above 1,500 m above sea level, where daily temperatures are below 20 ^{0}C and annual rainfall above 800 mm per annum. In Kenya wheat is mostly grown in the Rift Valley regions of Uasin Gishu, Narok, Trans Nzoia, Nakuru counties and some areas of Central province region of Nyandarua, Nyeri and Timau areas. These areas have altitudes above 1,500m above sea level and receiving annual rainfall above 800 mm per annum (USAID, 2010).

Wheat production in Kenya is done by medium to large scale producers using capital intensive technologies. The numbers of small scale farmers is relatively smaller when compared to medium to large scale farms (Monroy et al., 2013). The small scale producers complain of prohibitive production expenses and low production output caused by use of non-certified seeds and low use of inputs (MOA, 2013). There situation is made worse since the wheat varieties grown by the small scale farmers are susceptible to stem rust (Njau et al., 2009).

The cost of key inputs such as certified seeds, fertilizers and pesticides is way high for the resource poor farmer. This leads to farmers spraying their fields when infestation is already too high due to high costs of fungicides which lead to low application of fungicides as recommended. The sub-division of large commercial land between family members and competition from other land use projects is also a major problem to wheat production (GOK, 2010). A large portion of the current wheat planting materials are susceptible to stem rust and farmers will continue to register low production as yield losses associated with stem rust under farm conditions in Kenya is about 70% but up to 100% yield loss can be recorded under severe disease condition (Wanyera et al., 2008).

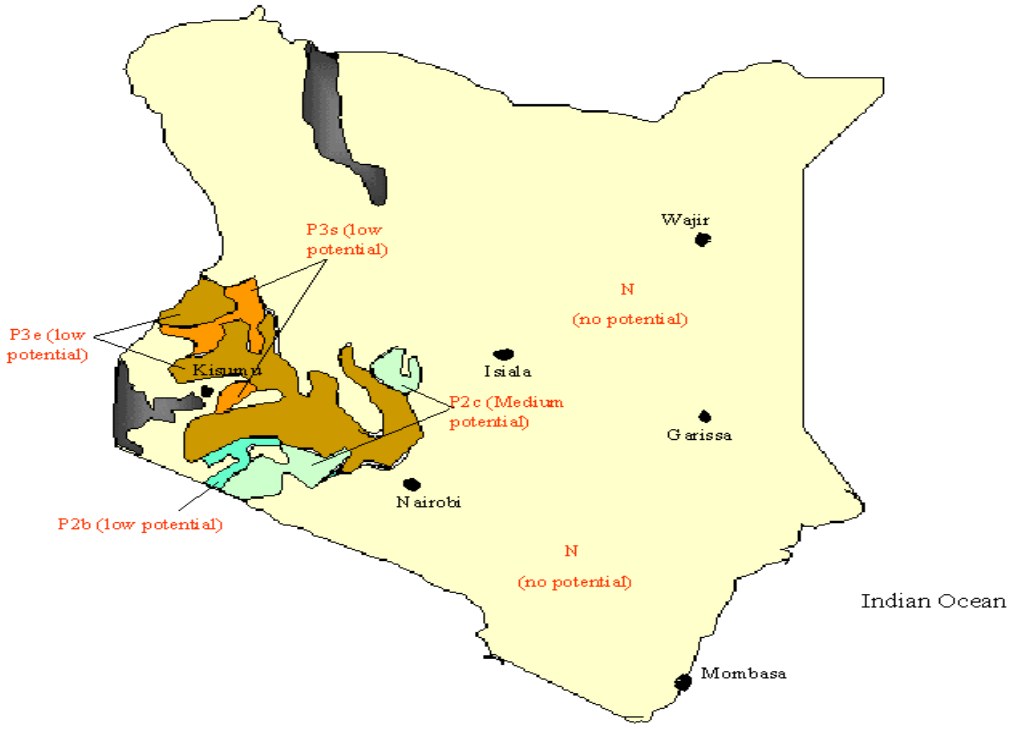


Figure 1: **wheat production in Kenya (Source: Survey of Kenya)**

Wheat is grown in approximately 170,000 Ha of Kenyan land. It's an important crop to achieving development in agriculture and is basically used for domestic and commercial baking (FAO, 2017). Land with potential for wheat production is available but the importation of close to two-thirds of Kenya's wheat requirement is a worrying trend. Wheat production in Kenya is faced by abiotic and biotic constraints including; drought, floods, outbreak of pest and diseases, soil infertility, inadequate machinery and equipments, rising cost of production and trends in the global wheat markets (MOA, 2013). But the most serious constraint facing wheat producers is the outbreak of pest and diseases, specifically stem rust race Ug99 and its variants (Njau et al., 2009).

Stem rust disease was initially identified in Uganda in 1999 where the name Ug99 came from. Ug99 was first found in Kenya in 2000. Currently, more than 15 races of Ug99 linage of stem rust disease have been confirmed and reported in Africa and beyond (Singh et al., 2015).

Kenya is known to be the hot-spot for new virulent races of stem rust race Ug99 and the disease severity observed is high in low to mid altitudes areas. This is because over 95% of local commercial wheat varieties grown in Kenya are partially susceptible to highly susceptible to stem rust race Ug99 and its variants. Globally, since the discovery of Ug99 race about 80% to 90% of the wheat grown are susceptible to stem rust disease and often it leads to up to 70% yield loses (Singh et al., 2015).

In Kenya, it has been reported that when an outbreak of race Ug99 occurs, up to 100% yield losses is realized (Njau *et al.,* 2010). This is because the environment in the wheat growing areas is so diverse with wheat producers practicing continued cropping seasons. There will be a wheat crop that is ready for harvest and in the next field another crop that has just emerged. This encourages the pathogen to jump from one crop to the next crop and therefore a continual cycle that favors the pathogen. There is also a lack of suitable crop to be used in wheat crop rotation. There is also a low level of surveillances in affected areas which encourages the pathogen to mutate quickly and take up other important genes. The situation is worsened by the wheat producers who continue to grow susceptible wheat varieties to stem rust race Ug99 and other newly identified races because of high cost of purchasing certified seeds encouraging producers to save their own seed for the next season crop (Singh et al., 2011).

In practice, wheat producers have been managing stem rust disease through the use of chemicals, biological, physical and cultural methods like crop rotation and burning of wheat straws. However, there are issues with the use of these methods including; cost involved, accessibility, pathogens resistance to the fungicides used, lack of knowledge on acquisition and application of fungicides and the harmful effects of fungicides to human health and the environment (Khokhar *et al.,* 2014). Breeding of resistant varieties remains the most effective and efficient way to control the pathogens as genetic resistance is economically viable, durable and environmentally friendly strategy. To date over 70 genes have been designated for resistance to stem rust in wheat (McIntosh et al., 2014). Some of them (e.g *Sr2,Sr5, Sr6, Sr21, Sr31, Sr33* and *Sr44*) were discovered in bread wheat and from other wheat species. Unfortunately, stem rust race Ug99 carries virulence against *Sr31* gene which was effective against all the existing stem rust races and the breakdown of *Sr31* gene caused the emergence of other newly identified races. Breeding for stem rust resistance in Kenya has been ongoing (Singh *et al.,* 2011).

Currently we have eight races of Ug99 and its variants showing that the pathogen is evolving at an alarming rate. Although some exotic genes have shown resistance to race Ug99 of stem rust, their introgression into the adapted local varieties has been complicated because *Sr* genes in these wheat relatives are not adapted to our local conditions. This requires many years to develop locally adaptable varieties with these exotic genes yet an urgent solution is required. It's therefore imperative that an alternative resistance is sought and generating variability within locally adapted varieties is one such option (Pretorius *et al.,* 2012).

Mutation technique is one of the breeding methods used in wheat breeding for developing mutant lines with disease resistance and increased agronomic value. Genetic variability as a result of induced mutation by various mutagens has contributed to modern plant breeding in the development of superior plant varieties with increased yield, early maturity, disease resistance, lodging resistance, improved quality among others (Kharkwal et al., (2009).Several achievements in wheat improvements through mutation breeding have resulted in improved varieties that are directly used for commercial cultivation or are available as genetic stocks with improved characters and better combining ability traits (Roychowdhury et al., 2013). Classical breeding and cross breeding requires five or more years to eliminate unwanted characteristics from the genotype and to develop the sought after desired traits. More than 3,222 mutant varieties including numerous crops, ornamentals and trees have officially been released for commercial use in more than 210 plant species from over 70 countries (IAEA, 2015). In Kenya, mutation breeding has been used successfully to generate drought tolerant wheat varieties (Njau et al., 2005).

In this study, through the use of gamma irradiation rays at Seibersdorf laboratories, Vienna Austria, seeds from two local wheat varieties Kwale (SP-K) and Njoro II (SP-N) were irradiated and resistant plants were selected to generate mutant lines. This study was carried out to evaluate selected mutants lines developed through mutation induction for yield gain, stem rust resistance and genetic diversity. Mutation induction on varieties Njoro II (SP-N) and Kwale (SP-K) generated mutant lines with both field and seedling resistance to stem rust. Induced mutations have contributed to significant increase in crop production at locations people could directly access ((Kharkwal et al., (2009).

1.2 Statement of the Problem

Wheat is the second most important cereal crop after maize in Kenya. It is a central crop to achieving development goals in agriculture contributing to food security (Mahagayu et al., 2007). The national annual wheat output is estimated at 350,000 MT grown in approximately 150,000 ha of land contributing about 40% of total annual consumption which stands above 900,000 MT per annum (USDA, 2013). Kenya imports about 60% of its wheat requirement for domestic consumption yet there is sufficient fertile land for wheat is production (FAO, 2012).

The decline in wheat production is attributed to unreliable weather conditions, high cost of production and infestation by pests and diseases (GOK, 2010). The re-emergence of stem rust race Ug99 and its variants have caused devastating crop losses (Njau et *al*., 2009). The pathogen is virulent to gene *Sr 31* and its variants are virulent to genes *Sr 24* and *Sr 36* which was used widely throughout the world to offer protection to wheat against stem rust disease. The current available wheat varieties are susceptible to stem rust race Ug99 and its variants. This disease is usually controlled by fungicides, cultural, biological and physical control methods. However the cost of fungicides is too high and it has hazards to the environment. Its accessibility and use have been a constraint to most wheat producers leading to low production (GOK, 2010). This has led to reduced acreages under wheat production as more wheat producers opt for other competing commercial enterprises leading to continued dependence of wheat importation to bridge the deficit and this remains a big threat to Kenya's food security. Wheat being the second important cereal staple food means that the pathogen has potential to affect food security (Singh et al., 2011).

The causal agent for stem rust (*Puccinia graminis* f.sp. *tritici)* has currently more than 15 confirmed races in the Ug99 lineage which have been reported in Africa and beyond. This shows that the pathogen is evolving at alarming rate and there is continued fear that stem rust race Ug99 would still mutate against previously resistant materials thereby increasingly loosing their resistance (singh et al., 2015). There is therefore need to widen methods for continued evaluation of wheat genetic materials against stem rust disease in order to avail new varieties to the farmers with good level of resistance to stem rust.

1.3 Justification

Over 95% of the local commercial wheat varieties are susceptible to stem rust while there are a few older varieties showing some level of adult plant resistance (APR) to stem rust (Njau et al., 2010). While chemical control and cultural practices have been used to manage stem rust, its high cost considering the poor resource wheat farmers and the harmful effects posed to the environment has been a major constraint to usage of fungicides. Breeding for resistant varieties remains the most economically durable and environmentally friendly method of controlling stem rust in wheat (Gamalat et al., 2015). Wheat stem rust being a major foliar disease of wheat grown in Kenya and since most of the fungicides being used is expensive and ineffective as a control measure of stem rust. This leaves introgression of rust resistant genes (*Sr*) as a strategy used to develop commercial varieties with broad and durable resistance. But, effective resistance over time has been difficult to achieve due to changing nature of stem rust pathogen (Ravi et al., 2011).

This study was undertaken as part of an ongoing wheat improvement programme at the University of Eldoret which utilizes mutation techniques as a tool to identify new wheat

varieties that are high yielding and resistant to stem rust. Genetic variability as a result of induced mutation has contributed to plant breeding in the development of superior plant varieties with characteristics such as high yielding, early maturity, lodging resistance, increased flag leaf area, improved nutritional quality and disease resistance (Kharwal et al., 2009).

The dynamic nature of stem rust pathogen provides a continuous threat to released wheat varieties where some are rescinded soon thereafter. Mutation induction through irradiation of seeds with good genetics backgrounds and the selection of disease resistant lines is an attractive approach in breeding for resistance (Roychowdhury et al., 2015). Screening for disease resistance in the greenhouse and in the field helps to detect other forms of resistance in seedlings and adult plants (Singh et al., 2006). Hence the needs for continuous evaluations on selected plant materials against stem rust and wheat grain yield before recommending stable genotypes for release to farmers for growing as new wheat varieties (Sharma et al., 2012).

Several molecular markers have been tagged for disease resistance genes including SSR markers which have been used to identify resistant genes and to study genetic diversity within varieties (Eagles et *al*., 2001). With the aid of stem rust races, green house and field screening, sources of resistance to stem rust can be identified and utilized in wheat breeding programmes. This will cut down production costs, increase yields and guarantee optimum returns to the farmers. This will act an incentive to them to increase wheat production acreages hence contributing to a stable wheat production in Kenya.

1.4 Objectives

1.4.1 Broad Objective

To contribute to stable wheat production in Kenya by evaluation of selected high yielding stem rust (*Puccinia graminis* f. sp. *tritici)* resistant elite bread wheat mutant lines.

1.4.2 Specific Objectives

1. To evaluate yield potential of selected wheat mutant lines under diseased condition

2. To determine the genetic diversity of selected wheat mutant lines in comparison to the parent varieties using morphological and molecular markers

3. To evaluate selected wheat mutant lines for stem rust resistance.

1.5 Alternative Hypotheses

H_1 Selected mutant lines are different from their parents varieties and other commercial check varieties based on their yield potential.

H_2 Selected mutant lines have genetic differences from their parent varieties based on morphological and molecular markers.

H_3 Selected mutant lines are different in stem rust resistance from their parent varieties and other commercial check varieties based on the greenhouse, field and laboratory tests.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wheat production in Kenya

Wheat (*Triticum aestivum.*L) is one of the key staple crops for global food security providing more than 35% of the cereal calories to the world population (FAO, 2017). Wheat is believed to have originated from the Fertile Crescent region (Feldman et al., 2007) but is now cultivated worldwide. In 2017, world production of wheat was estimated at 722.4 million tons against an estimated consumption of 692 million tons, making it the third most produced cereal crop after maize which had 1,016 million tons and rice 745 million tons. Wheat production in 2014 was 730 million tons against a consumption of 714 million tons showing an almost equilibrium production and consumption trend. Globally, wheat is the leading source of cereal protein in human food providing more than 20% of the protein (FAO, 2017).

Wheat is an important cereal in Kenya to achieving sustainability in agriculture. It contributes to food security and is the second most important cereal crop after maize. The area under wheat production in Kenya is estimated at 170,000 Ha with annual production estimates of 450,000 metric tons and average consumption estimated at 1,200,000 metric tons (FAO, 2017). Demand for wheat products is increasing due to increased population growth, increased urbanization and changing diets. Due to increased consumption and

demand for wheat products, wheat yields must be increased as an important strategy to achieving food security goals (KALRO, 2016).

Wheat has been grown in Kenya since early 1900s and is the second most produced crop after maize (USAID, 2010). Early development of wheat was confined to large scale farms, but small scale farmers have taken up wheat farming but on smaller plots when compared to large and medium scale farmers (Monroy et al., 2013). Wheat is essentially a temperate climate crop and requires an optimum temperature of between 10 to 24°C for development. When temperatures exceed 35°C photosynthesis stops and the wheat plant is killed by heat. Wheat requires minimum amount of rainfall of between 800 to 1000 mm per annum and does not grow well under very warm conditions with high relative humidity because wheat diseases are generally encouraged by such climatic conditions (Singh et al., 2011).

Soils suited for wheat production are aerated, well drained and rich in organic matter with a soil PH which ranges from between 5.5 - 7.0. Bread wheat cultivars in Kenya are categorized into four different groups depending on their baking characteristics: Group one is considered weak wheat not ideal for baking but can be used for fodder or blended with other superior wheat for baking. Group two has red wheat with fairly good baking qualities. Group three has strong dispensable wheat that has good baking quality and can be used for pasta. Group four has white wheat used for confectionary and pasta. Small scale farmers complain of high cost of key inputs such as fertilizers, certified seeds and pesticides. A grain bushel weight is a critical factor to millers as it affects the baking characteristics of their products. Grains with lower bushel are rejected by milers or given

a lower grade and are used for animal feed (MOA, 2013). Therefore, safer and cheap methods of crop protection like host resistance is increasingly becoming a better option to resource poor farmers to manage their cost of production.

2.2 Phylogeny and genepools of wheat

Cultivated wheat and their close wild relatives belong to the genus *Triticum* L., a member of the tribe Triticeae, which contains over 300 species (Clayton and Renvoize 1986). The wheat genus *Triticum* consists of six species: *Triticum monococcum* L. (AA genome); *Triticum urartu* Tumanian ex Gandilyan (AA genome); *Triticum turgidum* L. (AABB genome); *Triticum timopheevii* (Zhuk.) Zhuk. (AAGG genome); *Triticum aestivum* L. (AABBDD genome); and *Triticum zhukovskyi* Menabde & Ericz. (AAAAGG genome) (Huang et al., 2002). These species are grouped into three sections: Sect. Monococcon (consisting of diploid species); Sect. Dicoccoidea (consisting of tetraploid species); and Sect. Triticum (consisting of hexaploid species) (Dubcovsky and Dvorak, 2007).

Of these species, *T. urartu* exists only in its wild form, whereas *T. aestivum* and *T. zhukovskyi* exist only as cultivated forms. The other species, *T. monococcum*, *T. turgidum* and *T. timopheevii*, have both a wild and a domesticated form (salamini et al., 2002). Among these species diploidy, tetraploidy and hexaploidy are quite common and are derived through hybridizations between *Triticum* and diploid species of the genus *Aegilops* as well as interspecies hybridization (Tsunewaki, 2009). Some of the *Aegilops* species played an important role as being the secondary gene pools of wheat

domestication. The hexaploid *Triticum* wheat emerged through natural hybridization between the tetraploid cultivars and diploid *Aegilops* and *Triticum* species. *T. aestivum* (AABBDD genome) is thought to have arisen through hybridization of *T. turgidum* with the wild wheat species *Aegilops tauschii* Coss. (DD genome) (Kihara 1944).

2.3 Origin and domestication of wheat

The evolution of the *T. turgidum* lineage wheat as crops was initiated when wild emmer wheat (*T. turgidum* subsp. *dicoccoides*) was brought into the process of domestication. Natural stands of wild emmer wheat occur widely across the arc of the Fertile Crescent and they form two genetically distinct populations: southern (including Israel, Palestine, Lebanon and Syria) and northern (including Turkey, Iraq and Iran) populations (Valkoun et al. 1998, Luo et al., 2007). The domestication site of emmer wheat has been sought out through molecular marker analyses to detect amplified fragment length polymorphisms (AFLPs). The results agree that the northern populations had a role in the domestication of emmer wheat. The AFLP studies indicate that Diyarbakir region in southeastern Turkey is a likely place for emmer wheat domestication (Özkan et al,. 2002, 2005).

2.4 The Stem Rust pathogen

2.4.1 Pathogen taxonomy and nomenclature

The taxonomy of cereal rusts was complicated by the morphological variation and specialization of rust on numerous different hosts. From the morphological taxonomy and analysis, var. *stakmanii* and var. *graminis*, were separated based on urediniospore size and numbers of urediniospore germ pores (Urban et al., 1984). Three distinct

morphological forms: P. *graminis* subsp. *graminis* var. *graminis*; P. *graminis* subsp. *graminis* var. *stakmanii*; and P. *graminis* subsp. *graminicola*. The distinction of subsp. *graminis* and subsp. *graminicola* was justified based on morphological features, host ranges and distinct evolutionary histories (Urban and Markova, 1984).

However, currently the causal fungi are directly named and the sub spies designation is not included (Stakman *et al.,* 1962); for example, P. *graminis* f.sp. *triticii*. A formae speciales (f.sp.) may be further subdivided into numerous races or pathotypes. Races, or pathotypes, constitute a taxon below the forma speciales level. These are characterized by differences in pathogenic reactions, resulting in different virulence patterns on a selected set of differential resistance genotypes within a host genus. The differential set for P. *graminis* f.sp. *tritici* has been used as a worldwide standard and still provides the basis for race classification. The advantage of this system is that it provides a taxonomic base and a means of comparison and communication (Stakman *et al.,* 1962).

2.4.2 Host range of *Puccinia graminis* f. sp. *tritici*

Stem rust is caused by the fungus *Puccinia graminis* f.sp *tritici* is specialized to their host to produce formae specialis (Cagas B, 1975). A formae specialis does not produce pustules on a species outside its range (Knott, 1989). On this basis, we have; *Puccinia graminis* f.sp *tritici* which is specific to wheat, barley and the relatives of wheat. For example *Puccinia graminis* f.sp *secalis* is specific to rye and related grasses while *Puccinia graminis* f.sp *avena* is specific to oats and related grasses (Knott, 1989). Stem rust as the name implies, infects the stem and also infects the leaves, sheathes, glumes

and awns. Stem rust is also called black rust associated with the black teliospores produced towards the end of the growing season of an infected wheat plant. The genus *Puccinia* belongs to the family of Puccinae in the order Urinidales of the class Basidiomycetes. Meiosis occurs in a Basidium and results in the production of four, haploid, single-celled basidiospores (Roelfs, 1984).

2.4.3 Symptoms of Stem rust disease

The infections of stem rust mainly occur on the stem and the leaf sheathes but may also occur on the leaf blades and glumes. Rust symptoms initially appear 8-10 days after infection as spots on the surface of the leaves and stem. The small pustule then enlarge and rapture of the host epidermis forming a mass of brick-red urediospores containing thousands of microscopic uredinispores (Leonard and Szabo, 2005). These pustules are linear or diamond shaped and may enlarge up to 10 - 80 millimeters in diameter and they appear as cinnamon brown and turn almost black as they develop. With the disease progression, much of the stem becomes chlorotic. The black brown teliospores are produced as the host matures. Stem rust pustules are large than those of leaf and stripe rust and are oval shaped. Pustule size can be affected by overcrowding resulting from large numbers of infection points and by extreme temperatures. Under severe infections, the stems weaken and lodge (Leonard and Szabo, 2005).

2.4.4. Life Cycle of *Puccinia graminis* f.sp *tritici*

The life cycle of *Puccinia graminis* f.sp *tritici* consists of continual uredinial generation. The urediniospores are airborne and can move from one plant to another and from field to

field. Primary inoculums may originate locally (endemic) from volunteer plants or can be carried from long distances (exodemic) by wind and deposited by rain (Roelfs et al., 1992). Like most fungi, urediniospores are the most important spores produced in enormous numbers through repeated infections of host plant. Stem rust has both sexual and asexual stages (Figure 6). The asexual stages occur on wheat and related grasses and the sexual stages occur on an alternative host, barberry (*Berberis spp* or *Mahonia Spp).* Stem rust is referred to as macro cyclic since it has five types of spore in the life cycle and heteroecious since it has an alternate host. Uredospores, teleutospores and basidiospores develop on wheat plants and pycnidiospores. The alternate hosts are important in areas which have cold winters and where asexual stage cannot survive but teliospores do (Leonard and Szabo, 2005). Stem rust pustules seen on wheat during its growing cycle are called uredia and produces urediospores. Urediospores contain two genetically different nuclei which are oblong in shape and reddish brown in colour. Most of the spores remain within the crop canopy in the absence of a strong wind. Wind carries spores sometimes for long distances but a more common phenomenon involve movement of spores from field to field over short distances (Pretorius et al., 2009).

Under conditions of free water on the wheat plants and optimum temperatures urediospores germinate. This is followed by the development of an aspersorium over the stomata opening through which an infection peg pushes and a vesicle develops in the sub-stomata cavity. Infection hyphae grow from the vesicle and produce haustoria mother cells. Infections become visible in 5-8 days and sporulation begins 7-14 days under optimum conditions (optimum temperature for germination is 15–20^{o}C). Where

alternative host is not available, wheat stem rust can survive entirely in the asexual stage (Roelfs, 1985). Towards the end of the growing season, uredia begin to turn black as urediospore production stops and production of black teliospores follows. Teliospores are resistant to weather extremes and can germinate only after alternate period of freezing and thawing or wetting and drying to give rise to basidiospores which are small and slightly oval shaped. Basidiospores are forcibly discharged in to the air and carried to alternative host where they germinate (Roelfs, 1985).

Once the alternate host is infected, flask-shaped pycnia develop on the upper surface of the leaf. In 7-14 days after the infection, the pycnia open and a viscous liquid (honey dew) containing pycniospores appears. The pycniospores function as male gametes and the pycnia are of two mating types, (+) and(-.). Successful mating can only occur between opposite types where pycniospores are transferred from one pycnium to another by insects attracted to the 'honey dew' or by splashing raindrops. Ultimately, pycniospores give rise to aeciospores which are then carried by wind to nearby wheat fields where they mate and initiate heavy early infections. The infections on wheat develop into uredia, thus completing the life cycle (Leonard and Szabo, 2005).

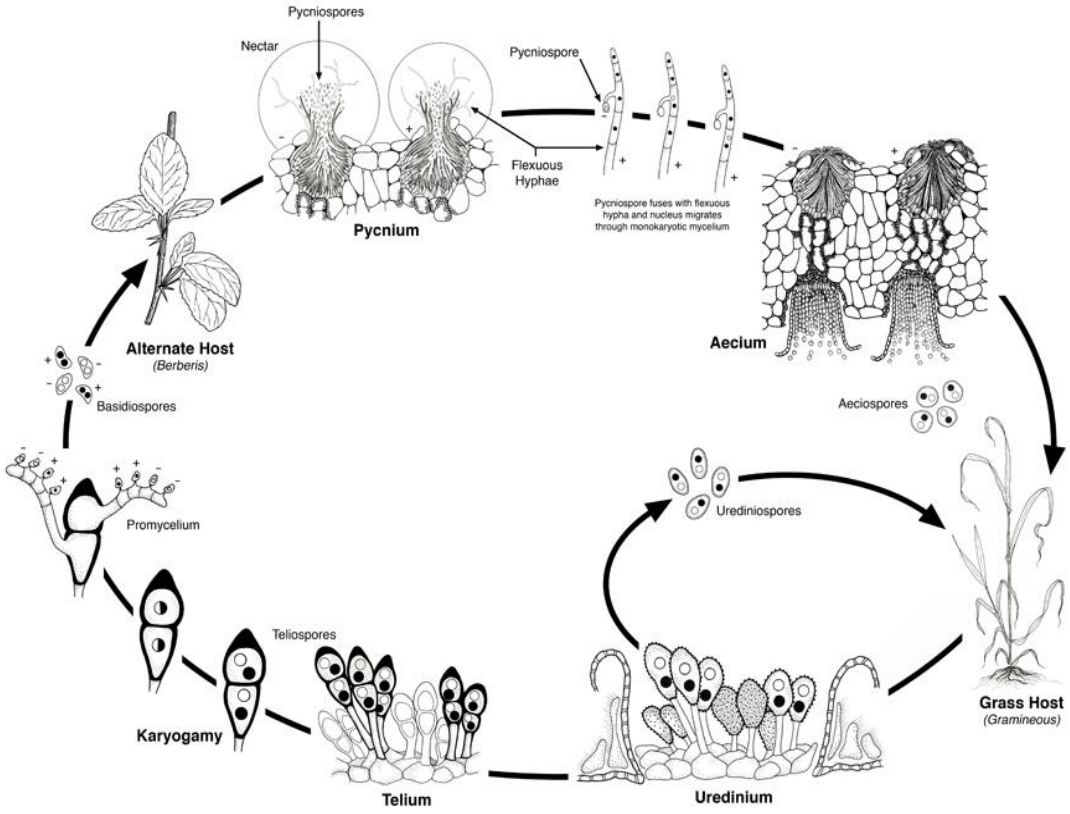


Figure 2: Reproduction cycle of *Puccinia graminis*(Leonard and Szabo, 2005).

2.4.5 Economic Importance of wheat stem rust

Stem rust reduces yields and quality of wheat grains and its ranked among the most important diseases of wheat. Stem rust associated yield losses of up to 100% have been reported in Kenya (NJau et al., 2010). Damage to wheat crop is experienced both as loss yield of yield in the field and loss of baking quality resulting from shriveled grains caused by poor grain formation. Introgression and deployment of resistant genes to commercial varieties circumvented stem rust production challenge. But the evolution and selection of new stem rust races with increased virulence has become more frequent

(Singh et al., 2011). Stem rust remains an important threat to wheat production worldwide and a threat to food security in Kenya.

2.4.6. Distribution and epidemiology of *Puccinia graminis* f.sp *tritici*

Stem rust is one of the most widespread diseases of wheat in all the wheat growing areas of the world including Asia, Europe, South and North America, Australia and Africa ((McIntosh et al., 1995). The distribution and ability of stem rust to move for long distances makes it an important disease of wheat worldwide. They have potential to develop rapidly under optimal environmental conditions, form new races that attacks previously resistant cultivars resulting in serious yield losses (Singh et al., 2011). It is only in the most extreme climatic regions such as very hot and dry tropical humid conditions that *Puccinia graminis* f.sp *tritici* does not occur (Prescott, 1984). Appropriate conditions such as source of inoculums, susceptible hosts, optimum temperature and humidity are required for rust germination and development (Roelfs et al., 1992). When these conditions occur, stem rust disease losses become more severe. New genes can also be introduced from outside a particular area and become established in new regions with subsequent spread to other regions where the new pathotypes will be different from those that were earlier introduced (McIntosh, 1995). Wheat rust urediniospores are principally disseminated by wind but migratory birds, animals, clothes, water, vehicles and implements also disseminate rust (singh et al., 2007).

2.4.7 Genetic variation and characterization of stem rust

The evolution and selection of new stem rust races with increased virulence has become frequent (Singh et al., 2013). There is new threat of stem rust owing to the re-emergences

of new set of stem races called the Ug99 family. A considerable variability in the races of stem rust manifested itself from year to year. *Puccinia graminis* f.sp *tritici* posses many physiological races and is also highly variable in pathogenicity (McIntosh et al., 1995). Discovery of races 1 and 2 by Stakman lead to the physiological races of stem rust which were based on the 12 differential host cultivars. The infection types were divided into 5 groups, with 0, 1 and 2 indicating host resistance and infection types 3 and 4 indicating susceptibility. The X infection type is considered separately as a heterogenous host response (Stakman et al., 1962). The physiological races were determined by a dichotomonous key of resistant and susceptible host responses with the heterogenous responses in a separate section of the key. The differential host selected by Stakman was used through the earlier years of the 80s (Roelfs et al., 1988). The method that has gained wide application is that developed and improved at the Cereal Rust Laboratory, St. Paul MN To designate a race, a coding system is employed. Twenty of the testers are divided into five sets of four lines (Jin *et al*., 2008).

When four lines are classified for resistance or susceptibility there are 20 possible combinations and are coded from B to T. The pathogenicity of a race is coded using five letters, each indicating one set of the four lines. Code BBBBB indicates the tester lines are resistant while TTTTT indicate the tester lines are susceptible (Jin et al., 2008).

Table 1: Pathogen code for the 20 North American differential hosts

Patho code	Infection Response (IR)			
B	Resistant	Resistant	Resistant	Resistant
C	Resistant	Resistant	Resistant	Susceptible
D	Resistant	Resistant	Susceptible	Resistant
F	Resistant	Resistant	Susceptible	Susceptible
G	Resistant	Susceptible	Resistant	Resistant
H	Resistant	Susceptible	Resistant	Susceptible
J	Resistant	Susceptible	Susceptible	Resistant
K	Resistant	Susceptible	Susceptible	Susceptible
L	Resistant	Susceptible	Susceptible	Susceptible
M	Resistant	Susceptible	Resistant	Susceptible
N	Susceptible	Resistant	Susceptible	Resistant
P	Susceptible	Resistant	Susceptible	Susceptible
Q	Susceptible	Susceptible	Resistant	Resistant
R	Susceptible	Susceptible	Resistant	Susceptible
S	Susceptible	Susceptible	Susceptible	Resistant
T	Susceptible	Susceptible	Susceptible	Susceptible

Source: (Jin et al., 2008)

2.5 Symptomatology

2. 5.1 Effects of stem rust on wheat

Stem rust reduces yields and quality of wheat and their products worldwide and its ranked among the most important wheat diseases affecting wheat production in Kenya, as up to 100% yield loses have been reported (Njau et al., 2010). Damage to wheat crop is both on yields loss and baking quality resulting from shriveled grains. Significant negative relationship between stem rust severity and yields, number of grains per spike, seed weight, grain yield per spike has been observed (Leilah and Al-Khateeb (2005).

In most wheat growing regions of the world, existing environmental conditions favor stem rust infection, which at times leads to epidemic build up (Singh et al., 2011). This situation is worsened by the fact that susceptible wheat varieties are grown over large areas and that large portion of the current breeding materials are susceptible to stem rust race Ug99 and other newly identified races. Stem rust disease will continue to have huge potential to cause wheat yield losses that would affect food security (Singh et al., 2011).

Over time the greatest challenge facing wheat production in Kenya and globally has been getting varieties that are resistant to the deadly Ug99 strain of stem rust. Breeding for stem rust resistance has been a big challenge by the advent of Ug99 and the new stem rust races. This is because most varieties earlier released had Sr31 which was effective against all the previous identified stem rust races. Most local commercial varieties grown in Kenya are susceptible while only a few older varieties showing some level of adult plant resistance has been identified (Njau et al., 2010). Currently, more than 15 confirmed races of Ug99 have been reported in Africa (Singh et al., 2015).

2.5.2 Evolution of stem rust race Ug99 and its effect on wheat crop

The first recognized variant in the family of Ug99 race was designated as TTKSK based on the effects on select host resistance genes differentials. It was first observed in a nursery in Uganda in 1999 (Jin et al., 2008). Similar virulence was observed in 2001 in Kenya and Ethiopia in 2003. Susceptible stem rust pustules were found on wheat lines known to have stem rust resistance gene Sr 31 and Sr38 resistance genes which was widely utilized in wheat breeding for resistance worldwide and for which virulence had not been reported previously anywhere in the world (Pretorius et al., 2000).

Since the discovery of Ug99 about 80% to 90% of wheat grown globally is susceptible to stem rust and often leading to up to 70% yield losses (Singh et al., 2006). In Kenya, stem rust associated yield losses of up to 100% have been reported (Njau et al., 2010). Susceptibility of wheat provide little barrier to the spread of this virulent race hence the risk of food security (USAID, 2010). Stem rust race Ug99 has mutated to overcome other known Sr genes, *Sr*5, *Sr*6, *Sr*7a, *Sr*8a, *Sr*8b, *Sr*9a, *Sr*9b, *Sr*9d, *Sr*9g, *Sr*10, *Sr*11, *Sr*12, *Sr*15, *Sr*16, *Sr*17, *Sr*18, *Sr*19, *Sr*20, *Sr*23, *Sr*30, *Sr*31, *Sr*34, *Sr*38 and *Sr* Wild (Jin *et al.*, 2008) have been overcome by race Ug99 (Singh et al., 2006). The additional new virulence has separated race TTKS into two different races: TTKST Virulent to Sr 31+Sr 24 and TTTSK Virulent to Sr 31+Sr 36 This makes the remaining Sr genes very vulnerable (Jin *et al.*, 2008). Through airborne transmission, Ug99 has reached Asia one of the main global wheat producing baskets. Stem rust has also been reported in Yemen in 2006, Iran in 2007 and Pakistan in 2009 (Hudson et al., 2009, Nazari et al., 2009). More than 15 races in the Ug99 linage have been reported in Africa (Singh et al., 2015).

2.6 Assessment of stem rust resistance on wheat

2.6.1 Inoculums production

Studies of cereal rusts require collection, isolation, bulking and preservation of inoculums. This procedure for spore increase is to select a susceptible host or a local host that is susceptible to the isolate to be increased but resistant to other isolates, this eliminates contamination problems. To initiate stem rust epidemic bulk stem rust fungi spores are collected from rust increase plots and spore suspension prepared.

2.6.2 Inoculation Methods

Spores can be placed on plants in a number of ways as illustrated in the Table 2 bel dow. The method adopted depends on the purpose of inoculation, number of plants to be inoculated, amount of inoculums and the occurrence of wet period during the inoculation process. Different carriers of inoculums are also available and the choice of the carrier will depend on the equipment and amount of spore's available (Roelfs et al., 1992).

Table 2: Methods of rust inoculation

Inoculation Method	Control of inoculums	Risk of Contamination	Spores	Equipment required	Cost	Labor
a)Dusting	Limited	High	Many	Duster	Low	Low
b)Brushing	Poor	High	Many	None	Low	Moderate
c)Infection	Excellent	Low	Few	Syringe	Low	Intensive
d)Spraying	Excellent	Moderate	Many	Sprayer	Low	Low

Source: (Roelfs et al., 1992).

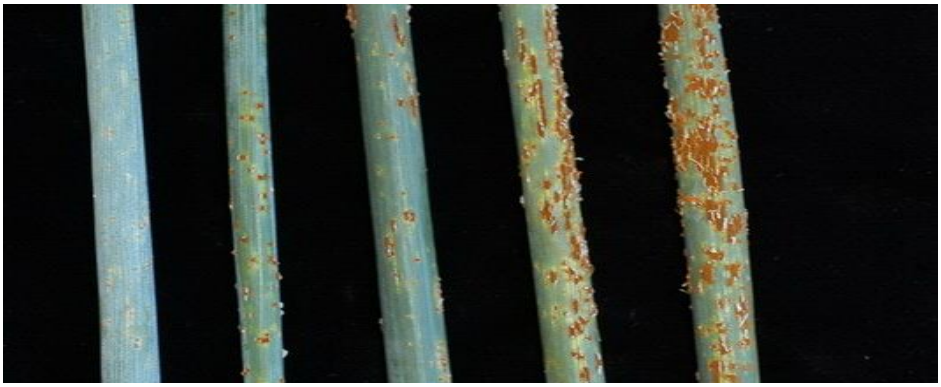
2.6.3 Disease Scoring

Scoring for adult plant resistance (APR)

Identification of sources of resistance and candidates genes remains important for breeding purposes. Breeding wheat varieties with durable resistance to stem rust disease is the best strategy to control stem rust (Singh et al., 2005). Host plant resistance to disease is effective, environmentally friendly and economically sustainable approach to control stem rust against wheat yield losses than the use of chemicals (Pretorious et al., 2007; Mccallum et al., 2016).

Modified Cobb scale (Peterson *et al.,* 1948) under field conditions was used in the adult plant resistance (APR) assessment of stem rust disease. It has two components; Diseases severity (DSS) and host response to disease infection (IR). Disease severity evaluation is an important decision support for adoption of strategies for disease control (Bade et al., 2011). Disease severity is used to determine the percentage of possible tissue infection expressed as a percentage 0 – 100% (Figure 4). This score is then weighed with infection response data (Figure 3) (McIntosh, 1995).

When presenting the results of severity and infection responses are presented together. Data comparisons are made with check cultivars, growth stage of test material and virulence of the pathogen population to the designated resistance. Screening for APR genes requires proper phenotyping to correctly detect their expression in the field. Often genotypes with APR may show susceptibility at the seedling stage, but at adult stage plant may show low disease severity scores (Herrera-Foessel et al., 2014).



R MR M MS S

Figure 3: Host Response to wheat stem rust (Roelfs *et al.*, 1992).

- Resistant (R)
- Moderately Resistant (MR)
- Combination of Moderately Resistant to Moderately Susceptible (M)
- Moderately Susceptible (MS)
- Susceptible (S)
- With other intermediate classes in between the major classes (RMR, MSS)

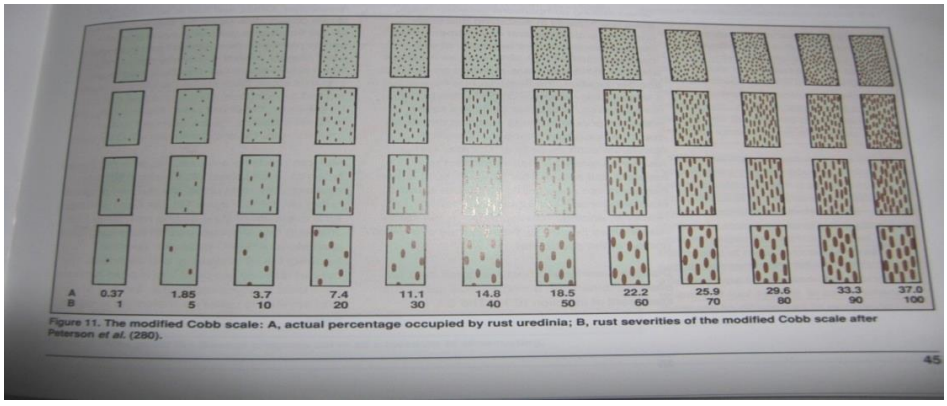
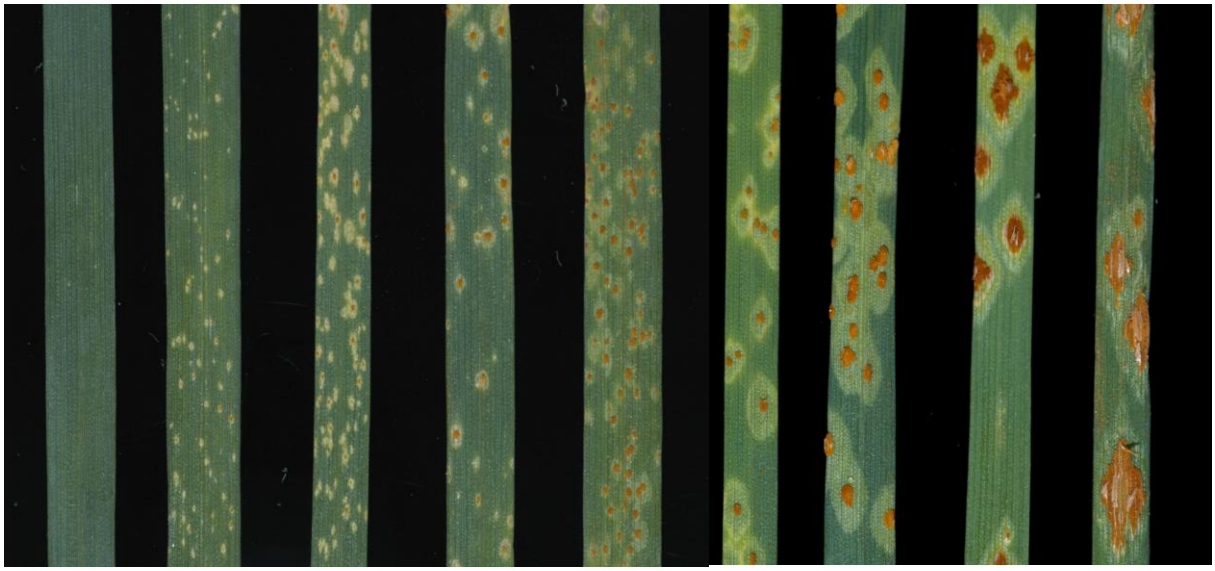


Figure 4 above: Wheat stem rust severity based on the modified Cobb's scale on 0-100% scale (Peterson *et al.*, 1972).

Scoring for seedling Infection Type (IT)

Infection types for uredinia on seedling leaves are commonly designated based on a scale developed by Stakman, (1962) which gives infection types as 0, 1, 2, 3 and 4. Where "0" is no disease and the genotype is resistant while "4" shows the highly susceptible genotype with large uredinia without necrosis or chlorosis. Flecking with small uredinia surrounded by necrosis or chlorosis was denoted "1". While small to medium sized uredinia surrounded by necrosis or chlorosis was denoted by "2", while moderate uredinia without necrosis or chlorosis was denoted "3". Variations with less or more than the average of a class was indicated by the use of superscript "+" for pustules that were slightly larger than expected, or "-" for pustules that was smaller than the normal size (Figure 5). Infection types "0", ";", "1", "2" or combination indicated low infection type. Infection type "3" to "4" was considered high infection types.



0 ; 0 ;1 1 2 2+ 3 3+ 4

Figure 5: Infection type of stem rust in wheat seedling leaves (Stakman *et al.*, 1962)

Table 3: Description of infection types of stem rust in wheat seedling leaves

Description	Infection type(IT)	Seedling Reaction
0	No uredinia or macroscopic signs of infection	Resistant
0;	No uedinia, but chlorosis or necrosis flecks present	Resistant
1	Small uredinia, surrounded by chlorosis or necrosis	Resistant
2	Medium uredinia surrounded by chlorosis or necrosis	Resistant
3	Medium sized uredinia without chlorosis or necrosis	Susceptible
4	Large uredinia without chlorosis or necrosis	Susceptible

(Adopted from Stakman *et al.*, 1962)

2.7 Wheat stem rust control

2.7.1 Cultural control method

The objective of cultural practices is to break the life cycle of stem rust. In Kenya, different regions have different growing periods and hence wheat is grown all year round and this leads to a green bridge which helps to carry over the spores from one season to the other (Monroy et al., 2013). Early infection arises from volunteer plants and delayed planting may help. Cultivation to remove volunteer plants, crop rotation, early planting and avoiding green bridge reduces sources of inoculums. Early maturing varieties have been used to control stem rust disease (McIntosh, 1995). Early planting was used to escape wheat stem rust epidemics before resistant varieties were introduced (Borlaug, 1954). Cultural practices have a small effect on stem rust disease severity but with other control measures, it plays a big role towards reducing severity (Monroy et al., 2013).

2.7.2 Chemical control method

Fungicides as foliar spray or seed treatment play an important role in the integrated management of wheat stem rust and effective control of stem rust have been achieved with standard application of fungicides (Njau *et al.,* 2009). The effectiveness of stem rust control using different fungicides for example Orious 25EC, Folicur 250EC, Silvacur 375EC and Amister Xtra 280SC amoung others have been tested. But, using fungicides to control stem rust is expensive, hazardous to the environment and the user (Wanyera et al., 2009). While breeding for resistance is an effective method of managing stem rust disease, most local commercial varieties of wheat are susceptible to stem rust (Njau *et al.,* 2010). Therefore, fungicides as foliar spray or through seed treatments still play a role in the integrated disease management (IDM) strategies until varieties with improved resistance are released. IDM combines biological, cultural, physical and chemical control strategies in a holistic way (Tadesse et al., 2010).

2.7.3 Biological control method

Biological control uses biological control agents for control of plant diseases. Biological control agents work by competing with the pathogen for space and nutrients through parasitism or predation. A large number of fungi have been identified as hyperparasites for rust fungi (Jeffries and Young, 1994). More than 100 strains of bacteria have been evaluated against rust under greenhouse conditions and the application of cultures of one strain each of *Pantaoe agglomerans* and *stenotrophononas maltophilia* is reported to have led to reduced rust severity comparable to the use of contact fungicides and there is potential for the use of biological control agents against rust fungi (Yuen et al., (2001).

2.7.4 Host plant resistance

Use of resistant varieties is an economically viable strategy of controlling stem rust when compared to the application of fungicides. To date there are over seventy genes that have been designated for resistance to stem rust (McIntosh et al., 2014). Resistant varieties are harmless to the environment and wheat producers do not need to incur high expenditure in purchasing expensive fungicides (Njau *et al.,* 2009). Some of the stem rust resistant (*Sr*) genes like *Sr*31, *Sr*36, and *Sr*24 have been widely deployed in commercial wheat cultivars. However, most varieties with these genes are now susceptible to stem rust race Ug99 (Singh et al., 2015).

Numerous studies on the inheritance of stem rust resistance have been undertaken and its generally acceptable that resistance to rust is controlled by a combination of major and minor genes (Steadman et al., 1995). The *Sr*2 gene, which is a slow rusting conditioner, has been introduced in the local varieties Kwale and Njoro II but these varieties do not have enough adult plant resistance (APR) against stem race Ug99 (Njau *et al.,* 2009). The gene *Sr*24 had been a good source of resistance to race Ug99 until 2007 when farmers started experiencing epidemics on wheat variety KS Mwamba (SP-M) which carries *Sr*24 (Singh *et al.,* 2007; Jin *et al.,* 2008). The effectiveness of *Sr*24 to new virulent variants of stem rust race Ug99 has been decreased (Jin *et al.,* 2008). The gene *Sr*31 had been widely used for long as a source of resistance against all known races of wheat stem rust until 1999 when the detection of race Ug99 was found to have overcome this resistant gene (Pretorius *et al.,* 2000; Macharia, 2009; Njau *et al.,* 2009). The gene Sr36 has been showing high to medium resistance to the original race Ug99 and was successfully used

in Africa to control stem rust until stem rust race Ug99 overcome this gene and rendered it ineffective against stem rust race Ug99 (Singh *et al*., 2007). Therefore, Sr36 is no longer recommended as a useful source of stem rust resistance against Ug99 (Jin *et al.,* 2009). The other new variants of stem rust race Ug99 have overcome most other *Sr* genes (Jin and Singh, 2006; Jin *et al.,* 2007; Pretoruis *et al.,* 2000; Pretorius *et al.,* 2010). Currently only a few other genes are still effective against stem rust race Ug99 such as *Sr*Tmp, which results in low infection type to race Ug99 (Singh *et al.,* 2007) and *Sr*1A/1R derived from *Secale cereale* has been detected on several hard red winter wheat cultivars (Jin and Singh, 2006). Other genes, including *Sr*22, *Sr*25, *Sr*35, *Sr*39, *Sr*40 and *Sr*44 confer moderate to high resistance to race Ug99 in seedling tests (Singh *et al.,* 2007; Jin *et al.,* 2008).

Wild relatives of wheat are important sources of new genes for cultivated wheat. In the past 40 years, several desirable genes, including approximately 20 stem rust resistance genes have been transferred into common wheat from its wild relatives by developing wheat-alien species chromosome translocations through chromosome engineering (Liu *et al.,* 2011). The limitation of these wild relatives' sources is the risk of introgression of undesirable characteristics in the highly adapted local varieties (Gill *et al*., 2011). Among the adult plant resistance genes, Sr2 gene has been studied widely. Combination of Sr2 gene and other known slow rusting resistance genes form the Sr2 complex which provides durable resistance to stem rust (McIntosh *et al.,* 2014). Breeding for durable resistance in wheat has led to global search for new genes or combination of genes to combat stem rust race Ug99 that could be realized as new varieties (Njau *et al.,* 2009).

2.7.5 Mutation breeding method

Mutation is one of the crop breeding methods that have been successfully used in wheat breeding to develop new mutant lines with disease resistance and increased agronomic value (Roychowdhury *et al.,* 2013). Mutation breeding is the application of mutation techniques for crop improvement. Through the use of induced mutations important traits with good agronomical such as shorter morphological mutants, increased tolerance to abiotic and biotic stresses have been develop (Shu *et al.,* 2012). The new stem rust race Ug99 and its variants have depleted most of the genetic resistance used to cover against stem rust disease. This has contributed to heavy losses in wheat crop production worldwide. Majority of Kenyan germplasm are known to be partially susceptible to susceptible Ug99 and its variants (Njau et al., 2009).

Nuclear technology through mutation induction offers the possibility to generate variations where the desired variations were lacking. If the desired genetic variability is not available in a crop, then mutation breeding is a logical step to generate it (Mba C., 2013). The range and number of desirable mutants induced will differ with the mutagen and genotype (Forster *et al.,* 2012). The genetic variability in the subsequent generations allows for the screening and selection of mutants with desirable traits which are then subjected to preliminary performance test for uniformity to evaluate the desired traits under selection. The best mutant with desired traits selected after agronomic evaluation will then be release as new a variety to growers for planting or to be used as parent in cross-breeding programs as source of particular alleles such as lodging resistance and disease resistance (Maluszynski *et al.*, 2009).

Over 232 crops and plants species have been subjected to mutation breeding, including wheat, rice, cotton and bananas. According to (FAO/IAEA, 2015), mutant varieties database (MVD) by the end of 2015 was 3,222 cultivars which have been obtained by mutation and officially released. Of these, 67% were directly selected from mutated generations following the use of radiation, mainly gamma rays.

Many useful mutants have been induced for various plant characters in wheat through mutagens. For proper expression of genetic yield potential, any wheat genotype should have proper combination of disease resistance, plant height, fertile tiller, spike length and seed weight. These characteristics could be genetically manipulated to permit maximum yield stability. To improve genetic gains and realize production increases in wheat mutation breeding, more efficient screening and selection methodologies and tools have been developed. Grain yields are dependent upon many phonological, morphological and physiological characters (Njau et al., 2005). Application of induce mutation through gamma rays lead to the development of eight mutant lines under review (SP-9, SP-16, SP-20, SP-21, SP-26, SP-29, SP-31 and SP-34). The wheat grains were selected from popular but susceptible wheat varieties Njoro II (SP-N) and Kwale (SP-K). They were sent to Seibersdolf labs of IAEA for irradiation with gamma rays and later mutant's population were generated from the irradiated seeds at the University of Eldoret screening laboratories. It's expected that the information generated from this study will guide wheat improvement programmes in Kenya and the selected mutants will be resistant to stem rust race Ug99 and its variants. This is expected to impact on wheat

production and positively improve food security status in Kenya and on other future scientific innovations.

2.8 Marker assisted selection

2.8.1 Application of modern technique in wheat improvement

Marker assisted selection is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical or molecular) linked to a trait of interest for example productivity, disease resistance, stress tolerance and quality. Use of biotechnology holds a great promise to increased food production through development of new crop varieties by generating and utilizing genetic variability (Shu et al., 2012). Its use has helped improve efficiency and speed up the breeding process. Long conventional process of wheat breeding up to six generations makes the system inconvenient where growers require urgent solutions. This application process has been used extensively in plant breeding (Ben-Ari et al., 2012).

2.8.2 Morphological markers

Morphological markers is one of the three (morphological, biochemical and molecular) types of genetic markers which have been utilized to study genetic diversity in wheat, inheritance studies and gene mapping. A genetic marker represents genetic differences between individual organisms or species (Collard *et al.*, 2005). Morphological markers are often detectable by eye, visible characters which are usually identified by simple visual inspection such as seed shape, presence or absence of awns, leaf sheath coloration, growth habits, grain colour or height. The major disadvantages of morphological markers are that, they may be limited in numbers and are influenced by the environmental factors

and the developmental stage of the plant. Despite these limitations, morphological markers are useful to plant breeders (Eagles *et al.*, 2001).

2.8.3 Biochemical markers

Biochemical markers include allelic variants of enzymes called isozyme. Isozymes markers are differences in enzymes that are detected by electrophoresis and specific staining. Despite the limitations of isozymes in numbers and influence by environmental factors or developmental stage of the plant, they have been used in plant breeding to identify valuable genes. The major applications of allozymes have been the elucidation of geographical patterns of genetic diversity, molecular linkage mapping, marker assisted selection and positional cloning of genes and gene clusters (Eagles *et al.*, 2001).

2.8.4 Molecular Markers

Molecular makers provide an efficient way to address problems faced in conventional breeding methods. Unlike morphological and biochemical markers DNA markers are practically unlimited in numbers and are not affected by environmental factors or developmental stage of the plant (Winter et al., 1995). DNA markers are abundant and arise from DNA mutations such as substitutions mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandem repeated DNA. These markers are selectively neutral because they are usually located in non-coding regions of DNA (Collards *et al.*, 2005).

Molecular markers have been used to monitor DNA sequence variation among species and create new sources of genetic variation by introducing new and favorable traits from land races and related species. Rust resistance genes can be tagged with tightly linked

DNA markers and selection based on these markers is used to improve the efficiency of breeding programs (Todorovska et al., 2009). Molecular markers are stable and have proven to be genetically informative and useful for genotype discrimination.

DNA markers are used for construction of linkage maps, assessing the level of genetic diversity within germplasm and variety identity. Gene pyramiding in which genes identified in different genotypes are deployed into a single cultivar that contains desirable alleles at more than one locus has become efficient (Joshi and Nayak, 2010).

Screening of breeding material for disease resistance genes using conventional approaches require time, as some genes express only at later stage of plant growth. Also disease innoculum has to be applied on plants which is dangerous in regions where particular pathogen race is not present. The development of molecular techniques for genetic analysis has lead to increase in knowledge of wheat genetics and understanding of the structure and behavior of wheat genome. Improvement in markers detection system used to identify markers linked to useful traits has enabled great advances to be made. The molecular markers provide an efficient way to address problems faced in conventional breeding methods (Joshi and Nayak, 2010). They are broadly divided into hybridization-based, polymerase chain reactions (PCR) and DNA sequence (Jahufer et al., 2003). Restriction fragment length polymorphism (RFLP) has been used to confirm patterns of genetic diversity earlier identified with biochemical markers.

But due to the long duration of procedure, the large amounts of DNA required, and the dangers of radioactivity, PCR markers such as Random Amplified Polymorphic (RAPDs) Simple Sequence Repeats (SSRs) and Sequence Characterized Amplified Regions (SCARs) are preferred. RAPDs markers have been used extensively to characterize

genetic diversity. The choice of markers to use will be dependent upon the objective under investigation (Farooq *et al.,* 1996).

Several RAPD markers associated with gene conferring resistance to rust have been identified but the applications of these markers have restricted because of reproducibility problems. SCARs markers have been used as alternative procedures to increase the reproducibility of RAPDs. SSRs are another PCR based molecular markers developed around segments of DNA in which a specific motif of one to six nucleotides is repeated in tandem and multiple times. These repeat regions have been found to be hyper variable possibly due to DNA polymerase slippage at repeats during the normal replications process. Normally the more repetitions of a repeat the more likely it is to be polymorphic. Length polymorphism is generally visualized by running products on polyacrylamide gels. Radioactive, florescent, silver staining or other techniques are used for detections (Maluszynski et al., 1995).

Nuclear SSRs show extreme high levels of allelic variation at individual loci. A high allelic variation makes SSRs the method of choice for studying gene flow, paternity and genetic bottlenecks in populations. The technique gives highly reproducible results and polymorphisms as it relies on specific primers. It can be used on lower quality DNA than dominant markers procedures. SSR analysis is the basis of modern forensic practice using small quantities of often poor quality DNA. An essential characteristic of SSRs vary in polymorphism depending on the length and sequence of the repeat motif they contain and their location in gene-coding or non-coding segments of the genome. In wheat SSR markers have been used to evaluate intra-specific diversity and fingerprint genetic diversity in wheat varieties (Blair *et al.,* 2003). Suitable DNA markers should be

polymorphic in the DNA level and have the capability of expression in all tissues, organs, and various developmental stages (Dudley J, 1993).

2.8.5 Gene mapping

Stem rust a damaging disease of wheat can be controlled by utilizing effective stem rust resistance genes. Major genes and quantitative trait loci for a wide variety of traits in wheat have been mapped (McCallum et al, 2008). Mapped stem rust resistance has been done in populations derived from crosses of a susceptible parent and developed resistant lines. The segregation of resistance in each population indicated the presence of a single gene. The new sequencing technologies have provided fast and cost-efficient strategies for mapping of complex genomes (Sourdille et al., 2004).

2.8.6 Molecular markers linked to stem rust resistance genes

Resistant varieties have effectively controlled stem rust for several decades but the emergence of stem rust race Ug99 has made these resistant varieties susceptible (Jin et al., 2007). Over 95% of Kenya's local commercial wheat varieties are partially susceptible or highly susceptible to Ug99 race of stem rust disease (Njau et al., 2010). Over 70 genes have been designated for resistance to stem rust (McIntosh et al., 2014). Of those 34 are ineffective against race stem rust race Ug99 (Singh et al., 2015). Among the adult plant resistant genes, Sr2 gene and other unknown slow rusting resistance genes forms the ''Sr2 complex'' which provide durable resistance to stem rust disease (McIntosh, 2008). Lr34 is another important disease resistant gene which confers resistance to stem rust (Singh et al., 2006). Several DNA markers linked to various stem rust resistant genes in wheat have been identified and developed. These genes include

Sr25 (Liu et al., 2011); Sr26 (Mago et al., 2005; Lui et al., 2010), Sr31 (Das et al., 2006), Sr35 (Zhang et al., 2010), Sr51 (Lui et al., 2011) and Sr53 (Lui et al., 2011).

2.9 Screening of stem rust disease and assessment of yield potential

2.9.1 Screening stem rust disease

Screening of wheat against stem rust race Ug99 in the greenhouse and field conditions has been undertaken in key wheat growing areas in Kenya (Singh et al., 2006). The long term strategy to control stem rust race Ug99 and its variants is to identify resistant sources among exciting materials and develop resistant varieties that can adapt to the prevalent high risk environments and release them to the farmers as new varieties after carrying out performance trials (Singh et al., 2006). Existing environmental conditions in most wheat growing areas favors infections of stem rust which leads to epidemic buildup. Genotype x Environment Interaction (GEI) need to be well characterized for better targeted genotype development and recommendation (Singh et al., 2011).

Rust resistant varieties have effectively controlled stem rust for several decades before the emergence of race stem rust race Ug99 and its variants whose virulence has been reported on most of the previously resistant varieties at both seedling and adult plant resistance stages (NJau et al., 2009). Seedling resistance to stem rust is regulated by major genes which can be completely effective against some races of the pathogen but can also be vulnerable to at least one other race of the pathogen hence not reliable. While Adult Plant Resistance (APR) is regulated by minor genes and can be used over a large area, for a long time especially when the host is exposed to a wide range of the pathogen but still remains resistant. This type of resistance is often undetectable at seedling stage

as opposed to adult plant stage and is known to be limited to specific physiological races of the pathogen (Lowe et al., 2011).

2.9.2 Assessment of yield potential

Screening of stem rust in the field allows the assessment of yield potential of genotypes in the presence of the disease. The development of durably resistance wheat varieties will help reduce farmers cost of production and the frequency of serious epidemics build up which will enhance wheat production. But durably resistant wheat varieties will be of less importance to the farmer unless it has other important traits such as high yield. Measurement of GEI for disease resistance and high yields enables breeders to identify adaptable genotypes that offer stable performance across a wide range of sites under high disease pressure. This will aid the development of an optimum breeding strategy for release of new wheat varieties adaptable to a particular target environment (Yan et al., 2005). Since the discovery of stem rust race Ug99, over 80% of wheat grown globally is susceptible to stem rust and often leadings to up to 70% yield losses (Singh et al., 2006). In Kenya, yield losses of up to 100% have been reported (Njau et al., 2010).

Superior advanced lines which combine both high yields and stem rust resistance will be selected to be included in regional and national performance trials for development of new varieties and those that can be used as parental lines for obtaining segregating population for stem rust resistance and yield related trials. Wheat grain yield is highly influenced by production environments and superior lines must be tested across different environments to determine their yield stability before it's recommended for release to the farmers as new varieties (Sharma et al., 2012).

CHAPTER THREE

EVALUATION OF YIELD POTENTIAL FOR SELECTED STEM RUST MUTANT LINES UNDER FIELD NATURAL INFESTATION

Abstract

Wheat (*Trticum aestivum* L.) production in Kenya is faced with a major threat of stem rust (*Puccinia graminis* f. sp *tritici*). The interaction between wheat and stem rust in the presence of a favorable environment can cause a complete crop failure. This study was conducted to assess the yield potential of selected stem rust resistant mutant lines in comparison with their parent varieties and commercial checks with an objective to select high yielding mutant lines with resistance to stem rust across three environments in Kenya. Eighth selected mutant lines, two parents and seven commercial checks were tested in two consecutive growing seasons in University of Eldoret, KALRO Njoro and KALRO Kitale using Randomized Complete Block Design (RCBD) with 3 replications. Yield data with their response to stem rust were collected and subjected to statistical analyses. Genotype response to stem rust was recorded based on modified cobb's scale while stem rust severity was recorded on a scale of 0 to 100%. The mean grain yield ranged from 0.8 t ha^{-1} to 4.3 t ha^{-1}. Results of coefficient of infection showed about 25% of the genotypes were moderately resistant. Genotype, location and genotype x location interaction for yield were significant ($p< 0.05$). Results showed two mutant lines SP-21 and SP-26 were high yielding and showed resistance to stem rust. These two mutant lines are potential candidates for future release as new varieties and yield improvement.

3.1 Introduction

Wheat (*Triticum aestivum*.L) is an important cereal crop in Kenya produced for domestic consumption. The area under wheat cultivation is estimated at 170,000 ha with an annual production estimate of 450,000 tons per year (FAO, 2017). Consumption is estimated at 1,200,000 tons per year and hence Kenya is a net importer of wheat as local production can only support one-third of total consumption while the remaining two-thirds of consumption is covered by imports (USDA, 2017).

With increasing population and continued dependence on wheat importation to satisfy domestic consumption, the countries role of food security and sustainable development is at risk. This calls for solutions to be directed diverting our attention towards maximizing wheat yield on sustainable foundations. It is implicit that grain yield in wheat is a factor of many contributing components and these factors include variety appropriateness, good husbandry practices, disease resistance, and ecological components among other factors. Yield losses associated with stem rust under farm conditions in Kenya is about 70% but up to 100% yield loss have been recorded under severe disease conditions (Wanyera et al., 2008). Stem rust disease in wheat had been contained worldwide through utilization of resistant genes. A number of these resistant genes had been identified in wheat which include; Sr2, Sr21, Sr24, Sr31, Sr36, Sr44 amoung others (McIntosh et al., 1998). But it was not until 1999 when the emergence of stem rust race Ug99 designated as TTKS (Roelfs et al., 1988) using the North American nomenclature system occurred in Uganda (Pretorius et al., 2000) that these resistant genes were overcome by the pathogen. This pathogen Ug99 become virulent to Sr31, its mutant race TTKST overcome Sr24 while

another mutant race TTTSK over Sr36 (Jin et al., 2008; Njau et al., 2010). Although wheat breeding in Kenya has attempted to develop resistant wheat varieties through utilization of these resistant genes, virulence has been reported in most of these varieties (Njau et al., 2009). Development of stem rust resistant varieties will be of less value to the wheat farmers if the varieties so developed are not high yielding. Hence an interaction between stem rust resistant varieties and high yields are a prerequisite to enable a breeder to identify staple genotypes. But quantitative traits such as yield are usually influenced by genotype, environment and genotype x environment interactions (Hunt et al., 2002). This can be managed by selecting genotypes that are broadly adapted to a range of environments. This will aid the development of an optimum breeding strategy for releasing varieties adapted to a target environment (Ahmad et al., 1996).

The development of resistant wheat varieties will reduce the cost of wheat production and enhance the production of wheat in Kenya. Wheat is the second most important crop in Kenya after maize but the country is a net importer of wheat. Efforts to tackle Stem rust related problems in this study were initiated through joint collaborations between University of Eldoret, Kenya and Seibersdorf laboratories, Vienna Austria. Where seeds of Kenya's local wheat varieties preferred by farmer's (Kwale, and Njoro II) were selected and irradiated using Gamma rays at Seibersdorf laboratories with the objective of generating Stem rust resistant mutant lines. The selection process was carried out at stem rust 'hot spot' in Kenya. The objective of this study was to evaluate yield performance of pre-selected stem rust resistant wheat mutant lines in comparison with their parent's varieties and other commercial checks across three sites in Kenya.

3.2 Materials and methods

3.2.1 Plant materials

Seventeen wheat genotypes comprising of eight mutants, two parent varieties and seven commercial checks were assed for yield performance and screened for stem rust resistance across the three sites. The eight mutants were selected from University of Eldoret research unit and these were mutant lines that had been developed and established to M_3 generations. While the two parents and the seven commercial checks were sourced from KALRO Njoro Seed Unit. The two parent used were Njoro II (SP-N) and Kwale (SP-K) while the seven commercial checks used were; Duma (SP-D), Pasa (SP-P), Simba (SP-S), Farasi (SP-F), Robin (SP-R), KS Mwamba (SP-M) and Chozi (SP-C). They were planted in three sites for two seasons (2012 and 2013) to assess their yield performance and host response to disease across the sites selected. The commercial checks used are popular commercial varieties grown in Kenya.

3.2.2 Experimental sites

The seventeen genotypes were planted in University of Eldoret farm. The farm is located at 0°34′N; 35° 18 ′E, with an altitude of 2,150 m above sea level and mean average temperatures of 17^{o}C. In 2012 and 2013 it received an average annual rainfall of 1,100 mm. The experimental plots were different in all the seasons. The soils were *rhodic ferralsols* (Jaetzold *et al.,* 2006). The second site was at KALRO, Kitale which is located at 0°33′S; 35° 55′E, 5 km from Kitale town with an altitude of 1,890 meters above sea level and mean annual temperatures of 18.3^{o}C with average annual rainfall is 1097 mm.

The soils are deep, well drained, fertile sandy loam. The third site was KALRO Njoro, located at 0°20′S; 35° 56′E, at an altitude of 2,185 meters above sea level and mean annual temperatures of 17^{o}C while the average annual rainfall is about 900 mm. The soils are deep, well drained, fertile *vitric Mollic Andosols* (Jaetzold and Schmidt, 1983). The sites were selected due to their significance in natural population of stem rust suitable for wheat stem rust screening and vast land sizes under wheat crop.

3.2.3 Field experiments

Land that was fallow was ploughed and the seventeen genotypes were planted in a Complete Randomized Block Design (RCBD) with three replications across the three selected sites in Kenya. Each experimental plots was measuring 2 m by 6 rows with inter row spacing of 20cm and intra row spacing of 5cm. The plots were separated by paths of 30 cm while blocks were separated by 2m paths. A spreader (a susceptible wheat cultivar) was planted along the border line of the trial plots to facilitate inoculums build up. Seeds were hand planted head to row with Di-ammonium Phosphate (DAP 18:46:0) at a rate of 125Kg/ha while planting, followed by an application of Urea (75 kg/ha) as a source of Nitrogen at late tillering and booting stages of the plant. Irrigation was done depending on soil moisture levels. Common wheat agronomic practices (except fungicides use) were carried out as described by Kinyua and Ochieng (2005). The study was undertaken for two growth seasons (March and September) in 2012 and 2013 season.

3.2.4 Data Collection

Whole plots for each entry were harvested, threshed and winnowed for grain yield assessment. Grain yield for each entry was adjusted to 13.5% moisture and then weight

before converting them to t ha^{-1} for statistical analysis.. The grain yield data, host response to stem rust and disease severity data were recorded in all the environments.

Table 4: The 17 wheat genotypes evaluated for yield potential and disease response

Sample No.	Sample Description
1	Duma
2	Pasa
3	Simba
4	Farasi
5	Robin
6	Njoro II (Parent)
7	KS Mwamba
8	Chozi
9	Kwale (Parent)
10	SP- 9 (Njoro II Mutant)
11	SP- 16 (Njoro II Mutant)
12	SP- 20 (Njoro II Mutant)
13	SP- 21 (Njoro II Mutant)
14	SP-26 (Kwale Mutant)
15	SP-29 (Kwale Mutant)
16	SP-31 (Kwale Mutant)
17	SP-34 (Kwale Mutant)

Plant disease response assessment was done from dough stage (Zadok's growth stage 65, 75 to 85) (Zadok et al., 1974) to grain development. Disease response observations were made three times on an interval of ten days. The plant disease responses to stem rust were recorded based on the modified Cobb's scale (Peterson et al., 1972). Plant disease response to infections combines several infection type categories scales; I immune, R resistant, MR moderately resistant, M moderately resistant to moderately susceptible, MS moderately susceptible S susceptible (Figure 3). The stem rust severity was recorded using modified Cobb's scale (Peterson et al., 1972), where the severities ranged from 0 – 100% scale where, 0% scale was considered immunity response while 100% scale was considered highly susceptible (Figure 4).

3.2.5 Data analysis

Yield and plant disease response to stem data was analyzed using Genstat computer software (Gensat 15th Edition, 2012). A combined analysis of yield performance and coefficient of infection (CI) was performed using linear mixed model following restricted maximum likelihood procedure (REML). The plant response to stem rust and the disease severity were converted to coefficient of infection (CI) by multiplying the disease severity with the arbitrary constant value for plant response (Roelfs et al., 1992). Where I=0, R=0.2, MR=0.4, M=0.6, MS=0.8 and S=1. Genotypes, location, replicate and Genotype x location were considered as fixed effects while incomplete blocks nested in replicates (Replicate x Block) were considered as random for coefficient of infection while blocks were fixed for yields. The following statistical model was used; $Y_{ijkl} = \mu + G_i + L_i + R_i + B_{)k} + GL_{il} + \mathcal{E}_{ijk}$

Where: Y_{ijkl} = observations; μ = mean of the experiment; G_i = effect of the i^{th} genotype; L_i = effect of the l^{th} location; R_i = effect of the j^{th} replicate; $B_{)k}$ = effect of k^{th} block nested in the j^{th} replicate; GL_{ij} = effect of the interaction of the i^{th} genotype with l^{th} location and $\mathcal{E}_{ijk}$ = the experimental error.

For analysis purposes, the relationship between yield performance and field disease response was done using simple linear regression using Genstat (Genstat 15th Edition, 2012). The least square difference was determined at $P<0.05$. The least square difference was calculated using the formula: LSD = average standard error of difference (REML output) x t/device degree of freedom (t-table).

3.3 Results

3.3.1 Yield performance for seventeen wheat genotypes across the three locations

Some mutant lines (table 5) gave higher ($P \leq 0.05$) grain yields than their parents and commercial check varieties. Highest grain yield was recorded from mutant lines SP-21 and SP-26. On location difference, Eldoret and Kitale recorded similar mean yields of 1.99 t ha^{-1} and 2.02 t ha^{-1} respectively while Njoro mean was 1.70 t ha^{-1} which was the lowest in yields (Table 5).

Table 5: Results of mean wheat yield for 17 genotypes evaluated in Eldoret (UOE), KARLO Njoro and KALRO Kitale in Kenya during 2012 – 2013 cropping season

EXP NAME	Eldoret (t/ha)		Njoro (t/ha)		Kitale (t/ha)		Eldoret mean (t/ha)	Njoro mean (t/ha)	Kitale mean (t/ha)	Grand mean (t/ha)
	2012	2013	2012	2013	2012	2013				
Duma	1.29h	0.79g	0.92h	0.39h	1.21i	1.01g	1.04h	0.66h	1.11h	0.94h
Pasa	1.21h	0.25i	0.92h	0.15i	1.28i	0.29i	0.73i	0.54i	0.79i	0.69i
Simba	2.66d	2.04d	2.38d	1.73d	2.68d	2.12d	2.35d	2.06d	2.40d	2.27d
Farasi	1.83f	1.21f	1.64g	0.62g	1.77g	1.09f	1.52g	1.13g	1.43g	1.36g
Robin	2.30e	1.84d	1.98f	1.47e	2.29e	1.91d	2.07e	1.73e	2.10e	1.97e
Njoro- II	3.45c	2.57c	3.24c	2.33c	3.36c	2.58c	3.01c	2.79c	2.97c	2.92c
Mwamba	2.60d	0.26i	2.36d	0.12i	2.63d	0.32h	1.43g	1.24f	1.48g	1.38g
Chozi	1.15h	0.25i	0.91h	0.39h	1.10i	0.38h	0.70i	0.65i	0.74i	0.70i
Kwale	2.55d	1.41e	2.34d	1.03f	2.54d	1.55e	1.98e	1.69e	2.05e	1.91e
SP- 9	2.75d	1.27f	2.54d	0.96f	2.74d	1.42e	2.01e	1.75e	2.08e	1.95e
SP- 16	2.51d	2.31c	2.23e	1.92d	2.48e	2.38c	2.41d	2.13d	2.43d	2.32d
SP- 20	1.62g	1.18f	1.12h	0.61g	1.51h	1.25f	1.40g	0.87h	1.38g	1.22g
SP- 21	4.53a	4.25a	4.27a	4.12a	4.58a	4.36a	4.39a	4.20a	4.47a	4.35a
SP- 26	4.13b	4.03a	4.05a	3.82a	4.16b	4.01b	4.08b	3.94b	4.09b	4.04b
SP- 29	2.76d	0.82g	2.39d	0.51g	2.70d	0.82g	1.79f	1.45f	1.76f	1.67f
SP- 31	2.01f	1.53e	1.93f	1.42e	2.01f	1.61e	1.77f	1.68e	1.81f	1.75f
SP- 34	1.45g	0.57h	0.75i	0.27h	1.42h	0.89g	1.01h	0.51i	1.16h	0.89h
Mean	2.40	1.57	2.11	1.29	2.38	1.65	1.99	1.70	2.02	

Mean separation was also performed for individual genotypes in order to study the differences in yields resulting from site, year, replication or genotype (Table 6).

Table 6: Mean separation on yield performance per replications for 17 genotypes evaluated in Eldoret (UOE), KARLO Njoro and Kitale in 2012 – 2013 seasons.

Code	**Genotype**	**Rep 1**	**Rep 2**	**Rep 3**
1	SP-D	0.945jk	0.936jk	0.943jk
2	SP-P	0.690l	0.697l	0.688l
3	SP-S	2.276cd	2.274cd	2.271cd
4	SP-F	1. 366gh	1.369gh	1.377gh
5	SP-R	1.983de	1.982de	1.985de
6	SP-N	2. 915c	2.929c	2.924c
7	SP-M	1.381gh	1.392gh	1.380gh
8	SP-C	0.693jk	0.705jk	0.694jk
9	SP-K	1.915de	1.912de	1.910de
10	SP-9	1.953de	1.960de	1.958de
11	SP-16	2.324cd	2.321cd	2.327cd
12	SP-20	1.220hij	1.219hij	1.225hij
13	SP-21	4.352a	4.350a	4.355a
14	SP-26	4.040b	4.048b	4.033b
15	SP-29	1.675efg	1.671efg	1.674efg
16	SP-31	1.752ef	1.751ef	1.753ef
17	SP-34	0.894jk	0.895jk	0.892jk
Cv		**12.91659**	**12.90950**	**12.91191**

Treatment means within columns followed by the same letter are not significantly different at ($P \leq 0.05$) according to Tukey's test.

3.3.2 Genotypic response to stem rust across the 3 locations

In this study, genotype x location interactions was significant for stem rust infection and this indicated variable infection of stem rust on genotypes across the different locations. Significant ($P<0.01$) variation for ACI was observed in genotype, location and genotype x location interaction. The coefficient of infection (ACI) for the seventeen wheat genotypes is presented in table 7. In this study wheat mutant lines with ACI values of 10 and below were considered stable and resistant against stem rust. Mutant lines SP-21 and SP-26 were considered resistant and stable against stem rust with the lowest ACI mean values of 5.1 and 6.0 respectively. From this study, Eldoret (ACI=31.3) and Kitale (ACI=30.8) locations mean were similar with the lowest disease response scores while Njoro (ACI=34.2) location mean was the highest in disease response scores (Table 7).

Weather related factors may have played a vital role in disease infection in one location to another. Rainfall and temperatures appear critical in disease infection because a small difference in these factors affects germination of the spores. The mean of Eldoret (ACI=31.3) was found not to be different from the mean of Kitale (ACI=30.8) but different from the mean of Njoro (ACI=34.2). Mutants SP-21 (ACI=.5.1)and SP-26 (ACI=6.0) were moderately resistant than the best parent SP-N (ACI=21.1). Mutant lines SP-31 and SP-16 showed considerable level of resistance against stem rust but ACI values were higher than 10. Mutants with ACI values of 30 and below namely; SP-31, SP-16 and SP-29 showed moderately resistances to moderately susceptible resistances against stem rust while mutant lines SP-9, SP-20 and SP-34 had ACI values of above 30 and were moderately susceptible to susceptible against stem rust (Table 7).

Table 7: Average coefficient of intersection (ACI) for 17 wheat genotypes evaluated in Eldoret, KALRO Njoro and Kitale in 2012 – 2013 cropping season

EXP NAME	Eldoret mean	CI	Njoro mean	CI	Kitale mean	CI	Aver. Eldoret mean	Aver. Njoro mean	Aver. Kitale mean	Overall (ACI) mean
	2012	**2013**	**2012**	**2013**	**2012**	**2013**				
Duma	42.3g	47.7g	46.2g	51.4g	43.4g	45.2f	45.0h	48.8h	44.3h	46.3h
Pasa	39.7f	59.9j	43.1f	62.7j	39.3f	58.4i	49.8i	52.9i	48.9i	50.5j
Simba	25.5e	32.6d	28.4e	35.1d	25.4e	31.6d	29.1e	31.8e	28.5e	29.7e
Farasi	39.2f	46.3g	44.5f	52.2g	41.3g	44.1f	42.8f	48.4h	42.7h	44.6h
Robin	15.1c	32.2d	17.5c	35.5d	15.2c	31.4d	23.6d	25.5d	23.6d	24.3d
Njoro II	16.1c	24.7c	18.1c	26.7c	17.4c	23.4c	20.5c	22.4c	20.4c	21.1c
Mwamba	25.2e	55.4i	27.5e	57.4i	24.6d	54.2h	40.3g	42.5g	39.4g	40.7g
Chozi	43.9g	55.5i	46.3g	58.9i	44.6h	52.7h	49.2i	52.6i	48.7i	50.2j
Kwale	26.8e	40.2f	28.9e	43.6f	26.9e	38.6e	33.5f	36.3f	32.8f	34.2f
SP- 9	23.6d	41.1f	25.8d	43.9f	23.8d	39.4e	31.5f	34.9f	31.9f	33.1f
SP- 16	22.5d	24.8c	25.8c	27.9c	23.1d	23.7c	23.7d	26.8d	23.5d	24.6d
SP- 20	38.3f	50.7h	43.8f	53.4g	39.6f	48.1g	44.5h	48.6h	44.0h	45.7h
SP- 21	3.3a	6.2a	6.3a	6.9a	3.3a	4.8a	4.8a	6.6a	4.1a	5.1a
SP- 26	5.5a	6.0a	5.7a	7.3a	5.5a	6.0a	5.8a	6.5a	5.7a	6.0a
SP- 29	15.5c	38.2e	19.3c	41.3e	16.2c	38.3e	26.9e	30.3e	25.2d	28.3e
SP- 31	9.2b	19.1b	9.3b	19.4b	9.4b	18.2b	14.1b	14.3b	13.8b	14.1b
SP- 34	40.2f	50.4h	48.3g	53.6h	40.7h	48.9g	45.3h	51.1i	44.8h	47.1i
Mean	25.4	37.1	28.5	39.9	25.9	35.7	31.3	34.2	30.8	

Treatment means within columns followed by the same letter are not significantly different at P≤0.05 according to Tukey's test. A simple regression analysis revealed a significant linear and inverse relationship (P≤0.01) between grain yield and coefficient of infection (CI) (Y = -0.00825x +4.7315, s.e = 0.13, R^2 = 0.3524). There was a significant (P≤0.01) genotype and location effect. There was (P≤0.05) genotype x location interaction across sites for grain yield. Mutants of SP-N recorded highest means for yield when compared to the mutants of SP-K. But mutants of SP-K recorded lower means for ACI when compared to mutants of SP-N. Its only mutant lines SP-21 and SP-26, which recorded higher yields and lower ACI values when compared to their respective parent's varieties or the best commercial check.

3.4 Discussions

From this study, none of the genotypes evaluated was high yielding and completely resistant to stem rust. However, two genotypes were found to be high yielding and moderately resistant to stem rust. Results from this study showed that genotypes with moderate resistance had also low infection rate and probably these could be due to partial resistance which is attributed with additive or epistatic genes (Nzuve et al., 2013).

Results from this study revealed that high yielding genotypes were also relatively resistant to stem rust which explains the effects of disease on yield. Stem rust causes grains to shrivel thereby reducing their seed weight and overall grain yield. It was also found that yield and disease infection were independent of each other. Mutant SP-9 was stable for yield but unstable for disease infections while mutant SP-29 and SP-31 were stable for disease infection but unstable for grain yield. However, mutants SP-16, SP-21 and SP-26 were stable for both yield and disease. From this study, there was a negative correlation between grain yield and host disease response which revealed a linear and an inverse relationship between grain yield and ACI. This relationship between grain yield and ACI though weak, indicates that apart from stem rust, there must be other factors other than disease which influences yield in wheat production. Yield variability existed across the three locations due to diverse genetic background of the genotypes, location and genotype x location interaction. There was difference in yield and disease pressure across the environments. The disease pressure influenced the yield performance of individual genotypes across the three environments. The significance of genotype x location was an indication of inconsistency in genotypes in response to the environment. Similar results were observed by (Mohammed et al., 2009). Njoro had the lowest mean

for grain yield of 1.70 t ha^{-1} and the highest mean for disease response of 34.2 while Eldoret and Kitale locations recorded almost similar mean yields of 1.99 t ha^{-1} and 2.02 t ha^{-1} respectively with the lowest host disease response mean of 31.3 and 30.8 respectively. The highest disease pressure was in Njoro location and this could be attributed to a buildup of stem rust inoculums in this location. Similar relationship has been reported by Macharia and Wanyera, (2012). Stem rust is favored by warm and moist environment which is the characteristic of Njoro location (wanyera et al., 2006). Eldoret and Kitale locations had almost equal mean for both stem rust response and grain yield and this similarity showed that, while carrying out a multi-locational trial, one of these location can be selected as a representative of the other when resources are limited and insufficient to be used in the two locations. From this study, Njoro location could be chosen as the best site for evaluation of wheat in response to stem rust. These results were in agreement with Hintsa and Fatien (2013).

Considering grain yield and stem rust resistance, mutants SP-21 and SP-26 combined both high yields and stem rust resistance. These two mutants can be considered as potential candidates for variety release. Mutants SP-16 and SP-9 had on average yields above of 1.90 t ha^{-1}, but its ACI scores were high above the average ACI line of 10 hence moderately susceptible to susceptible. These mutants could probably have some tolerance to stem rust but are likely to be broken down in future. Mutant SP-31 had ACI value of 14.1 which was below 20 considerably low for disease resistance but its grain yields 1.75 t ha^{-1}, was lower, and hence it could be considered for gene pyramiding in future to improve on its yields. Mutants SP-20 and SP-34 were susceptible to stem rust.

3.5 Conclusion

The assessment of genotypes yield potential and the screening of stem rust in the field across the three locations, allowed for the evaluation of genotypes for yield potential and response to stem rust across the different environments. The results of this study revealed two superior mutant SP-21 and SP-26 which combined both high yields and disease resistance. They were ranked the best among the eight mutants evaluated with good potential for variety release. These outstanding mutants can be included in the National performance trials (NPT) for further evaluation in readiness for release to farmers as new varieties for growing. They can also be used as parental lines for obtaining segregating population for yield related trials and stem rust resistance. These mutants had higher grain yields than their parent varieties and higher than all the commercial checks used in this study.

3.6 Recommendations

Therefore, on the basis of yield performance and disease resistance across the three sites used in this study, the selected mutant lines SP-21 and SP-26 are superior to their respective parent varieties and all commercial checks used in this study. The selected mutant displayed yield stability across the three geographical sites and the two growth seasons. Therefore the two mutants meet the necessary conditions for release as new wheat varieties to be recommended to farmers for growing in Eldoret, Njoro and Kitale and future studies for their advancement.

CHAPTER FOUR

GENETIC DIVERSITY OF SELECTED WHEAT MUTANTS LINES USING MORHOLOGICAL AND MOLECULAR MARKERS

Abstract

Morphological characteristics and molecular markers have been used to study genetic diversity. Genetic diversity is important for crop improvement because crosses involving parents that are genetically divergent are a suitable way to bring forth greater genetic variability in segregating generations. The objective of this study was to determine the genetic diversity of selected mutant lines in comparison with their parent varieties using morphological characteristics and molecular markers. Seventeen wheat genotypes were assessed at seedling stage in a controlled environment using (CRD) and at adult stages in the field using (RCBD) designs. Laboratory work was conducted at KALRO Njoro while field experiments were conducted in Eldoret, Njoro and Kitale for two seasons in 2012 and 2013. Twenty five agro-morphological traits were evaluated to determine morphological diversity and ten SSR markers were used to study genetic diversity of selected mutant lines in comparison with their parent**.** Results showed mutants and their parents have greater genetic variability being separated majorly by their grain yield per spike, 1000 seed weight, grain colour/texture, number of tillers and days to maturity. A dendrogram based on 10 SSR markers grouped the genotypes into two major clusters and four sub clusters with the mutants clustering with their respective parents. There was significant genetic diversity among genotypes that can be selected for breeding purposes

4.1 Introduction

In the study of genetic diversity, morphological traits and molecular markers play an important role in the analysis of variance in crop species and their relatives (Stoilova *et al*., 2005). Morphological traits are used to assess genetic diversity among varieties although these traits under analysis are greatly influenced by the environment. A genotype may exhibit different morphological characteristics for two different locations and hence the combination of morphological traits and molecular markers is recommended to complement each other and increase the resolving power of genetic analyses. Randomly Amplified Polymorphism DNAs (RAPD), Restriction Fragment Length Polymorphisms (RFLP), and Simple Sequence Repeats (SSRs) have widely been used to asses genetic diversity. SSRs are considered the best markers of choice as they are the most efficient markers for assessing genetic diversity (Roubos *et al.*, 2010).

(SSR markers have an essential characteristic to detect polymorphism in a large sample of germplasm depending on the length and sequence of the repeat motif they contain and their location in the genome (Blair *et al*., 2006). The variability at SSR loci results from high rates of mutation, making SSR markers ideal for genetic mapping and characterizing genetic diversity in crop species and hundreds of SSR markers have been developed for wheat breeding programs. Evaluation of SSR polymorphism in different wheat varieties is important to establish similarities and differences among them and understand their genetic diversity. The objective of this study was to determine genetic diversity of selected wheat mutants in comparison with their parent varieties and other commercial checks using morphological traits and SSR markers.

4.2 Materials and methods

4.2.1 Plant materials

Seventeen wheat genotypes comprising of eight mutant lines, two parents and seven commercial checks were used in this study to determine the genetic diversity of the genotypes across the three selected environments. The eight mutants were selected from University of Eldoret screening laboratories and these were mutants that had been developed and established to M_3 generations. The nine commercial checks were sourced from KALRO Njoro Seed Unit. The two parent were Njoro II (SP-N) and Kwale (SP-K) while the seven commercial checks were; Duma (SP-D), Pasa (SP-P), Simba (SP-S), Farasi (SP-F), Robin (SP-R), KS Mwamba (SP-M) and Chozi (SP-C). They were planted in three sites for two seasons (2012–2013) to assess their genetic diversity across the sites. The commercial checks are among popular wheat varieties grown in Kenya.

4.2.2 Experimental sites

The seventeen genotypes were planted in University of Eldoret farm. The farm is located at 0°34′N; 35° 18 ′E, with an altitude of 2,153 m above sea level and mean average temperatures of 17^{o}C. In 2012 and 2013 it received an average annual rainfall of 1,100 mm. The experimental plots were different in all the seasons. The soils were *rhodic ferralsols* (Jaetzold *et al.,* 2006). The second site was at KALRO, Kitale which is located at 0°33′S; 35° 55′E, 5 km from Kitale town with an altitude of 1890 meters above sea level and mean annual temperatures of 18.3^{o}C with average annual rainfall is 1097 mm. The soils are deep, well drained, fertile sandy loam. The third site was KALRO Njoro, located at 0°20′S; 35° 56′E, at an altitude of 2,185 meters above sea level and mean

annual temperatures of 17^{o}C while the average annual rainfall is about 900 mm. The soils are deep, well drained, fertile *vitric Mollic Andosols* (Jaetzold et al., 2006). The sites were selected due to their significance in natural population of stem rust suitable for wheat disease screening.

4.2.3 Field experiments

Land that was fallow was ploughed and the seventeen genotypes were planted in a Complete Randomized Block Design (RCBD) with three replications across the three identified sites (University of Eldoret, KALRO Njoro and KALRO Kitale) in Kenya. These regions are the wheat growing areas in Kenya. KALRO-Njoro also hosts the global phenotyping facility for characterizing and selection of wheat genotypes resistant to stem rust (Singh et al., 2006) under the auspices of the BGRI project. Experimental plots were measuring 2 m by 6 rows with inter row spacing of 20cm and intra row spacing of 5cm. The plots were separated by paths of 30 cm while blocks were separated by 2 m paths. A spreader (a susceptible wheat cultivar) was planted along the border line of the trial plots to facilitate uniform inoculums build up. Seeds were hand planted head to row with Di-ammonium Phosphate (DAP 18:46:0) at a rate of 125Kg/ha while planting, followed by an application of Urea (75 kg/ha) as a source of Nitrogen at late tillering and booting stages of the plant. Irrigation was done depending on soil moisture levels. Common wheat agronomic practices (except fungicides use) were carried out as described by Kinyua and Ochieng (2005). The study was undertaken for two growth seasons (March and September) in 2012 and 2013.

4.2.4 Data collection

Data collection was done from selected mutant lines from M_4 population which were planted in a completely randomized block design (RCBD) with each experimental unit having three replications. Each mutant line entry formed an experiment unit and both parents (SP-N and SP-K) and the other commercial varieties were included as control. Twenty five agro-morphological traits were evaluated to determine morphological diversity. They were divided into qualitative and quantitative trait categories. Description of how the traits were measured or observed is detailed as follows; Growth habit was assessed visually from the attitude of the leaves and tillers. Germinations rate was measured from the number of seeds planted and those that germinated, plant height (cm) at maturity was measured from the ground to the tip of the spike excluding the awns, Spike length was measured from the spike base to the tip of the spike, flag leaf area was calculated by measuring the surface area, seed shape, recurved flag leaf, spike shape, flag leaf attitude, straw pith, spike density, grain colour, lower glume (shoulder shape), pre-harvest sprouting tendency, awndness, degree of seed shriveling were visually observed, Number of tillers was measured by counting the tillers, Lodging was measured by counting the number of stem that lodged, number of grains per spikelet, spikelet's per spike, Awn length, seed diameter, Days to 50% heading were determined from days of sowing to when 50% of the plants had one head formed, Days to 50% maturity were determined from days of sowing to when 50% of the plants in a plot had fully matured, grain yield per spike, 1000 seed weight at 13.5% MC were weight to obtain seed weight

and total yield per plot in order to determine the performance of the genotypes. Numerical values were assigned to aid in data analysis.

4.2.5 Design and statistical data analyses

Analyses of variance were performed on the quantitative traits using Genstat computer software (Gensat 15th Edition, 2012). The data were subjected to general linear model for individual season. The data were analyzed as a RCBD with the following general linear model for individual seasons considering a triple lattice design as follows: $\mathbf{Y_{ijkl} = \mu + \lambda_i + \pi_{(j)i} + t_k + \lambda t_{jk} + \varepsilon_{ijkl}}$ Where:

$\mathbf{Y_{ijkl}}$ = plot observations. $\boldsymbol{\mu}$ = overall mean of experiment.

$\boldsymbol{\lambda_i}$ = Season effect. $\boldsymbol{\pi_{(j)i}}$ = Replication within season effect.

$\mathbf{t_k}$ = genotype effect**.** $\boldsymbol{\lambda t_{jk}}$ = Interaction of genotype effect.

$\boldsymbol{\varepsilon_{ijkl}}$ = Residual effect

Analysis of variance was conducted on the spike yield using the number of plants harvested per plot. A sample of 10 plants per plot was used. Mean separation was done using Tukey's test at 95% confidence level using GenStat edition 12. Qualitative data were subjected to frequency distribution analyses. Estimate of variability for each qualitative trait were assigned numerical values and computed in excel using the standardized Shannon-Weavers diversity index (H) using the formula as ollows: $H'=-\sum Pi(\log_e Pi)/\log_e n$ Where: H' = Shannons-weaver diversity index, Pi = Frequency proportion of each qualitative trait, n = Total number of classes per qualitative trait. The standardized Shannon-weavers index has a value ranging from 0-1, where 0 indicates

absences of diversity and 1 indicates maximum diversity. Dendrogram was derived showing similarities in 17 genotypes based on the 25 agro-morphological traits.

4.2.6 Molecular evaluation

Seeds of plants used for DNA extraction were planted in a greenhouse and maintained. From 3 weeks old, leaves from 10 plants per genotype were harvested and crushed to make a smooth homogenate using a mortar and a sterile micro-pestle. 500 µl of SDS extraction buffer was added and mixed with the help of the micro-pestle. It was allowed to stand for 10 minutes. The mixture was then centrifuged at 1,000 rpm for 5 minutes to separate and form DNA pellet. The supernant was gently discarded by pouring out leaving the DNA pellet in the eppendoff tube. The 500 µl of 70% ethanol was added to the tube to wash the DNA. The tube was gently tapped to dissolve the DNA pellet and allowed to stand for a few minutes before centrifuging at 10,000 rpm for 5 minutes to re-pellet the DNA. The supernatant was discarded by pouring out the ethanol and dried using a clean paper towel by draining away any remaining excess liquid. The tube were allowed to stand for 30 minutes and 100 micro-litre of 1 XTE buffer was added to re-suspend the DNA. The remaining DNA suspension was stored at 4^{o}C for further analysis.

Polymerase Chain Reactions (PCR)

A stock solution of PCR mix was made which comprise of PCR buffer, taq polymerase, water and DNA template DNTP mix was used. Two sets of SSR primers Wmac031 and Wmac 167 which amplify a fragment of 196 bp which is a marker for wheat tem rust and another set of primers Wmag 217 and Wmac 900 which amplify a fragment of 200 bp which is a marker for the same gene were used. Amplification was performed in a total of

10 µl reaction. The PCR profile amplification was conducted in a thermal cycler (EPPENDORF) using the following temperature profile: Initial denaturalization was 94^{o}C for 3 minutes followed by Denaturalization at 94^{o}C for 30 seconds, Annealing temperatures at 47^{o}C - 55^{o}C for 60 seconds, Extension at 72^{o}C for 40 seconds and final extension at 72^{o}C for 3 minutes and then hold at 4^{o}C.

Gel electrophoresis

After amplification, a volume of 4 µl of 6X loading buffer (10 mM tris-HCL, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA) was added to each PCR reaction. The content was loaded in 6% non-denaturing polyacrlamide gel and run at 10 V/cm for 3 hours before they were stained with ethidium bromide (0.5 µg/mL) for 30 minutes. The gel was removed from the gel tank and taken to a dark room to view it under UVP GelDoc-it system box to view the DNA under UV light.

Selection of SSR markers for diversity

The SSR markers for diversity were selected based on clear polymorphic bands. They were identified by screening all the 10 SSR markers against the DNA obtained from the 17 samples. The band sizes were determined using 100 bp molecular ladder.

SSR allelic scoring

The SSR allele sizes were scored for all loci on the basis of comparison to 100-bp molecular ladder and an allele matrix was prepared from this dataset. The average number of alleles and gene diversity were observed and expected heterozygosis and

polymorphism information content were calculated for each SSR marker locus. Genetic distances among accessions were calculated using chord distance.

4.3 Results

4.3.1 Morphological diversity of the wheat genotypes

Qualitative traits

From the results, 94% of the genotypes evaluated exhibited erect growth habit while 6% exhibited semi-erect, (94% had low frequency of recurved flag leaves while 6% had medium to high frequency. 24% of the genotypes had plum grains, 40% had intermediate grains while 43% had shriveled seeds. 24% of the genotypes had hard red grain in texture, 40% had intermediate and 34% had white grains. 94% of the genotypes at maturity had thin to medium straw pith in cross section while 6% had thin straw pith. In 94% of the genotypes awns were present and were medium to long in length while 6% had short awns. 64% of the genotypes had oval grain shape, small to medium in size, medium in length and width, rounded with angular cheeks with short brush hairs while 24% had elliptical grain shape, small to medium in size, medium to long in length, medium width with angular cheeks and short brush hairs. 82% of the genotypes had parallel sided spike shape, dense with erect attitude at maturity, absent or very sparse hairiness of the convex surface of the apical rachis while 12% had tapering spike shape.

The computed diversity indices for qualitative traits ranged from 0.27 – 0.85. The low diversity values for straw pith at maturity (0.27) indicated a low variation, flag leaf attitude(0.53), growth habit (0.57) and spike density (0.67), brush hair length(0.61),

frequency of recurved flag leaves (0.65) showed medium variation while the rest traits showed high variation (0.70 – 0.85) amoung the varieties.

Table 8.: Frequency distribution for qualitative traits

Trait	Frequency %	H'[a]	Trait	Frequency%	H'
Flag leaf(recurved)		**0.65**	**Awns length**		**0.34**
Medium to high	6		Medium long	97	
Low to medium	94		Long awns	3	
Shriveled grains		**0.85**	**Grain colour**		**0.71**
Plump	24		white	30	
Intermediate	40		Intermediate	46	
Shriveled	34		Red	30	
Grain shape		**0.75**	**Spike shape**		**0.65**
Oval	64		Tapering	12	
Oblong	12		Parallel sided	82	
Elliptical	24		Semi clavate	6	
Flag leaf attitude		**0.53**	**Straw**		**0.27**
Erect	6		**maturity**	94	
Semi-erect	74		Thin	6	
Drooping	20		Medium		
Sprouting tendency		**0.79**	**Brush hair**		**0.61**
No	6		Short	64	
Low	42		Medium	30	
Medium	52		Long	6	
Spike density		**0.67**	**Growth habit**		**0.57**
Sparse	6		Erect	94	
Medium	18		Semi-erect	6	
Dense	75		Intermediate	0	

H'[a] – Shannon-Weaver index for qualitative traits

Quantitative traits

The biggest flag leaf area was on mutant SP-21 (42.8 cm^2) while mutant SP-21 recorded the longest spike length (12.5 cm per spike). Mutant SP-26 had the highest number of mature tillers (5 tillers per plant). The highest number of grains per spike was recorded in

mutant SP-26 (45 grains per spike). The highest grains yield per spike was in mutant SP-21 (2.12 grams per spike), the highest 1000 grain weight was in mutant SP-21 (47.8 grams) and the highest yield per genotype was from mutant SP-21 (4.34 t/ha) (table 9).

The values for yield (Y) ranged between 0.69 - 4.35 t/ha with some mutant lines recording significant increase in yield when compared to their respective parent varieties. Values for 1000 seed weight (SW) ranged from 24.6 to 47.8 grams and the means of some mutants were higher than those of their respective parents. Some mutant lines showed increase in their seed diameter (SD) when compared to their respective parent varieties. Values for seed diameter ranged from 0.20 to 0.28 cm per seed. Values for number of tillers per plant (GT) ranged from 1 – 5 mature tillers per plant. Some mutant lines showed increase in number of tillers per plant when compared to their respective parent varieties. Values for maturity period (M) ranged from 115 to 145 days with mutants of SP-N and SP-K showing reduced maturity periods when compared to their parent varieties which had longer maturity periods when compared to their mutant lines.

Values for spike length (SL) ranged from 7.1 to 12.5 cm per spike. Values for lodging (L) ranged from 3 to 8 in a scale of 1 - 9 (where 1 is prone to lodging while 9 are resistant to lodging). Values for plant height (H) ranged from 75.45 to 88.76 cm and there was a reduction in plant height for mutants of SP-K when compared to their parent while mutants of SP-N had increased plant height when compared to SP-N parent. Values for time of ear emergence (EE) ranged from 65 to 70 days and there was no difference in the time of ear emergence for mutants when compared to their respective parent varieties. Values for grain yield per spike (GY) ranged from 0.82 to 2.12 grams per spike and there

was an increase in grain yield per spike for some mutants when compared to their respective parent varieties. Values for flag leaf area (FLA) ranged from 27.7 to 42.8 cm^2. Values for flag leaf area showed increase for some mutants when compared to their respective parent varieties.

Values for awn length (AL) ranged from 6.5 to 7.3 cm and there was no difference in awn length for mutants when compared with their respective parent varieties. Values for number of spikelets per spike (S) ranged from 8.9 to 20.1 per spike. There was a increase in the number of spikelets per spike for some mutants of SP-N and SP-K when compared to their parent varieties. Values for the number of grain per spike (GS) ranged from 20.3 to 45.1 per spike and there was an increase in the number of grain per spike for some mutants when compared with their respective parent varieties.

Table 9: Mean of difference of quantitative traits of 17 wheat genotypes

Exp name	1000 SW (gms)	SD (cm)	GT (no)	M (days)	SL (cm)	L (1-9)	H (cm)	EE (day	GY (gms)	FLA (cm^2)	AL (cm)	S (nos)	GS (nos)
Duma	29.7f	0.22c	3c	115d	9.1d	4e	83d	65a	1.15h	29.5g	6.9a	13d	29d
Pasa	25.3g	0.21d	1d	135b	8.9e	3f	88a	70a	0.84i	28.6h	7.0a	11e	24e
Simba	38.5d	0.25b	3c	115d	10.7b	6c	82e	65a	1.55e	36.4d	6.8a	15c	36b
Farasi	37.2d	0.25c	3c	120d	9.6c	5d	82e	65a	1.24g	32.1f	7.0a	13d	33c
Robin	33.8e	0.24b	3c	120d	9.7c	6c	83d	65a	1.32f	35.3d	7.2a	13b	34c
Njoro II	41.5c	0.26a	3c	125c	11.1b	5d	82e	70a	1.78c	38.6c	6.9a	16c	40a
Mwamba	24.6h	0.20d	2d	125c	7.1g	3f	76h	65a	0.82j	27.7i	6.5a	9f	20f
Chozi	25.8g	0.21d	1e	135b	8.7e	3f	87b	70a	0.91i	28.4h	7.1a	12d	25d
Kwale	35.4e	0.23c	2d	145a	9.5c	5d	89a	70a	1.37f	32.8e	7.3a	13d	32c
SP- 9	37.3d	0.24b	2d	115d	9.9c	5d	86c	65a	1.45e	35.1d	6.8a	14d	34c
SP- 16	39.7c	0.25b	4b	115d	10.8b	7b	86c	70a	1.67d	38.5c	7.0a	16c	38b
SP- 20	30.5f	0.23c	2d	115d	9.3d	3f	86c	65a	1.15h	34.5e	6.8a	12d	28d
SP- 21	47.8a	0.28a	4b	115d	12.5a	7b	86c	70a	2.12a	42.8a	6.8a	18b	44a
SP- 26	44.6b	0.26a	5a	130c	10.9b	8a	80f	70a	2.04b	40.3b	6.8a	20a	45a
SP- 29	28.9f	0.21d	3c	130c	9.5c	4e	79g	70a	1.13h	32.2f	6.7a	13d	30c
SP- 31	35.1e	0.23c	4b	130c	9.6c	7b	79g	70a	1.32f	35.8d	6.8a	15c	35b
SP- 34	27.5g	0.21d	4b	130c	9.3d	4e	79g	70a	0.97i	33.1e	6.8a	12e	28d
Mean	34.3	0.234	2.9	124.5	9.8	5.0	83.0	67	1.34	34.2	6.8	13.8	32.5

Pearson's moment Correlation

The relationship between yield components is under genetic control which can vary depending on the environment. But the high value of heritability would indicate the less influence of the environment on a particular trait. This genetic relationship is based on the gene effects or linkages of genes. Such genetic relationship is expressed through correlations between the trait and the environment. Pearson's correlation coefficients (r) showed significant positive correlation between spike length and grain yield per spike (r = 0. 71**), number of spikelet's per spike and spike length (r = 0.60**), seed weight and seed diameter (r = 0. 77**), seed weight and number of grains per spike (r = 0. 96**), number of grains per spike and spike length (r = 0.73**), seed weight and grain yield per spike (r = 0. 93**), seed weight and flag leaf area (r = 0.87**), seed diameter and grain yield per spike (r = 0.74**), seed diameter and flag leaf area (r = 0.67**), grain yield and flag leaf area (r = 0.61**), grain yield per spike and number of grains per spike(r = 0. 92**) and number of spikelet's per spike and number of grains per spike (r = 0. 89**), grain yield per spike and number of spikelet's per spike (r = 0. 88**).

However there was significant negative correlation between seed weight and number of tillers per plant (r = -0. 67**), seed weight and maturity time period (r = -0.63**), number of spikelets per spike and spike length (r = -0. 58**), seed diameter and number of tillers per plant (r = -0. 55**), spike length and number of tillers per plant (r =- 0. 53**), grain yield per spike and number of tillers per plant (r = -0. 56**), grain yield per spike and maturity period (r = r = -0.53**) and between seed diameter and maturity period (r = -0.51**).

Cluster analysis

The method of aggregation hierarchical called cluster analysis was used to classify the studied traits. Classifying by clustering technique was performed based on similarities and distances.

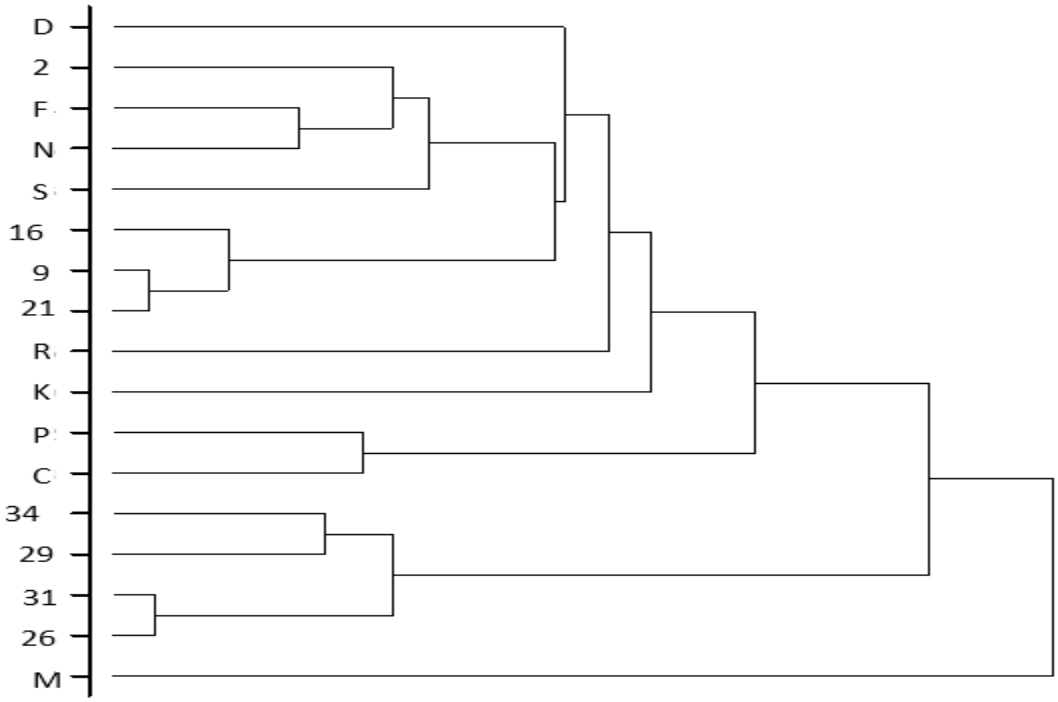


Figure 6. Dendrogram illustrating similarities based on 25 morphological traits

The dendrogram based on Euclidean similarities among the seventeen genotypes divided the genotypes into four clusters with a similarity distance ranging between 75% and 95%. The first cluster was made up of majorly hard red to intermediate red grains, medium in size, length and width. This cluster was composed of SP-D, SP-20, SP-F, SP-N, SP-S, SP-16, SP-9, SP-21 and SP-R. They were almost similar in plant height across the cluster and their maturity period was shorter when compared to the other genotypes.

Genotype SP-K, separated from the other red grains genotypes and formed the second cluster. It had hard red grains, small to medium in size, medium in width, elliptical with angular cheek and short length brush hairs. The third cluster was made up of the two white seeded genotypes SP-C and SP-P. They were both similar in height, maturity period and with fewer grains per spike. The intermediate red to white grains genotypes were made up of SP-26, SP-31, SP-29, SP-34 and SP-M all of which were mutants of SP-K except SP-M and they formed cluster four. They had small to medium size grains, medium in length and width, oval rounded with narrow shallow crease. They were shorter in height than the other genotypes and with medium maturity period. The respective parent wheat varieties and there mutants clustered into two major clusters with mutants clustering with their respective parents.

The similarities in clustering of genotypes were observed in the grain texture, size and number of grain per spike. These similarities are as a result of targeted breeding towards the achievements of certain goals in plant breeding. During the process of plant breeding and selection of new wheat varieties, a lot of attention is paid to the improvement of the main components of grain yield increase. This would include number of grains per spike and 1000 seed weight even with lower density would mean that a high grain yield would be achieved and even with lower costs. Even though grain size influences final grain yield, it’s important to note that small seeded varieties frequently compensate by producing more spikes and more grains. Grains per spike are simply the product of the number of seeds per spikelet and the total number of spikelets per spike. It was observed that genotypes bred from the same institution tented to cluster together for example SP-26, SP-31, SP-29, SP-34; SP-C and SP-P; SP-20, SP-N, SP-16, SP-9, SP-21.

Principle component analysis

The purpose of principal components analysis was to analyze available variance in the data into different components in order for the first component to explain the cause of available variance and the next components to justify slowly the cause of variations. Each component was independent of each other and there was no correlation between the resulting components. Variance is an important feature of each major component and the principal components are ordered by decreasing of variance.

Basing on results of Eigen loading for the traits as presented in Table 10.0 and the choice of components according to Figure 7.0, the principal component analysis can explain 5 elements having the most variance in the data. The data demonstrated that the increase in the number of components was associated with a decrease in Eigen values. When Eigen value decreased, the numbers of important traits in that component also decreases as the first two components have more traits. The traits responsible for separation along PC1 included: number of spikelets per spike (0.429817), number of seeds per spike, (0.391089), 1000 seed weight (0.365159) and grain yield per spike (0.359653). Separation along the PC2 was influenced by plant height (-0.53183), spike length (-0.49995), maturity time period (-0.38764) and number of tillers per plant (-0.36885). In PC3, the genotypes were separated majorly according to seed diameter (0.416595), seed weight (0.397916), flag leaf area (0.360768) and grain yield per spike (0.338100). The principal component analysis showed that the grain characteristics per spike were an important component of yield that majorly separated between the different genotypes. A change in grain weight per spike drastically influences the final yield.

Table 10: Correlation matrix Eigen values, variation percentage, and accumulated variation percentage of the obtained components

Eigen vectors					
Variable	**Prin1**	**Prin2**	**Prin3**	**Prin4**	**Prin5**
SW	0.365159	0.244609	0.397916	-0.21018	-0.13925
SD	- 0.21307	-0.35081	0.416595	0.471827	0.536414
GT	-0.05784	-0.36885	-0.28382	0.034051	0.248694
M	0.269849	-0.38764	-0.23694	-0.40854	-0.01333
SL	0.320312	-0.49995	-0.07169	-0.07447	0.124526
L	-0.01360	0.277474	0.240800	0.251810	0.278394
H	-0.12800	-0.53183	0.157220	0.186502	0.184076
EE	0.264795	0.068995	0.203835	0.001985	0.372317
GY	0.359653	-0.01544	0.338100	0.436681	-0.39230
FLA	0.112532	0.279153	0.360768	0.024231	0.264948
AL	-0.22311	0.077622	0.246337	0.179696	0.228642
S	0.429817	0.262533	0.151131	-0.04574	-0.01688
GS	0.391089	-0.06140	0.114662	-0.30823	-0.29776
Eigen V	2.799	2.307	2.047	1.457	1.298
Proportion	0.215	0.178	0.158	0.112	0.100
Cumulative	0.215	0.392	0.550	0.662	0.762

Where, 1,000 seed weight= SW, Number of tillers=GT, Maturity period (days) =M,Spike length=SL, Lodging=L, Plant height=H, Grain yield per spike=GY, Flag leaf area=FLA, Awn length=AL, Spikelet's per spike=S, Seed diameter=SD, Ear emergence=EE, number of grains per spike = GS**.**

4.3.2 Genetic diversity based on SSR markers

Microsatellite diversity in wheat

The level of polymorphism in the seventeen wheat genotypes in terms of numbers of alleles, major allele frequency, gene diversity, weavers' diversity index is presented in table 11.0. A total of ten polymorphic markers were detected after screening twenty markers on the seventeen wheat genotypes. Most primers had two alleles and the alleles sizes were within the expected range. The ten SSR primer pairs yielded a total 13 polymorphic loci with a percentage of 92.86 per cent among the seventeen wheat genotypes considered relatively informative (PIC > 25%). The mean number of different alleles per locus in each group was 2.0 and the mean number of polymorphic alleles per locus was 1.9286. This is consistent with previous studies which made similar observations. Salem et al. (2008), found that the number of alleles per locus ranged from 2 alleles to 7 alleles with an average of 3.2 alleles per locus while Jain et al. (2004) also reported similar observation of number allele per locus ranged from 3 to as high as 22 with an average of 7.8 alleles per locus. The gene diversity ranged from 0 to 0.4893 for each sample, with an average range mean of 0.3361.

To estimate the number of heterozygous loci between two randomly chosen gametes in the population, expected and observed moments of heterozygosity was calculated. The expected heterozygosity (HE) and observed heterozygosity (HO) ranged from 121.53 to 1.49 and from 22.75 to 0.642 respectively. Values for expected heterozygosity (HE) and observed heterozygosity (HO) for the population are presented in table

Table 11: Summary of 10SSR markers analyzed on 17 wheat genotypes

Locus	Sample Size	na*	ne*	h*	I*
A	17	2.0000	1.7101	0.4152	0.6058
B	17	2.0000	1.9931	0.4983	0.6914
C	17	2.0000	1.8408	0.4567	0.6492
D	17	1.0000	1.0000	0.0000	0.0000
E	17	2.0000	1.8408	0.4567	0.6492
F	17	2.0000	1.5622	0.3599	0.5456
G	17	2.0000	1.2620	0.2076	0.3622
H	17	2.0000	1.2620	0.2076	0.3622
I	17	2.0000	1.1245	0.1107	0.2237
J	17	2.0000	1.9396	0.4844	0.6775
K	17	2.0000	1.9931	0.4983	0.6914
L	17	2.0000	1.5622	0.3599	0.5456
M	17	2.0000	1.4098	0.2907	0.4660
N	17	2.0000	1.5622	0.3599	0.5456
Mean	17	1.9286	1.5759	0.3361	0.5011
St. Dev		0.2673	0.3281	0.1545	0.2023

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

The genetic diversity within the 17 wheat genotypes was done to estimate the in formativeness of the microsatellites used in this study. The gene frequency data presented varied from 0.8824 for Sr2 allele 2 to as low as 0.05882 for Sr 21 allele 3

Table 12: Genetic diversity within the genotypes on the informativeness of the SSR markers

Gene Frequency :

Allele \ Locus	sr22A	sr22B	sr22C	sr26D	sr26E	sr26F	sr2G	sr2H
Allele 1								
Allele 2	0.7059	0.4706	0.6471	1.0000	0.6471	0.7647	0.8824	0.8824
Allele 3	0.2941	0.5294	0.3529		0.3529	0.2353	0.1176	0.1176

Allele \ Locus	sr2I	sr28J	sr28K	sr25bfL	sr25bfM	sr25gbN
Allele 1						
Allele 2	0.9412	0.4118	0.5294	0.7647	0.8235	0.7647
Allele 3	0.0588	0.5882	0.4706	0.2353	0.1765	0.2353

Cluster analysis

All the seventeen wheat genotypes could easily be distinguished and the UPGMA cluster tree analysis divided the seventeen wheat genotypes into three clusters with six major sub-clusters with 0.1 genetic distances. Cluster analysis classified the genotypes into three groups and the first cluster composed of genotypes SP-26, SP-N, SP-K, SP-31 and SP-21. These genotypes had similar characteristics, they had bigger seed diameter, high number of tillers per plant, longer spike length and higher 1000 seed weight as compared to the other genotypes. The second cluster was composed of SP-16, SP-R, SP-20, SP-F, SP-P, SP-34, SP-S, SP-C, SP-M and SP-D. Except for SP-16 and SP-S the other genotypes comprised of genotypes with smaller seed diameter, low number of tillers per plant, shorter spike length and lower 1000 seed weight as compared to the other genotypes. But this cluster has shown that SP16 and SP-S are distantly related to SP-R, SP-20, SP-P, SP-34, SP-C, SP-M and SP-D.

The third cluster comprised of SP-29 and SP-9 these are genotypes which have medium seed diameter, medium in number of tillers per plant, medium spike length and 1000 seed weight as compared to the other genotypes. These were further grouped into six major sub-clusters. Most small diameter seed genotypes SP-C, SP-M, SP-P and SP-D were grouped together away from the large diameter seed genotypes. The large diameter seed genotypes SP-26, SP-N, SP-K, SP-21 and SP-31 sub-clustered together. This cluster was high value for the evaluated traits, as it accounted for the highest average in terms of performance. So to increase yield performance, this clusters genotypes can be used as hybrids in the selection criteria to improve wheat genotypes grain yields.

Principal coordinates analysis

The principal coordinate analysis uncovered a similar genetic structure as the cluster analysis. There was a clear separation of the genotypes between one another on the basis of grain characteristics. Similar to the dendrogram the large seed diameter grains, long spike length and high grain yield per spike genotypes SP-26, SP-29, SP-31, SP-N, SP-9, SP-21 and SP-K clustered together while the small seed diameter, low in tillering, spike length and low grain yield per spike genotypes like SP-D, SP-C, SP-M, SP-C34 and SP-P clustered together.

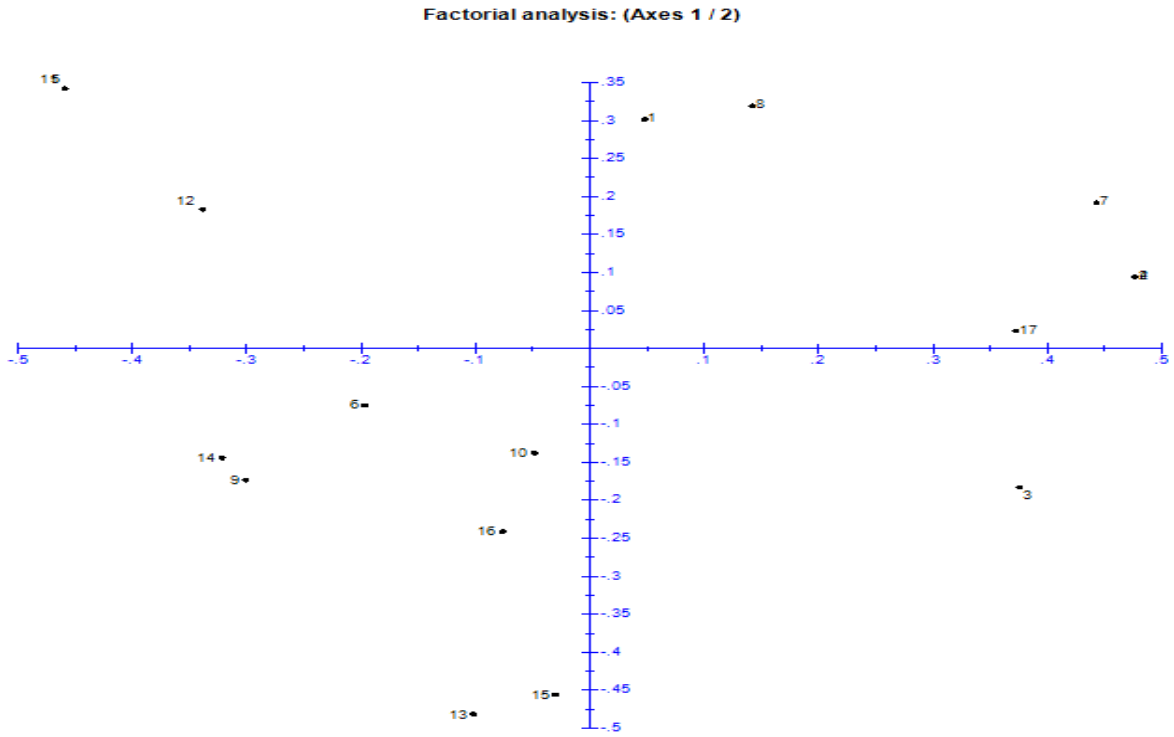


Figure 7: Principal coordinate of 17 genotypes based on analysis of 10 SSRs

The wheat genotypes segregated into 4 distinct groups with each group having discrete individuals. A. 1, 8, 7, 2, 17, B. 4, 3, C. 13, 15, 16, 10, 6, 14, 9, D. 12, 11, R.

4.4 Discussion

4.4.1 Morphological diversity of wheat genotypes

Results of the present study showed that morphological diversity existed between the mutant lines tested when compared to their parent varieties and other commercial checks. Both the qualitative and quantitative traits tested showed that diversity existed among the genotypes. Significant difference between the mutant lines and their parent varieties were observed on number of grains per spike, spikelet's per spike, maturity time period, number of tillers per plant and 1000 seed weight. The genotypes tested had erect growth habits which are related to accelerated heading and ripening time in wheat. With the necessity of breeding early maturing varieties, there exist a high correlation between growth habits, heading time and ripening time period. The largest grain heads are found at the top of the canopy at the harvest time. Those found below the top canopy have smaller heads and smaller grain size. And this could be the reasons for the preference of these commercial varieties by growers of wheat due to erect growth habits.

Two major types of wheat texture/colour were exhibited by the genotypes tested in this study, even though some genotypes exhibited an intermediary textures/colour between the two major types of wheat texture/colour. Genotypes SP-D, SP-M, SP-P, SP-C, SP-20, SP-29, SP-31 and SP-34 where soft to semi-hard white genotypes while SP-N, SP-K, SP-R, SP-S, SP-F, SP-16, SP-9, SP-21, and SP-26 were hard red to intermediate semi hard red grains in textures/colour genotypes. The hard red wheat genotypes stood out has the best wheat for baking bread and were the wheat varieties most preferred by farmers for growing than the soft white wheat.

Wheat millers prefer hard red wheat grains to blend with the white grain since the white grains to increase the gluten content in the flour. The gluten elastic nature helps to increase loaf volume by trapping carbon dioxide gas produced in the fermentation process. This means that bakers can make more bread from a given quantity of flour and the end product has improved moistness, softness and increased shelf life. The wheat texture correlates with the wheat grain weights. A lower grain weight equals lower value wheat flour and a higher grain weights brings the best price to the grower and provides the best quality wheat flour to the millers. From this study, the hard red wheat weighed between 35 – 45 grams per 1000 grains weight while soft white wheat grains weighs between 30 – 35 grams per 1000 seed weight with moisture content of 13.5%. SP-21 had the highest 1000 seed weight at 45.8 grams. From this study SP-21, SP-16, SP-26, SP-S, SP-R and SP-F (Table 8.0) meet the criteria set by the wheat millers.

Results from this study, showed that grain yield can be expressed as the product of three variables namely; number of tillers per plant, number of grains per spike and 1000 seed weight. The impact of each of these yield components on final grain yield will be determined at the different plant growth stages during the growing season. But for prospective realize of new wheat varieties, evaluation will be on the basis of milling, baking, yield and farmer preferred agronomic traits. The correlation that existed between the different traits contributed to the clustering of the seventeen wheat genotypes (Figure 6.0). Results from this study, showed that of the four criteria used for evaluating prospective new wheat varieties for realize to growers, baking qualities and increase in grain yield is perhaps the two most important parameters that are considered. SP-21, SP-26 and SP-N meet these conditions in terms of yields and baking qualities.

Results from this study showed that spike traits were a major component that separated the mutants from their respective parents. Spike traits were perhaps an important traits in determining expected grain yield. Increase in grain yield per genotype was a factor of the number of mature tillers forming heads, number of grains per spike and the 1000 seed weight. The number of mature tillers matters most because each tiller produce a mature head. It was observed that yield losses start at the tillering stage and continue to grain filling period when the grain size was determined. The number of tillers and florets initiated by the wheat plant were usually far in excess of the number of heads and grains that were supported to maturity. Results from this study showed that SP-S, SP-F, SP-R, SP-16, SP-21 and SP-26 had between 3 – 5 tillers per plant. The number of grains per spike was a function of the number of healthy shoots which was greatly influenced by the amount of nutrients supplied and the environmental conditions. It was observed that as many as 12 florets were initiated per spikelet under favorable conditions but only 2 – 4 florets actually set seed in each spikelet and the rest of the other florets aborts prior to flowering. It was observed that on average between 2 – 3 grains per spikelet's was formed and the spikelet's ranged from between 8 – 12 spikelet's per head though we had high yielding varieties like SP-21 and SP-26 which had between 18 – 20 spikelet's per head. This on average gave between 20 – 30 grains per head though the largest grain number was achieved with the genotype SP-26 and SP-21 with had 45 grains per head and 43 grains per head respectively. The timing of application of nutrients was also observed as an important aspect to tillering, 1000 seed weight and number of grains per spike. Though other factors such as genotype, population density, foliar diseases such as stem rust, insect infestation, moisture and weed control affects number of seed per head.

Analysis of results from this study showed that seed weight per plant, seed texture, number of tillers per plant and number of grains per spike were the major traits separating the mutant lines and their parent varieties. Major separation on the principal components analysis were on the spikelet's per spike, number of grains per spike, seed weight, grain yield per spike, maturity time period, number of tillers per plant and seed diameter. The difference in the wheat seed texture and the spike traits between the genotypes contributed significantly to the variability between the mutant lines and their respective parent variety. Soft white grain genotypes like SP-D, SP-P and intermediate SP-C, SP-29, SP-31, SP-34 and SP-M clustered together. Genotype SP-K had hard red intermediate to medium size grain size, medium to length in length and with elliptical grain shape clustered furthest from the other hard red grain genotypes (Figure 6.0).

The genotypes with high seed weight also had hard red grains in texture/colour and it comprised of SP-N, SP-K, SP-9, SP-16, SP-21, SP-26 and SP-R (table 8.0). They also had longer spike lengths when compared to the soft white genotypes. Mutants of SP-K had the longest maturity periods when compared to SP-N mutants, but they had more number of grains per spike and more number of tillers per plant. SP-N mutants had wider seed diameter and high grain yield per spike when compared to mutants of SP-K which were lower. The parents and the mutants clustered into two major clusters with mutants clustering with their respective parents an indication of closer relationships between parents and mutants. There was a positive correlation between seed weight, spike length and seed diameter between the genotypes which can explain the clustering. There were high interactions observed between seed weight, number of tillers per plant, grain yield per spike, spikelet's per spike and number of grains per spike.

4.4.2 Correlation coefficient of different traits

There was a highly significant positive correlations (P<0.05) that was observed between grain yields per spike (GS) and 1000 seed weight (SW) (r = 0. 93**). Grain yield per spike significantly influence the final yield. In this study the genotype with the highest grain yield per spike was SP-21 with 2.10 grams compared with its parent SP-N which had 1.78 grams. Mutant line SP-26 was the second with grain yield per spike of 2.04 grams compared with its parent SP-K which had 1.37 grams per spike (Table 8.0). These differences were statistically significant and it showed that it is possible to achieve high grain yield by simultaneously increasing the seed weight.

The mutant line with the highest 1000 seed weight SP-21 (45.7g) had lower number of grains per spike (42 grains) when compared to SP-26 (42.6 g) with higher grains per spike (45 grains) although SP-21 grain yield per spike was higher (2.10g) than in SP-26 (2.04g). This could be attributed to their difference in spike length and their seed diameter. SP-26 had shorter spike length of 9.9cm with a seed diameter of 0.25 cm while SP-21 spike length was 11.9cm with a seed diameter of 0.25 cm. This was contributed by higher number of tillers for SP-26 (5 tillers) while SP-21 had (4 tillers). Significant correlation between grain yield and number of grains per spike, spike length, seed weight and grain yield per spike have been documented by Leilah et al., (2005), who's observed a negative correlation between number of grains per spike and seed weight. And this would be expected from the fact that more grains per spike would tend to reduce the size of grains. Results from this study showed a combination of spike length, number of tillers and grain weight per spike contributed significantly to final grain yield.

Significant correlation was also observed between flag leaf area and grain yield per spike (r= 0.61**) which indicated the importance of assimilatory surface in productivity. Significant negative correlation was however observed between spike length and number of tillers per plant (r = -0. 53**). This could be attributed to reduction in food in the plant to cater for grains per spike. Significant negative correlation was also observed between maturity time period and grain yield per spike (r = -0.53**). Genotypes with longer maturity time periods showed reduced seed weight per spike. And this could be attributed to unreliable weather conditions for late maturing genotypes which contribute to the magnitude of losses in yield potential.

The genotypic coefficients of the different traits indicated greater genetic diversity in terms traits such as total number of tillers per plant, spike length, days to maturity, seed diameter, spikelets per spike, number of grains per spike and seed weight. Significant correlation existed between the different traits of the mutant lines and their respective parent varieties and these can be utilized by combining the main components which could lead to successful selection of suitable donors of one or more important traits for breeding and this also confirmed that indeed mutation changed the genotypes traits.

4.4.3 Molecular and Morphological diversity of the wheat genotypes

The use of morphological markers alone in wheat evaluation was found to be limited due to environmental influence on the genotypes traits though the use of SSRs markers will also depend on the type and number of markers available and their lineage relationship. Identification of groups of genotypes based on morphological traits was made possible by the existence of the differences that existed among the genotypes used.

This study found out that morphological and molecular markers were both useful in separating the genotypes along the seed weight, number of tillers per plant, spike length, grain yield per spike, flag leaf area, spikelet's per spike and number of grains per spike. The high correlation observed between the morphological and molecular dendrograms showed that molecular markers were able to explain the morphological groupings though not significantly. The complex genetic control of many morphological traits and the environmental influences often prohibit the determination of the precise genotype underlying each phenotype. This therefore emphasizes the importance of combining both morphological and molecular markers in germplasm evaluation.

Knowledge on relationship between grain yield and its components under mutation conditions would improve significantly the efficiency of breeding programs by identifying appropriate indices to select wheat varieties. Based on the correlation coefficient analysis, most of the variables had a high contribution to grain yield but grain yield per spike was the most effective variable separating the mutant lines from their parent varieties. According to Leilah and Al-Khateeb (2005), grain yield had a high positive correlation with the number of spikelets per spike, number of grains per spike, seed weight and grain yield per spike. The differential relations among the different traits may be attributed to the effects of mutation and environmental on plant growth. Grain yield per spike, flag leaf area, spike length, spikelets per spike and seed weight were variables that significantly separated the mutants from their parents. But lodging, plant height, and time of ear emergence, were not significantly different among the mutants and their parents. These results showed that mutation affected these traits and this was useful information for the purpose of future breeding programs.

Findings of this study are relatively similar to the studies of Leilah and Al-Khateeb, 2008, which showed that spike length, number of spikes/m^2, grain weights per spike and the biological yield contributed significantly to grain yield. The differences between the traits of the parent varieties and that of the respective mutant lines showed the effectiveness of mutation on interrelationship of the variables. These findings on the high yielding mutant lines in comparison to their parent varieties and other commercial checks was an important step to breeding programs as this mutant lines can be utilized for a broad choice of hybridization and breeding programs for new wheat varieties.

4.5 Conclusion

The seventeen wheat genotypes evaluated showed genetic diversity from one another by the difference exhibited by their traits. Average genetic diversity was observed between the selected mutants, their respective parent varieties and the commercial checks. The seventeen genotypes were morphologically categorized according to seed weight, number of grains per spike, number of tillers per plant, seed texture/colour and grain yield per spike. There was low genetic distance between the varieties in each sub-cluster and this was attributed to the high genetic similarity between the selected mutant lines, their parent varieties and other commercial checks. The results analyzed showed the observed heterozygosity was higher than the expected heterozygosity and this was attributed to the high genetic variations between the genotypes within the groupings. There were high similarities between the genotypes attributed to the close relationships and the effects of intense selection. The association between the different spike traits and the spike yield was observed and this is useful information in the selection of high yielding genotypes.

The SSRs markers gave useful information about the genetic variations and similarities between the genotypes which was an important step for breeding purposes. The results confirmed that mutation did change the relationship among the morphological traits between the selected mutant lines and their respective parent varieties. The selected mutant lines were distinct from their respective parents and therefore qualify to be released to growers as different varieties. There were also high similarities between the selected mutant lines, their respective parent varieties and the commercial checks a sign of search of preferred attributes by the industry. This study contributed to stable wheat production in Kenya fulfilling the objective of meeting the food security goals.

The study concludes by discovering traits relationship that can be used in the breeding programs for high yield gains. The selection of potential genotypes for breeding programs ought to be considered on the basis of number of grains per spike, spike length, seed weight, grain yield per spike and number of til0lers per plant. These variables were considered important contributors that distinguished one genotype from another and had greater influence to grain yield gain. Though the grain number is a function of number of fertile shoots per unit area and the number of grains per spike, the growers needs to consider plant nutrition has this showed to have greater impact on the number of grains per spike. The diverse genotypes could be selected for breeding purposes and the informative SSR markers can be used to map out important traits and consequently aid marker assisted selections.

CHAPTER FIVE

DIVERSITY IN STEM RUST RESISTANCE OF SELECTED BREAD WHEAT ELITE MUTANT LINES

Abstract

Stem rust (*Puccinia graminis* f sp *tritici)* of wheat is one of the important diseases that threaten wheat production in Kenya. Over 95% of Kenyan varieties are susceptible to stem rust. The aim of this study was to screen selected wheat mutant lines for stem rust resistance. Seventeen wheat genotypes were screen against stem rust at seedling stage in a controlled environment using (CRD) and at adult stages in the field using (RCBD) designs. Field experiments were conducted in three locations in Kenya (Eldoret, Njoro and Kitale) for two seasons in 2012 and 2013. Ten SSR markers linked to wheat resistant genes were used. Host reaction to stem rust was evaluated based on modified Cobb scale. Disease severity score, Average Coefficient of Infection and Area Under Disease Progressive Curve were used to characterize the genotypes for stem rust resistance. Genotypic, location and seasonal effect were recorded in the field. Two mutant lines SP-21 and SP-26 showed resistance to moderate resistance to stem rust at seedling and adult stages. Mutant lines SP-9, SP-20, SP-29 and SP-34 showed susceptible reactions while SP-16 and SP-31 were moderately susceptible. *Sr*2 gene linked to gwn533 was the most polymorphic marker while *Sr*28 gene linked to wPt-7004 was the least polymorphic marker. Mutant lines SP-21 and SP-26 were the most resistant and are recommended for further consideration for released as new wheat varieties and their genes could also be used to develop durable stem rust resistant wheat varieties.

5.1 Introduction

Wheat (*Triticum aestivum* spp. *Aestivum* L.) is the second important food grain to Kenya after maize. Its demand in Kenya is rising and it's expected to reach up to 60% increase by 2050 (FAO, 2017). However, Kenya is a net importer of wheat, with annual wheat consumption of 1,200,000 tons per annum against an average wheat production of 450,000 tons per year (USDA, 2017). The limitation to production included abiotic and biotic constraints but recent the latter, specifically stem rust have contributed to heavy crop losses because over 95% of Kenyan germplasm are known to be susceptible or partially susceptible to stem rust (Njau et al., 2009). Strategic introgression and deployment of resistant genes in commercial varieties greatly circumvented major stem rust epidemics but in more recent years evolution and selection for new races with increased virulence has become undesirably frequent (Singh et al., 2013).

East Africa is known to be the "hot spot" for origin of new virulent races of stem rust (Singh *et al.,* 2006). The race Ug99, first identified in Uganda in 1998 (Pretorius et al., 2000) is the only known race of *Pucccinia graminis* f. sp. *tritici* that has virulence for *Sr31* and Sr38 resistance genes which were effective against all previous stem rust races. A variant race Ug99 with added virulence on stem rust gene *Sr24* and *Sr36* further increased the vulnerability of wheat to stem rust disease (Jin *et al.,* 2008), Jin *et al.,* (2009). Currently, more than 15 confirmed races in the Ug99 linage have been reported in Africa (Singh et al., 2015). The resurgence of Ug99 and its mutants has depleted most of genetic resistance to stem rust and stem rust is at present one of the biggest threats to wheat production as most local commercial varieties are susceptible (Njau *et al.,* 2009).

While chemicals can be used to manage stem rust, the main challenge is the high cost of chemicals and its detriments to the environment. Breeding for stem rust resistance *(Pucccinia graminis f sp tritici)* is considered the most reliable way to combating the effects of this pathogen. Mutation breeding is one of the methods that has been used successfully to breed for stem rust resistance and increased yields in wheat (FAO/IAEA, 2015). Testing for stem rust resistance has been done using races of *Pucccinia graminis* f. sp. *tritici.*

Out of the few varieties showing some level of adult plant resistance *Sr2* is the only gene that provides broad spectrum resistance. However, this gene only reduces the severity of stem rust by slowing the latent period. But a combination of *Sr2* gene and other slow rusting resistant genes forms the *Sr2* complex which provides durable resistance to stem rust (McIntosh et al., 2014). Simple Sequence Repeat markers (SSR) with clear polymorphic bands linked to *Sr24, Sr31* and *Sr36* have been identified and developed. These molecular markers can complement the workload in the greenhouses and laboratory by helping to reduce the risk of 'escape' during the inoculation procedure when virulent races are unavailable. Given the devastating nature of Ug99 family of stem rust races to wheat productivity in Kenya, efforts to explore for resistance sources and incorporation of effective genes into new high yielding commercial varieties is important. Ug99 threat can be reduced to low levels by identifying, releasing and providing new varieties to farmers that are high yielding and disease resistant. The aim of this study was to screen selected mutant lines for stem rust resistance in comparison with their parent varieties and other commercial checks.

5.2. Materials and methods

5.2.1 Plant materials

Seventeen wheat genotypes comprising of eight selected mutant lines, two parental varieties and seven commercial checks were screened for stem rust resistance across the three environments in Kenya (Eldoret, Njoro and Kitale) for two seasons in 2012 - 2013. Two Kenyan high yielding and adopted commercial varieties (Kwale (SP-K) and Njoro II (SP-N) but are susceptible to stem rust were used as parents in this study. Two mutant populations selected in University of Eldoret stem rust screening nurseries were generated from these local varieties through mutagen exposure. Mutation induction was done using gamma rays from Cobalt source (CO^{60}) in Seiberdsorf Laboratories Vienna, Austria and $M_1 – M_3$ seeds had been planted and pre-selected for stem rust.

From the mutant populations, eight mutant lines were selected in the University of Eldoret. The mutant lines selected for screening included SP-9, SP-16, SP-20, SP-21 all of which are mutants of SP-N and SP-26, SP-29, SP-21 and SP-34 which are mutants of SP-K. While the seven commercial checks used for comparison purposes included; SP-D (Duma), SP-P (Pasa), SP-S (Simba), SP-F (Farasi), SP-R (Robin), SP-M (Mwamba) and SP-C (Chozi). Seed materials for the parents and commercial checks were sourced from KALRO Njoro Seed Unit. The seed materials were planted in three sites for two seasons (2012–2013) to evaluate their response to stem rust across the sites selected. The commercial checks used are among popular commercial varieties grown in Kenya. Seeds for each entry were first multiplied and purified in the green house for one season prior to planting.

5.2.2 Experimental sites

This research was carried out in three sites. The first site was University of Eldoret. The farm is located at 0°34′N; 35° 18 ′E, the site had an altitude of 2,153 m above sea level. In 2012 and 2013 it received an average annual rainfall of 1,100 mm with mean average temperatures of 17.5^{o}C. The experimental plots were different in all seasons to ensure there was no wheat planted in the last two seasons. The soils were *rhodic ferralsols* (Jaetzold *et al.,* 2006). The second site was KALRO Kitale which is located at 0°33′S; 35° 55′E, 5 km from Kitale town with an altitude of 1890 meters above sea level and mean average temperatures of 18.3^{o}C with average annual rainfall was 1,097 mm. The soils are deep, well drained, fertile sandy loam. The third site was KALRO Njoro, located at 0°20′S; 35° 56′E, at an altitude of 2,185 meters above sea level and mean annual temperatures of 17^{o}C while the average annual rainfall was about 900 mm (Ooro et al., 2009). The soils were deep, well drained, fertile *vitric Mollic Andosols* (Jaetzold et al., 2006). The sites were selected based on their significance in wheat production and high frequency of natural population for suitable for wheat stem rust disease screening

5.2.3 Field experiments

Land that was fallow, relatively flat in topography with well drained soils and free from tree stumps and shades was elected from available land in the three locations. It was ploughed to fine tilt and the seventeen genotypes were planted in a Complete Randomized Block Design (RCBD) with three replications in University of Eldoret, KALRO Njoro and KALRO Kitale in Kenya. Experimental plots were measuring 2 m by 6 rows with inter row spacing of 20cm and intra row spacing of 5cm.

Plots were separated by paths of 30 cm while blocks were separated by 2 m paths. A spreader (susceptible wheat cultivar) was planted along the border line to facilitate uniform inoculums. Seeds were hand planted head to row with Di-ammonium Phosphate (DAP 18:46:0) at a rate of 125Kg/ha while planting, followed by an application of Urea (75 kg/ha) as a source of Nitrogen at tillering and booting stages. Irrigation was done when soil moisture levels were low. The study was undertaken for two growth seasons (March and September) in 2012 and 2013. Common wheat agronomic practices (except fungicides use) were carried out as described by Kinyua et al., (2005); Weeds were controlled using Buctril MC (Bromoxynil Octanoate 225g/L and MCPA Ethyl Hexyl Ester 225g/L) at a rate of 1.25 L/ha mixed with Puma complete (Fenoxaprop-P-ethyl 64g/L+ Iodosulfuron-methyl-sodium 8g/L+ Mefenpyr-diethyl 24g/L) for both grass and broad leaf weeds. Russian Wheat Aphids (RWA) and other insects were controlled using Bulldock star EC (Beta-Cyfluthrin 12.5g/L+ Chlorpyrifos 250g/L) at a rate of 0.75l/ha.

5.2.4 Field data collected

Host response assessment was done from dough stage (Zadok's growth stage 65, 75 to 85) (Zadok et al., 1974) to grain development. Plant response observations were made three times on an interval of ten days. The host responses to infection were recorded based on (Roelfs et al., 1992). Which combines several infection type categories; R = resistant, RMR = resistant to moderately resistant, MR = moderately resistant, M = moderately resistant to moderately susceptible, MS = moderately susceptible and S = susceptible. Stem rust severity was recorded using modified Cobb scale (Peterson et al., 1948), in a scale of 0 – 100% where, 0 was immune while 100% was highly susceptible.

5.2.5 Greenhouse inoculation

The seventeen genotypes consisting of two parents, eight selected mutant lines and seven commercial checks were planted and evaluated in the rust-free greenhouse (20 to 28 ^{o}C) in a complete randomized design (CRD) replicated three times in KALRO Njoro. Ten seeds of each entry were planted in a 5 cm diameter pots filled with soil mixed with vermiculite media (six parts peat moss, four parts vermiculite, two parts perlite, three parts Roxana silt loam soil and three parts sand). The pots were then irrigated to field capacity and left to germinate in a germination chamber fitted with plastic trays with each pot in a fixed position.

When the seedlings were 10 days old, (two leaf stage) they were inoculated with bulk urediospores suspended in Soltrol 170 light mineral oil at a concentration of 6x106 spores/ml of oil. The inoculated plants were then air dried for 2 hours to allow for full evaporation of the oil from leaf surfaces. The plants were then incubated for 24 hours in a dark dew chamber and kept moist by frequent misting with distilled water using a small bowler to maintain a relative humidity of about 90% (80-100%). Temperatures were maintained of between 18-20^{o}C which is favorable for infection to take place. The seedlings were then transferred to a growth chamber where temperatures were maintained at between 22-30^{o}C after incubation for sporulation to occur. The seedlings were kept moist by misting with water at 2hr intervals after transfer to maintain about 80 - 100% relative humidity. Misting was done three times a day and was stopped when signs of infection was observed on the leaves and infection types on the plants were read 14 days after inoculation where there was maximum uredinia were observed.

5.2.6 Greenhouse data collection

Fourteen days after inoculating, the seedlings were scored on a "0" – "4" scale a procedure proposed by Stakman Figure 5 (Stakman *et al.,* 1962). Where "0" is no disease and the genotype is resistant while "4" shows susceptible genotype with large uredinia without necrosis or chlorosis. Flecking with small uredinia surrounded by necrosis or chlorosis was denoted "1". While small to medium sized uredinia surrounded by necrosis or chlorosis was denoted by "2", while moderate uredinia without necrosis or chlorosis was denoted "3". Variations with less or more than the average of a class was indicated by the use of superscript "+" for pustules that were slightly larger than expected, or "-" for pustules that was smaller than the normal size (Figure 5). The experiment was repeated three times to exclude the possibility of disease escape. Data collection was done when pustules were well developed. Its "0", ";", "1", "2" or combination indicated low infection type. Infection type "3" to "4" was considered high infection types.

5.2.7 Design

Plant disease response to stem rust data was analyzed using Genstat computer software (Gensat 15th Edition, 2012). For comparison purposes between the two parents, their respective mutants and the commercial checks on stem rust resistance, disease severity and infection response were used. The mean disease severity was used to calculate the area under disease progressive curve (AUDPC) using the formulae indicated below.

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

The data collected was entered in excel worksheets. The AUDPC values were generated on an excel worksheet using the formula above. Where, Xi is the cumulative disease severity expressed as a proportion at the *i*th observation; ti is the time (days after planting) at the *i*th observation and n is total number of observations.

The plant response to stem rust and the disease severity were converted to coefficient of infection (CI) by multiplying the disease severity with the arbitrary constant value for plant response (Roelfs et al., 1992). Where I=0, R=0.2, MR=0.4, M=0.6, MS=0.8 and S=1. Genotypes, location, replicate and Genotype x location were considered as fixed effects while incomplete blocks nested in replicates (Replicate x Block) were considered as random for coefficient of infection while blocks were fixed for yields. The following statistical model was used;

$$Y_{ijkl} = \mu + G_i + L_i + R_i + B_{)k} + GL_{il} + \mathcal{E}_{ijk}$$

Where: Y_{ijkl} = observations; μ = mean of the experiment; G_i = effect of the i^{th} genotype; L_i = effect of the l^{th} location; R_i = effect of the j^{th} replicate; $B_{)k}$ = effect of k^{th} block nested in the j^{th} replicate; GL_{ij} = effect of the interaction of the i^{th} genotype with l^{th} location and $\mathcal{E}_{ijk}$ = the experimental error.

For analysis purposes, the relationship between yield performance and field disease response was done using simple linear regression using Genstat (Genstat 15th Edition, 2012). The least square difference was determined at $P < 0.05$. The least square difference was calculated using the formula: LSD = average standard error of difference (REML output) x t/device degree of freedom (t-table).

5.2.8 Evaluation of genotypes using SSR markers linked to rust resistance genes

DNA extraction

Seventeen genotypes were evaluated for stem rust resistance genes using SSR markers. The seed for each entry to be used for DNA extraction were planted in a greenhouse and maintained. Three weeks after planting, leaves from 10 plants per genotype were harvested and crushed to make a smooth homogenate using a mortar and a sterile micro-pestle. 500 µl of SDS extraction buffer was added and mixed with the help of the micro-pestle. It was allowed to stand for 10 minutes to mix the contents. The mixture was then centrifuged at 1,000 rpm for 5 minutes to separate and form DNA pellet. The supernant was discarded out leaving the DNA pellet in the eppendoff tube. The 500 µl of 70% ethanol was added to the tube to wash the DNA. The tube was tapped to dissolve the DNA pellet and allowed to stand for a few minutes before centrifuging at 10,000 rpm for 5 minutes to re-pellet the DNA. The supernatant was discarded by pouring out the ethanol and dried wth a clean paper towel by draining away any remaining excess liquid. The tube was allowed to stand for 30 minutes for remaining liquid to evaporate and then 100 micro-litre of 1 XTE buffer was added to re-suspend the DNA before using it. The remaining DNA suspension was stored at 4^{o}C for polymerase chain reaction (PCR).

Amplification of DNA fragments

A stock solution of PCR mix was made which comprise of PCR buffer, taq polymerase, water and DNA template DNTP mix was used. Two sets of SSR primers Wmac031 and Wmac 167 which amplify a fragment of 196 bp which is a marker for wheat tem rust and another set of primers Wmag 217 and Wmac 900 which amplify a fragment of 200 bp

which is a marker for the same gene were used. Amplification was performed in a total of 10 µl reaction. The PCR profile amplification was conducted in a thermal cycler (EPPENDORF) using the following temperature profile: Initial denaturalization was 94^{o}C for 3 minutes followed by Denaturalization at 94^{o}C for 30 seconds, Annealing temperatures at 47^{o}C - 55^{o}C for 60 seconds, Extension at 72^{o}C for 40 seconds and final extension at 72^{o}C for 3 minutes and then hold at 4^{o}C.

Gel electrophoresis

After amplification, a volume of 4 µl of 6X loading buffer (10 mM tris-HCL, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA) was added to each PCR reaction. The content was loaded in 6% non-denaturing polyacrlamide gel and run horizontally at 10 V/cm for 3 hours before they were stained with ethidium bromide (0.5 µg/mL) for 30 minutes. The gel was removed from the gel tank and taken to a dark room to view DNA under (UVP GelDoc-it system)

Selection of informative SSR markers

The SSR markers were selected based on clear polymorphic bands. They were identified by screening each one of them using DNA from the controls for each gene. The band sizes were confirmed using 100 bp molecular ladder or 1 kb molecular ladders depending on the allele sizes of each marker. The bands were recorded as presence (+) or absence (-) of a linked allele and examples of bands banding patterns obtained and gel photos to show a general view of each marker.

5.3 Results

5.3.1 Field resistance

In this study, the effects of location (L), season (S), genotype (G), location by season (L x S), genotype by season (G x S) and location by season by genotype (L x S x G) interactions were significant ($P \leq 0.05$) for wheat stem rust infections. The disease severity scores (DSS) for the seventeen genotypes is presented in table 14.0. Wheat mutant lines with severity values of 20% and below were considered stable and resistant to moderately resistant against stem rust. Mutant lines SP-21 and SP-26 were considered moderately resistant and stable against stem rust with the lowest severity mean values of 8.5% and 10.0% respectively. From this study, Eldoret mean (DSS=35.8) and Kitale mean (DSS=35.2) were similar and lowest in disease severity scores while Njoro had a mean of (DSS=39.7) was the highest in disease severity scores (Table 14.0)

Mutant lines with severity values of 30% were SP-31 (23.4%) and SP-16 (30.8%) which showed moderately resistant to moderately susceptible resistances to stem rust and their severity scores were within almost that of the best parent SP-N which had 26.4%. The other mutant lines SP-29 (40.5%), SP-9 (41.2%), SP-20 (45.7%) and SP-34 (47.1%) which had severity values above 30% and showed moderately susceptible to susceptible resistances against stem rust (Table 14). Disease severity (DSS) was based on Modified Cobb's scale of 0-100% (Peterson et al., 1948) (Figure 4.0) while host response to infection type (IT) was based on (Roelfs et., 1992) where, TR = Trace, R = Resistant, MR = Moderately Resistant, MRMS (M) = Moderately Resistant to Moderately Susceptible, MS = moderately susceptible and S = susceptible (Figure 3.0).

Table 13: Stem rust severity scores of 17 wheat genotypes

EXP NAME	Average Eldoret DS mean		Average Njoro DS mean		Average Kitale DS mean		Eldor (DS) grand mean	Njoro grand mean	Kitale (DS) grand mean	Overall (DS) grand mean	Overall Infection type (IT)
	2012	2013	2012	2013	2012	2013					
Duma	42.3h	47.7d	46.2h	51.4e	43.4k	45.2e	45.0h	48.8g	44.3h	46.3g	S
Pasa	39.7g	59.9g	43.1g	62.7h	39.3i	58.4h	49.8i	52.9h	48.9i	50.5i	S
Simba	31.9f	40.7c	35.5f	43.9d	31.7g	39.5d	36.3e	39.7e	35.6f	37.2e	MS
Farasi	39.2g	46.3d	44.5g	52.2e	41.3j	44.1e	42.8g	48.4g	42.7h	44.6g	S
Robin	18.8d	40.3c	21.9c	41.9d	19.1d	39.4d	29.6c	31.9d	29.5e	30.3d	MS
Njoro II	20.2d	30.9b	22.6c	33.4b	21.8e	29.2b	25.6b	28.0c	25.5d	26.4c	MS
Mwamba	31.5f	69.2h	34.4f	71.8i	30.8g	67.8i	50.4i	53.1h	49.3i	50.9i	MS
Chozi	43.9h	55.5f	46.3h	58.9g	44.6k	52.7g	49.2i	52.6h	48.7i	50.2i	S
Kwale	33.5f	50.2e	36.2f	54.5f	33.7h	48.2f	41.9g	45.4f	41.0g	42.8f	MS
SP- 9	29.5e	51.3e	32.2e	54.9f	29.8f	49.3f	39.4f	43.6f	39.8g	41.2f	MS
SP- 16	28.1e	31.0b	32.2e	34.8c	28.9f	29.6b	29.6c	33.5d	29.3e	30.8d	MS
SP- 20	38.3g	50.7e	43.8g	53.4e	39.6i	48.1f	44.5h	48.6g	44.0h	45.7g	S
SP- 21	5.3a	10.3a	10.5a	11.5a	5.5a	8.0a	7.8a	11.0a	6.8a	8.5a	MR
SP- 26	9.1b	10.0a	9.5a	12.2a	9.0a	10.0a	9.6a	10.9a	9.5a	10.0a	MR
SP- 29	19.4d	47.7d	24.1d	51.6c	20.2e	47.9d	33.6d	37.9d	34.1d	35.3e	MS
SP- 31	15.3c	31.7b	15.2b	32.3b	15.7c	30.3c	23.5b	23.8b	23.0c	23.4b	M
SP- 34	40.2g	50.4e	48.3h	53.6e	40.7j	48.9f	45.3h	51.1h	44.8h	47.1h	S
Mean	28.6	42.9	32.7	46.4	29.2	41.1	35.8	39.7	35.2		

Treatment means within columns followed by the same letter are not significantly different at P≤0.05 according to Tukey's test.

From the above results, disease severity scores in some cases did not reflect on the infection type. An example is SP-F whose severity was 44.6% with an infection type of susceptible (S) compared to SP-M whose disease severity scores was 50.9% with an infection type of moderately susceptible (MS).

5.3.2 Epidemic Analysis

Area Under Disease Progress Curve (AUDPC) was calculated using a computer programme developed by CIMMYT following the formula given by Roelfs *et al.,* (1992). It calculates the area of the curve created by the disease scores taken at various growth stages (GS 65, GS 77 and GS 85) (Lal Ahamed *et al.,* 2004). Mutant line SP-26 of SP-K resulted to a significantly (P≤0.05) high level of resistance which led to a significantly (P≤0.05) high level of disease control among evaluated SP-K mutant lines. Mutant line SP-26 had the lowest level of wheat stem rust severity of 0.0% at GS 65, 10.00% at GS 77 and 10.00% at GS 85 and an AUDPC of 122.61 (Table 15.0). The highest level of disease severity among SP-K mutant lines was observed from mutant line SP-34 with a severity rating of 20.33% at GS 65, 40.0% at GS 77 and 50.0% at GS 85 and an AUDPC of 461.50. SP-K had disease severity rating of 10.67% at GS 65, 20.33% at GS 77 and 50.00% at GS 85 and an AUDPC of 416.67 (Table 15.0).

Mutant line SP-21 of SP-N resulted to a significantly (P≤0.05) high level of resistance which led to a significantly (P≤0.05) high level of disease control among selected SP-N mutant lines. Mutant line SP-21 had the lowest level of stem rust severity of 0.0% at GS

65, 5.00% at GS 77 and 10.00% at GS 85 and an AUDPC of 112.5. The highest level of disease severity among SP-N mutant lines was observed from mutant line SP-20 with a severity rating of 20.3% at GS 65, 30.7% at GS 77 and 50.0% at GS 85 and an AUDPC of 435.00. SP-N had disease severity rating of 10.33% at GS 65, 20.67% at GS 77 and 30.7% at GS 85 and an AUDPC of 227.67. SP-N had disease severity rating that was lower than mutant lines SP-9, SP-16 and SP-20 but was above SP-21 (Table 15.0).

Table 14: Mean Disease Severity scores, Infection Types and AUDPC of 17 wheat genotypes evaluated in Eldoret, KALRO Njoro and Kitale during 2012–2013

SP	70 DAP (GS 65)	82 DAP (GS 77)	96 DAP (GS 85)	IT	AUDPC
SP-D	25.00 *c*	40.00 *d*	50.00 *e*	4	455.50 *i*
SP-P	20.00 *d*	40.67 *d*	60.33 *f*	4	521.67 *k*
SP-S	10.33 *c*	20.33 *c*	40.33 *d*	3	389.83 *g*
SP-F	25.00 *c*	40.00 *d*	50.00 *e*	4	416.00 *h*
SP-R	10.00 *c*	20.00 *c*	40.00 *c*	2	286.00 *d*
SP-N	10.67 *c*	20.33c	30.00 *d*	2	227.67 *e*
SP-M	10.67 *d*	25.33 *d*	70.00 *f*	3	490.00 *l*
SP-C	20.00 *d*	35.00 *d*	50.00 *e*	4	511.00 *j*
SP-K	10.67 *c*	20.33 *c*	50.00 *e*	3	419.67 *h*
SP-9	10.00 *c*	30.33 *c*	50.67 *e*	3	391.33 *g*
SP-16	10.00 *c*	20.00 *c*	30.00 *d*	2	295.00 *f*
SP-20	20.00 *c*	30.33 *d*	50.67 *e*	4	435.00 *i*
SP-21	0.00 *a*	5.00 *a*	10.00 *a*	1	112.50 *b*
SP-26	0.00 *a*	10.00*b*	10.00 *a*	1	122.61 *a*
SP-29	10.00 *c*	20.00 *c*	50.00 *d*	3	397.50 *e*
SP-31	5.00 *b*	15.67 *c*	30.33 *c*	2	246.50 *c*
SP-34	20.33 *d*	40.00 *d*	50.00 *e*	4	461.50 *j*

From the results, it was also observed the AUDPC values seemed not to depend on whether a genotype was resistant or susceptible. For example SP-M had a higher AUDPC (490.00) than SP-20 (435.00) though SP-20 was susceptible (IT-4) but SP-M was moderately susceptible (IT-3). SP-21 had the lowest AUDPC of 112.5 while SP-P had the highest AUDPC of 521.67.

5.3.3 Infection type of wheat seedling of 17 genotypes grown in the greenhouse against stem rust races

Disease reactions at the seedling stage

The disease reactions of the seventeen wheat genotypes grown at the greenhouses in University of Eldoret and KALRO Njoro are summarized in Table 16. The infection type was scored on the 0 - 4 scale as proposed by Stakman *et al*. (1962). Infection types, 0, 0+, 1 and 2 were considered resistant (R), while 3 and 4 were considered susceptible (S). Variations with less or more than the average of a class was indicated by the use of superscript "+" for pustules that were slightly larger than expected, or "-" for pustules that was smaller than the normal size (Figure 5).

The genotypes showed different virulence reactions to *Puccinia graminis* f sp *tritici* race used in this study. The infection type ranged from 0 – 4, where mutant lines SP-9, SP-16, SP-21, SP-26, SP-29 and SP-31 exhibited resistant to moderately resistant reactions in the greenhouse while mutant lines SP-20 and SP-34 were susceptible at the seedling stage in the greenhouse. The greenhouse results were not different from the field results. However, clear results were exhibited in the greenhouse than in the field. Mutant lines

SP-9 and SP-29 exhibited moderately susceptible to susceptible reactions in the field but the two mutat lines exhibited moderately resistant reactions in the greenhouse. Mutant line SP-29 exhibited susceptible reaction in the field but showed moderately resistant reactions in the greenhouse. Mutant lines SP-16, SP-21 and SP-26 were the only genotypes that showed resistant to moderately resistant reactions in the field and in the greenhouse against the bulk stem rust race of Ug99 used in this study.

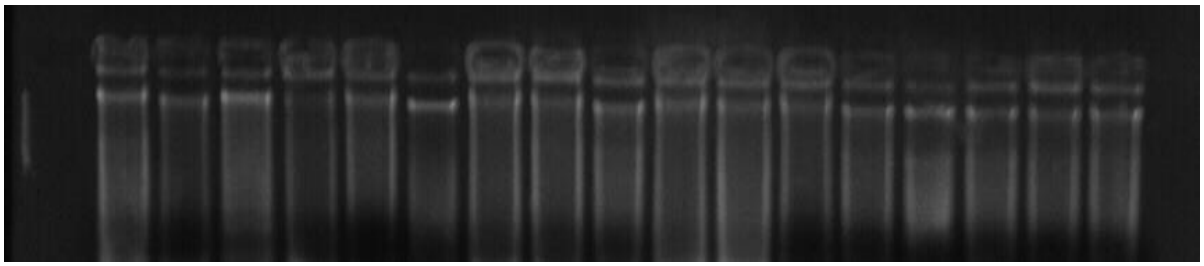
Table 15 IT of 17 wheat genotypes grown in the greenhouse against stem rust

Sample	Genotype	Infection type (IT)	Resistant/ Susceptible
1	SP-D	1(;C, 3)	Susceptible (S)
2	SP-P	1(+, 4C)	Susceptible (S)
3	SP-S	; 2+	Resistant (R)
4	SP-F	2(+,-, 4, 1C)	Susceptible (S)
5	SP-R	; 2+	Resistant (R)
6	SP-N	1,0	Resistant (R)
7	SP-M	; 2+	Resistant (R)
8	SP-C	2(+, 4C)	Susceptible (S)
9	SP- K	2+ C	Resistant (R)
10	SP-9	; 1+C, 3	Susceptible (S)
11	SP-16	; 1+	Resistant (R)
12	SP-20	2(+,-, 4, 1C)	Susceptible (S)
13	SP-21	; 0	Resistant (R)
14	SP-26	; 0	Resistant (R)
15	SP-29	; 2+	Resistant (R)
16	SP-31	; 1+C,	Resistant (R)
17	SP-34	1(;C, 4)	Susceptible (S)

IT, 0, 0+, 1 and 2 are considered resistant while 3 and 4 are considered susceptible

5.3.4 Detection of markers linked to rust resistance in wheat

Genotypic characterization to confirm presence of rust resistance gene *Sr* was done at KALRO Njoro. DNA extracted from the wheat samples were of good quality.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Plate 1. Above is a representation of an electrophoresis image for DNA quantification.

Table 16: Sample identities of the 17 wheat genotypes evaluated for *Sr* genes

Sample No.	Sample Description
1	Duma
2	Pasa
3	Simba
4	Farasi
5	Robin
6	Njoro II (Parent)
7	KS Mwamba
8	Chozi
9	Kwale (Parent)
10	Njoro II Mutant
11	Njoro II Mutant
12	Njoro II Mutant
13	Njoro II Mutant
14	Kwale Mutant
15	Kwale Mutant
16	Kwale Mutant
17	Kwale Mutant

Ten SSRs markers were used in this study which amplified DNA from the 17 wheat genotypes. The banding patterns of each molecular marker are indicated in table 18.0 below either as positive (+) /presence or negative (-)/absence of the allele linked to resistance. *Sr-26 and Sr-25* genes were the most common of the 17 wheat genotypes evaluated while *Sr-28* gene was the least common of the 17 wheat genotypes evaluated.

Table 17: Presence (+) or absence (-ve) of SSR markers linked to *Sr* genes in 17 wheat genotype

Marker/ Genotype	WMC633 *Sr*22	Sr26#43*Sr*26	gwn533 *Sr* 2	wPt0704*Sr*28	BF145935 25BF	Gb25Gb	csSr32#1 *Sr*31	Xbarc152 *Sr*33	csLr34 *Lr*34	Barc80 *Lr*67
Duma	-	-	-	-	-	+	+	+	-	-
Pasa	-	-	-	-	-	+	-	+	-	-
Simba	+	+	-		-	+	-	+	-	-
Farasi	-	-	-	-	-	+	-	+	-	-
Robin	+	-	+	-	-	+	-	+	-	-
Njoro II	-	+	+	+	+	+	-	-	+	+
Mwamba	-	-	-	+	-	+	+	-	-	-
Chozi	+	-	-	-	-	+	+	-	-	-
Kwale	+	-	-	-	+	+	+	-	-	-
N9	-	-	-	-	-	+	-	+	+	+
N16	+	-	+	-	-	+	-	-	+	-
N20	-	-		-	-	+	_	+	-	+
N21	+	+	+	-	+	+	-	+	+	+
C26	+	+	+	+	+	+	+	-	+	-
C29	-	-	-	+		+	+	-	-	+
C31	+	+	+	-	-	+	+	-	-	-
C34	-	-	-	-	-	+	+	+	-	+

WMC633$_{170}$ linked to *Sr-22*

This molecular marker is a dominant marker represented by 170 bp resistant allele. Amplification of 170 – 260 bp in diverse lines is associated with presence of *Sr-22* gene.

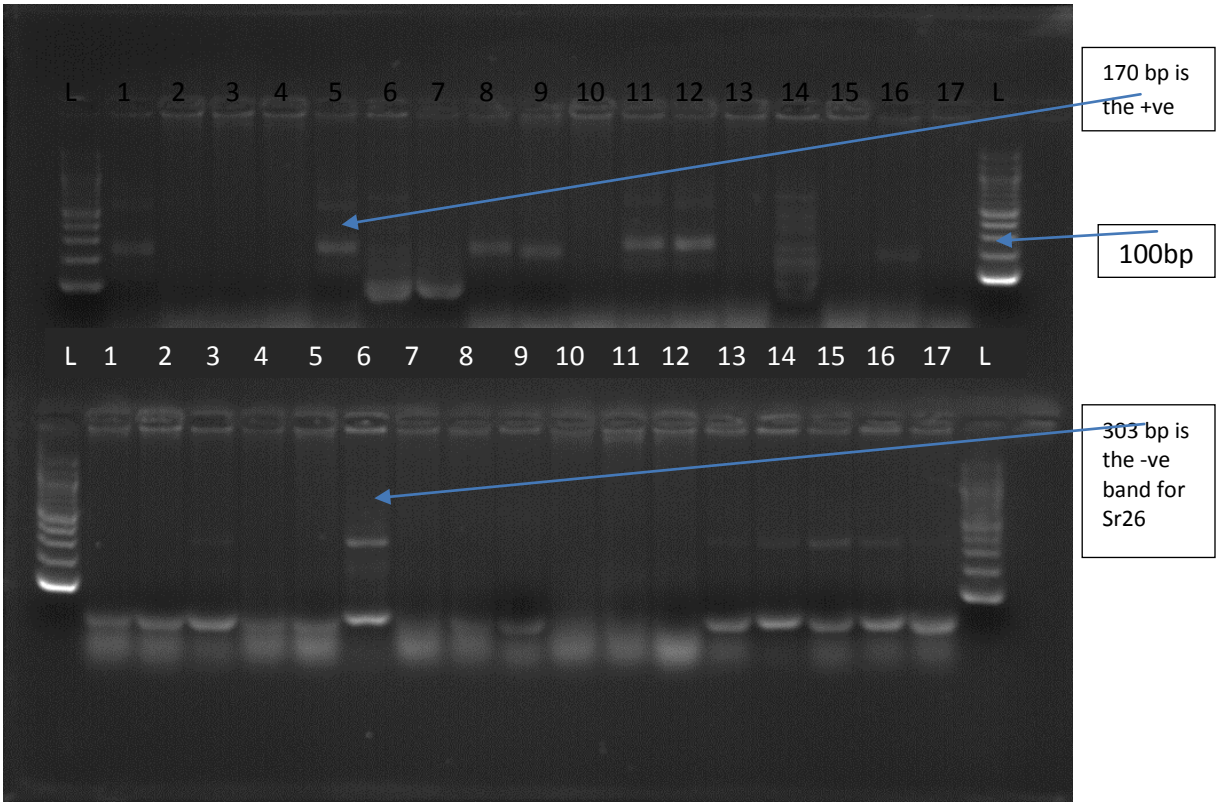


Plate 2: Gel picture indicating polymorphism revealed by the wheat WMC633 linked to *Sr-22* gene (above) and marker *Sr*26#43 linked to *Sr-26* gene (below) primer set amplification

Sr*26#43$_{303}$ linked to *Sr-26

This molecular marker is a co-dominant marker represented by 303 bp resistant allele. *Sr*26#43 amplifies a fragment of 207 bp when *Sr*26 is present and yields a fragment of 303 bp when *Sr*26 gene is absent in the genotype.

Gwn533$_{115}$ linked to *Sr-2*

This molecular marker is a dominant marker represented by 115 bp resistant allele. *Sr2* provides partial resistance which is difficult to select under field conditions. The markers associated with *Sr*2 gene amplify at 115 – 122 bp in diverse lines.

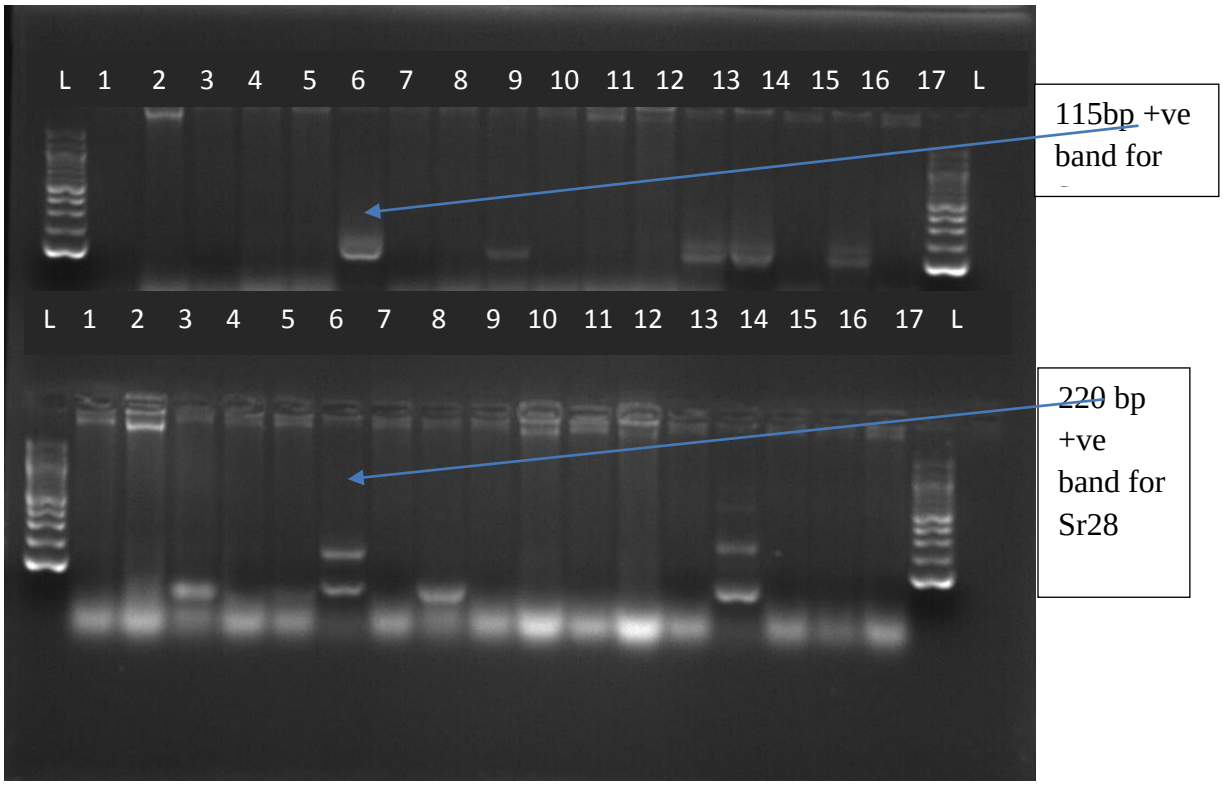


Plate 3: Gel picture indicating polymorphism revealed by the wheat marker Gwn533 linked to *Sr-2* (above) and marker Wpt7004 linked to *Sr-28* (below) primer set amplification

Wpt7004$_{220}$ linked to *Sr-28*

This molecular marker is a dominant marker represented by 220 bp resistant allele. Amplification product of 214 – 220 bp in diverse lines is associated with the presence of *Sr*28 gene.

BF145935$_{198}$ linked to *Sr-25BF*

This molecular marker is a co-dominant marker represented by 198 bp resistant allele. Marker BF145935 linked to *Sr-25BF* produced the fragment sizes of 198 and 180 bp in *Sr*25 lines and 202 and 180 bp in wheat lines without *Sr*25. The dominant marker Gb amplified at 130 bp fragment only in the *Sr*25-positive lines and no PCR product was obtained in wheat lines that lack *Sr*25

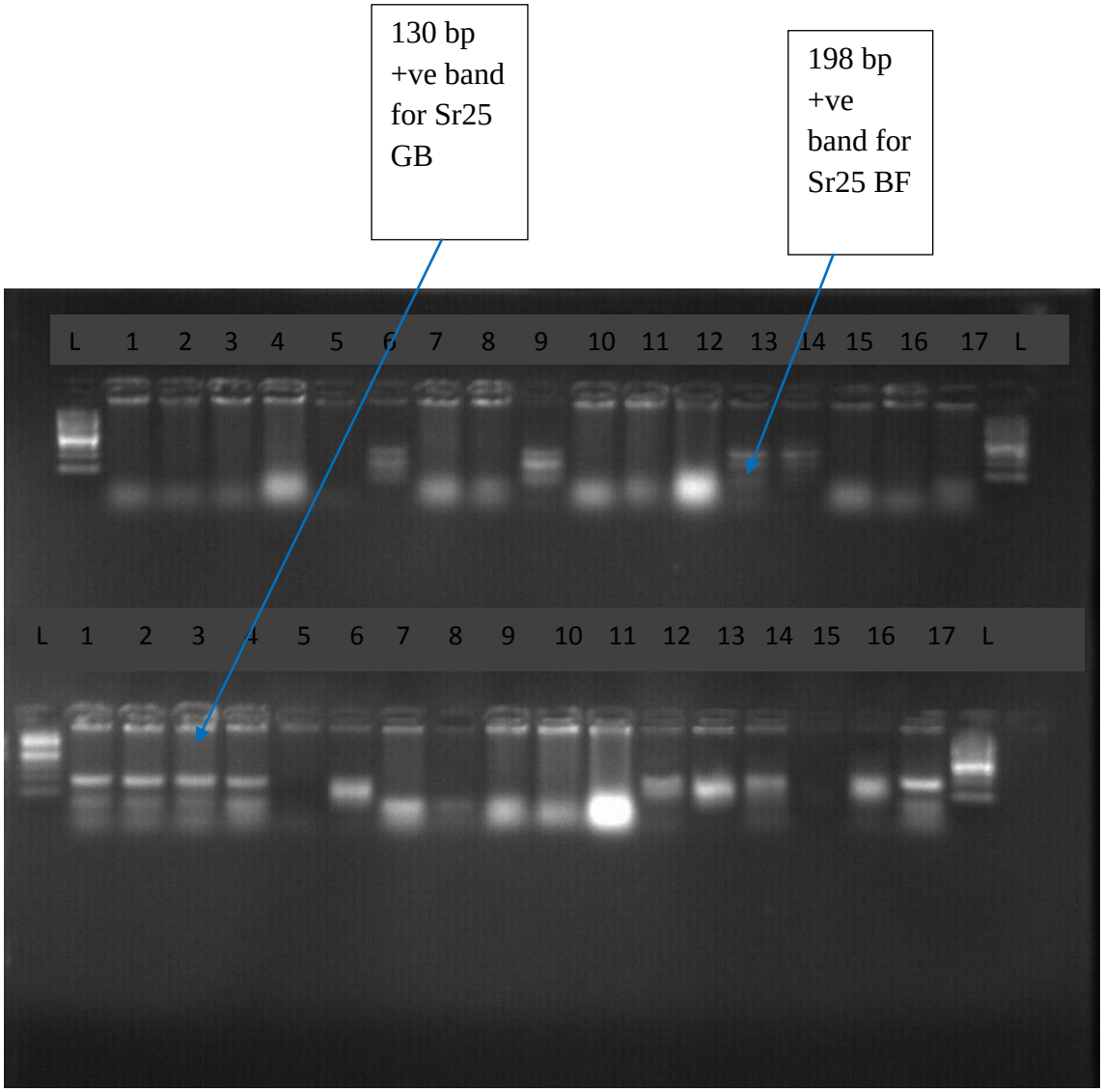


Plate 4: Gel picture indicating polymorphism revealed by the wheat marker BF145935 linked to *Sr-25BF* (above) and marker GB linked to *Sr-25GB* (below) primer set amplification. GB_{130} linked to *Sr-25GB*

$CSSr32\#1_{150}$ linked to *Sr-31*

This molecular marker is a dominant marker represented by 150 bp resistant allele. Amplification of 150 bp in diverse lines is associated with the presence of *Sr*31 gene.

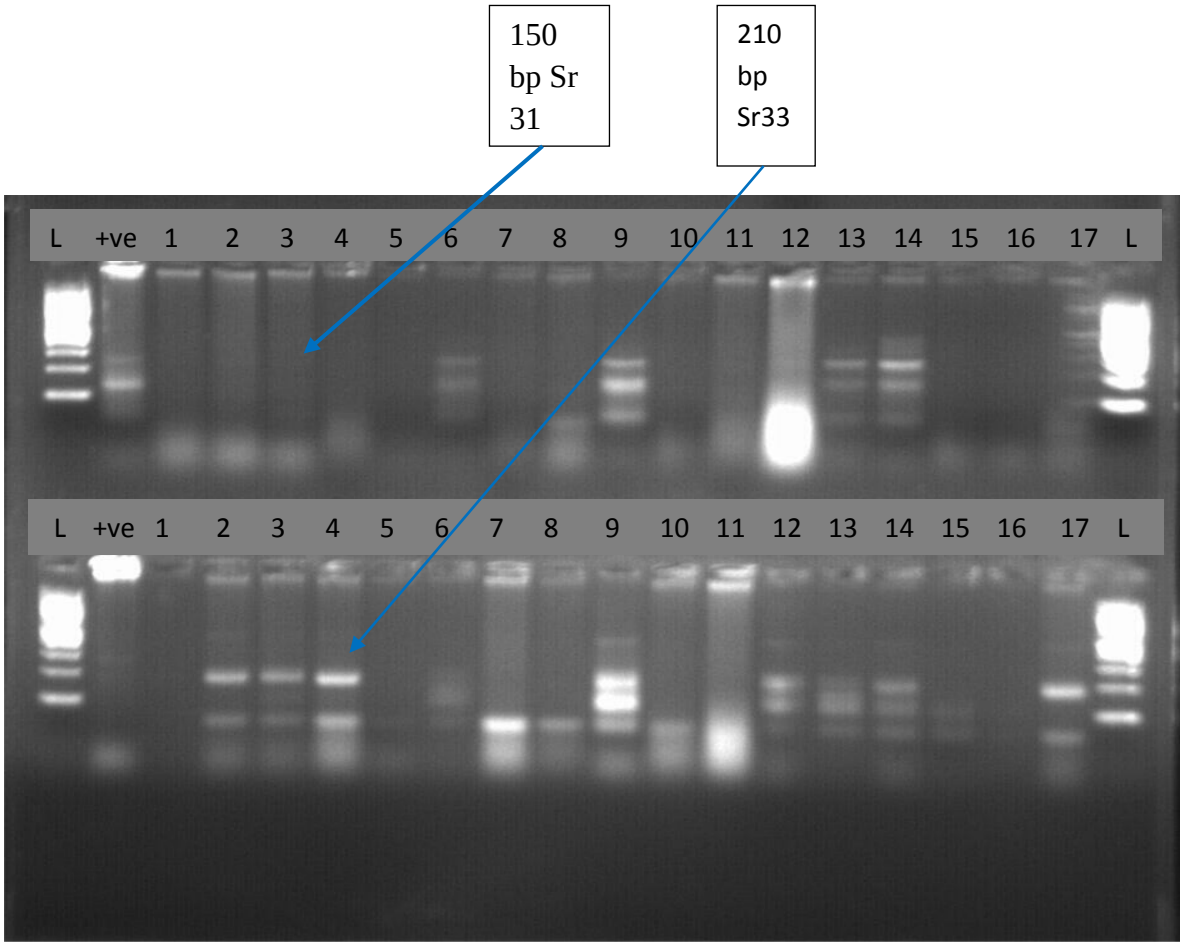


Plate 5: Gel picture indicating polymorphism revealed by the wheat marker CSSr32#1linked to *Sr-31* (above) and marker XBARC152 linked to *Sr-33* (below) primer set amplification

XBARC152$_{210}$ linked to *Sr-33*

This molecular marker is a dominant marker represented by 210 bp band. Amplification product of 210 bp in diverse lines is associated with the presence of *Sr*31 gene. This gene provides an intermediate level a resistance to several *Puccinia graminis* sp. *tritici* races. Sr33 gene is flanked by Xbarc152 and Xcfd15 markers.

CSLr34$_{150}$ linked to *Lr-34*

This molecular marker is a dominant marker represented by 150 bp resistant allele. Amplification of 150 bp in diverse lines is associated with the presence of *Lr-34* gene.

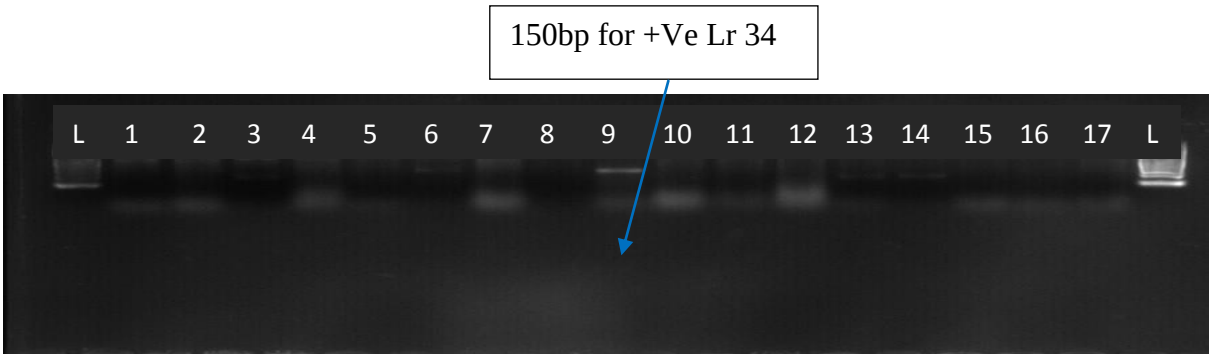


Plate 6: Gel picture indicating polymorphism revealed by the wheat marker CSLr34 linked to *Lr-34*

BARC80$_{214}$linked to *Lr-67*

This molecular marker is a dominant marker represented by 214 bp resistant allele. Amplification of 214 bp in diverse lines is associated with the presence of *Lr67* gene.

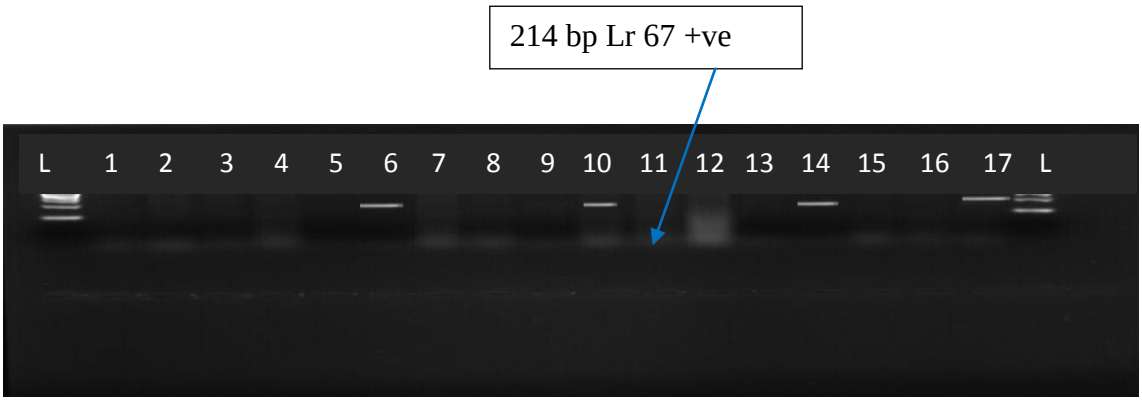


Plate 7: Gel picture indicating polymorphism revealed by the wheat marker BARC80 linked to *Lr-67*

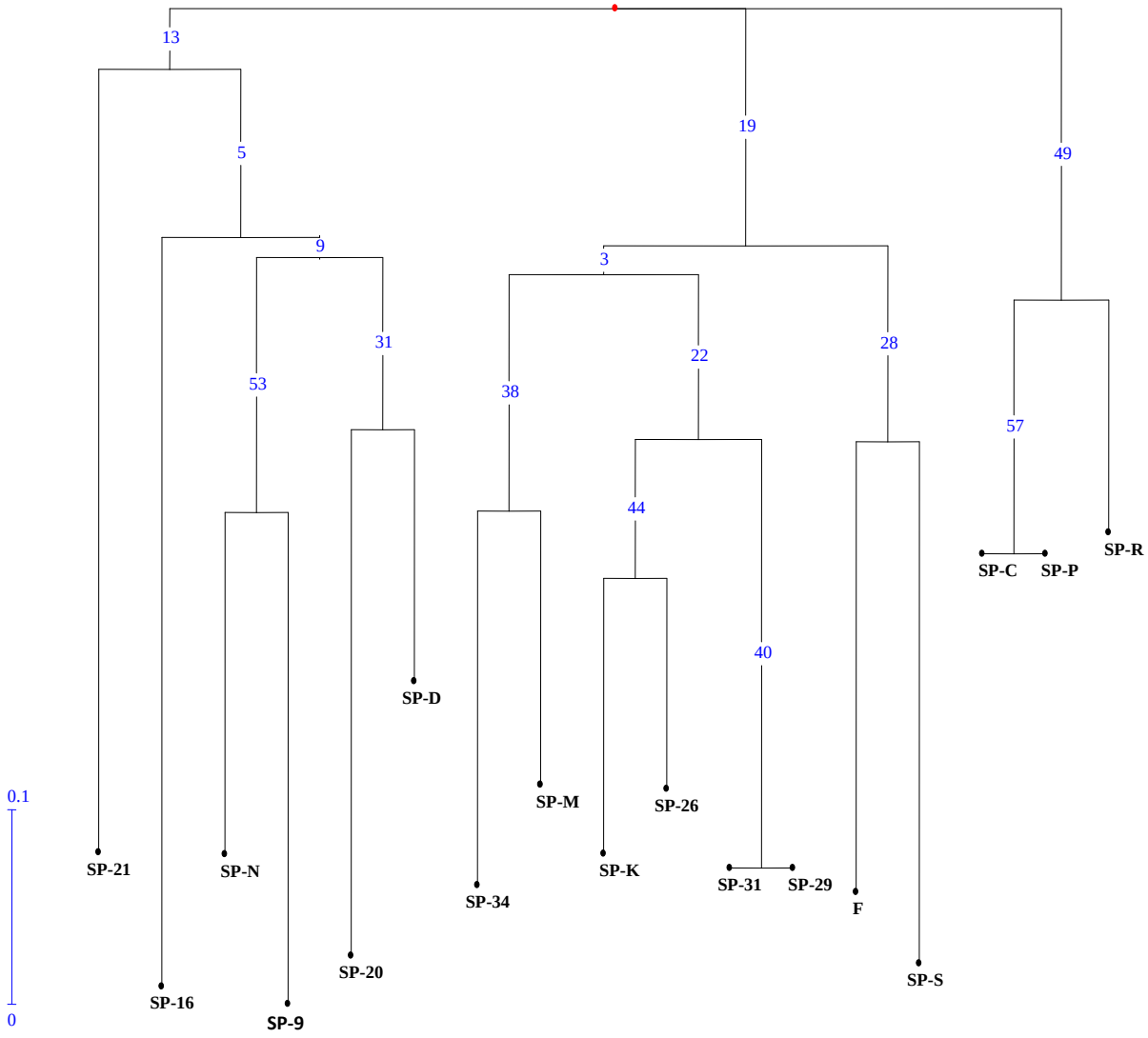


Figure 8: Dendrogram illustrating similarities among 17 wheat genotypes using UPGMA tree based on reactions to stem rust.

The dendrogram generated from the results (Table 14) showed the evaluated wheat genotypes segregate into three major clusters and six sub-clusters. The mutants clustered with their respective parental varieties as their resistance profiles were similar or related significantly all the mutants segregated with their parental varieties and hence resistance profiles of parents can be used as references to characterize resistance of the mutant lines.

5.4 Discussion

5.4.1 Reaction of Wheat genotype to wheat stem rust and field infection

Out of the four SP-K mutant lines evaluated against stem rust races, it is only mutant line SP-26 which showed the lowest level of stem rust at final disease score of 11.5%. This level of stem rust resistance translates to infection types 1, similarly, Area Under Disease Progress Curve was at 122.61. The other mutant lines of SP-K had final disease score as follows; SP-31 (23.4%), SP-29 (35.3%) and SP-34 (47.1). Therefore of all the four mutant lines of SP-K evaluated its only mutant line SP-26 that showed acceptable levels of wheat stem rust resistance and severity of an IT ≤ 2 and final disease score of ≤ 10. Therefore SP-26 a mutant line of SP-K can be recommended to farmers for growing as new wheat variety that is resistant against stem rust races.

Out of the four SP-N mutants evaluated against stem rust races, only SP-21 showed acceptable levels of stem rust severity and can therefore be recommended to the farmers for growing as new wheat varieties that are resistant against stem rust races. Mutant line SP-21 showed the lowest level of stem rust with a final disease score of 8.5%. This level of stem rust resistance translated to an infection type 1, similarly, Area Under Disease Progress Curve was at 112.5. The other mutant lines of SP-N had final disease score as follows; SP-16 (32.8%), SP-9 (41.2%) and SP-20 (45.7). Therefore of all the four mutant lines of SP-N evaluated only SP-21 showed acceptable levels of wheat stem rust resistance and severity of an IT ≤ 2 and final disease score of ≤ 10. Therefore SP-21 can be recommended to the farmers for growing as a new wheat variety that is resistant against stem rust races.

The other commercial checks were as follows; SP-D (46.3%), SP-P (50.5%), SP-S (37.2%), SP-F (44.6%), SP-R(32.3%), SP-M (50.9%), SP-C (50.2%) and SP-K (42.8%) showed susceptibility of varying degree ranging from Moderately Susceptible (MS) to Susceptible (S) with the most susceptible genotype being SP-P which had a severity score of 50.5. The above commercial checks were amongst the old varieties of wheat that are susceptible to stem rust races but still grown by farmers. This is because majority of them have stem rust resistance genes which are now ineffective against stem rust but still poses some genes which were used in most of the world's wheat varieties before it was broken down by the new stem rust races.

On comparison with the two parent varieties, SP-N showed moderately susceptible infection with a disease infection score of 26.4% while SP-K had disease infection score of (42.8%). The two parent varieties are commonly grown wheat varieties in Kenya which showed moderately susceptible infection though in some locations they showed moderately resistant infection type. This indicates a possibility of these varieties to carry several stem rust resistance genes some of which are effective while others may be ineffective against stem rust races. There is also a possibility that they may have effective stem rust resistance genes but their expression levels of these genes are low to provide sufficient resistance against stem rust. The resistance showed by SP-N and the resistant mutant lines can be attributed to the presence of resistant genes. *Sr2* gene is amoung resistant genes that are well characterized with known APR gene that provides a broad-spectrum resistance to all known variants of stem rust races (Njau et al., 2009). Four mutant lines SP-16, SP-21, SP-26 and SP-31 showed varying levels of resistance with the most resistant mutant line being SP-21 with a severity score of 8.5% (MR).

These moderately resistant mutant lines have major (race-specific resistance) and minor (adult plant resistance) genes or a combination of both. Analysis of these mutant lines reveals possibilities of them carrying the APR gene *Sr2* and further research can be done to confirm this finding. These genes provide varying resistance to stem rust races.

From this study, important sources of wheat rust resistance were identified. Wheat genotypes SP-21 and SP-26 can give impending protection against wheat stem rust under natural conditions. The two evaluated mutant lines can be recommended to the farmers for planting as new wheat varieties in most of the wheat growing areas of Kenya. SP-N being one of the parents that developed SP-21 mutant line was moderately susceptible showing that it is still exhibiting some resistance to stem rust unlike SP-K parent which is now susceptible and whose resistance could have been wearing out over time. The genotypes (SP-21 and SP-26) exhibited resistant reactions at seedling stage in the greenhouse and showed resistant to moderately resistant reactions in the field conditions considering Eldoret and Njoro locations as the 'hot spots' areas for stem rust in Kenya.

From the results of this study it can be concluded that the resistance in the high yielding SP-N is still available when compared with the other commercial checks varieties evaluated which showed susceptible reactions to stem rust. Mutant lines SP-21 and SP-26 are promising to be important sources of wheat stem rust resistance. The addition of other commercial checks in this study was to give more understanding on the types of resistant genes available in these commercial varieties and compare them with the evaluated mutant lines in terms of disease resistance.

The analysis of mutant lines SP-9 and SP-20 for SP-N parent showed they have fewer adult plant resistance minor genes than SP-N and this is a negative mutation resulting in inferior resistant plants compared to the parent variety. This may have resulted from deletion of some resistance genes due to exposure to mutagens. Results show mutant lines SP-21 and SP-26 having more adult plant resistance minor genes than the parents and therefore the mutants exhibited higher levels of resistance than their parental varieties. This is a case of beneficial mutations resulting in superior mutants than the parents in resistance to stem rust.

Results from these study show that resistant mutant lines have at least more than four to five *Sr* genes. Genotypes SP-21 and SP-26 contain multiple *Sr* genes and therefore the kind of resistance they carry could be of value to local breeding programmes. This is because there is need for identification and characterization of *Sr* genes contained in locally adapted resistant sources to enable easy introgression of these genes during breeding. Based on results of this study, mutant lines of interest for further studies and characterization are SP-21 and SP-26. Other genotypes that might require gene pyramiding to improve on their resistance are mutant lines SP-16 and SP-31. Mutant lines SP-16, SP-21, SP-26, SP-29 and SP-31 showed resistance at seedling stage implying these varieties may contain major genes that are effective against stem rust races at seedling stage since they had a score of 0, 1 and 2. Mutant lines SP-9, SP-20 and SP-34 showed susceptible reaction at seedling stage with a score of 3 and 4. This may imply that these mutants may not have major genes that are effective against stem rust races at seedling stage or these major genes may be present but not effective against stem rust races at seedling stage.

5.4.2 Genotype x environment

The significant difference observed between the two seasons in Eldoret, Kitale and Njoro contributed to the variable infection types exhibited by the genotypes across the seasons. The difference could have been due to the relative humidity that resulted from rainfall amounts received in the two seasons (2012 and 2013). Disease severity was higher in 2013 than was in 2012 in the three locations evaluated.

Seasonal effects of temperature and precipitation on disease development were discernible. Temperatures ranging from between 18–25 ^{o}C and relative humidity > 95% for more than seven days is ideal for rust development (Stavely, 2005). In season one when the temperatures were higher and precipitation was lower, the infection rates were lower. In season two, there were relatively lower temperatures and higher precipitation and the disease pressure was higher. Similar findings were extensively reported in rust epidemiology findings GRDC (2011). Results from this study showed the genotype x environmental interaction was significant for rust infection and this indicated variable infection of rust on genotypes across the three locations. High temperatures induce plants defense mechanisms increasing plant resistance to stem rust (Mittler, 2006). Njoro area had higher disease severity than was in Eldoret and Kitale. This result underscores KALRO-Njoro as an environment conducive to stem rust proliferation. Different reactions to stem rust observed between genotypes across the different locations, suggested that the environment contributed to the level of disease infections. The implication of genotype x location interactions is that the genotypes can be recommended for a specific location.

5.4.3 Disease severity and Area Under Disease Progressive Curve (AUDPC)

Out of the seventeen genotypes evaluated eleven genotypes showed susceptibility of varying degrees ranging from moderately susceptible, moderately susceptible-susceptible to susceptible with the disease severity being from SP-M which had disease reaction score of 70%. Most of the eleven genotypes that showed susceptibility to stem rust may be having ineffective stem rust resistance genes against stem rust races. An example is SP-M and SP-C which are known to carry *Sr*31 resistant gene. Six genotypes showed varying levels of resistance with the most resistant being SP-21 with a final disease reaction score of 8.5%. Most of this recent genotypes are known to carry major (Race-specific Resistance) and minor (Adult Plant Resistance) genes or a combination of both. An example is SP-R which is known to carry the minor resistant gene *Sr*2.

The Area Under Disease Progressive Curve (AUDPC) showed there was variation in the mean AUDPC values among the different genotypes evaluated. The lowest AUDPC was SP-26 with a mean AUDPC of 112 and SP-P with the highest AUDPC value of 521 (Table 15). It was also observed that AUDPC values do not necessarily depend on whether a genotype is resistant or susceptible. For example susceptible (S) genotype SP-F (416.00) had lower AUDPC values than moderately susceptible (MS) SP-K (419.67). AUDPC values can be used to measure the phenomena of slow rusting which is majorly attributed to the slow rusting APR gene *Sr*2. Results showed genotypes with *Sr*2 gene had severity scores of below 30% and AUDPC value below 300. These observations agrees with previous findings that genotypes know to have *Sr*2 gene have slow rusting effect as a form of broad spectrum resistance to stem rust disease (Njau et al., 2010).

5.4.4 Molecular characterization

The ten SSR primers used amplified the bands linked to the *Sr* genes for stem rust resistance and showed presence of alleles linked to stem rust resistance but the size of bands found on each genotype varied. Mutant lines SP-21 and SP-26 showed presence of most *Sr* genes linked to stem rust resistance an indication that induction of mutations on SP-K and SP-N generated mutant lines with resistance against stem rust. Mutant lines SP-9 and SP-20 had less *Sr* genes than the parent. This is considerer negative mutation resulting in inferior resistance compared to the parent which may have resulted from deletion of some resistance genes due to exposure to mutagens. Meanwhile mutant lines SP-26 and SP-31 had more *Sr* genes than the parent SP-K. This is considerer positive mutation resulting in superior resistance when compared to the parent variety.

Different reactions to stem rust observed between the genotypes suggest that the materials had diverse genetic backgrounds. The two mutant lines SP-21 and SP-26 that showed resistant to moderately resistant reactions could either be carrying single effective major genes or a combination of those. Singh et al. (2005) reported that a combination of 4-5 minor effect genes with race non-specific responses provided near immunity reaction to leaf rust. Similar results were reported by Leornard and Szabo (2005) who suggested that the presence of effective major genes in a variety limit infection process by triggering necrosis of the host cells in the neighborhood of the ineffective structures. The high frequency of susceptibility among commercial checks suggests presence of ineffective stem rust resistance genes in their background, probably Sr31 and Sr33 to which the current family of stem rust races are highly virulent.

5.5 Conclusions and recommendations

The molecular characterization, greenhouse and field experiments allowed the assessment of the response of genotypes to stem rust and stem rust resistance as a desirable output was achieved. The results showed the wheat genotypes evaluated in this study had high genetic diversity regarding their response to stem rust showing that the materials used were from diverse genetic backgrounds. The effects of genotypes, location and season were noted as this lead to different responses of the genotypes to different environments brought about by their differences in genetic makeup. Effective resistant genotypes across the environments were identified. The resistant mutant lines SP-21 and SP-26 can therefore be recommended to farmers for growing as new wheat varieties with stem rust resistance. They could also be used in wheat breeding as sources of resistance genes to wheat stem rust. Nakai et al., (1990), Taura et al., (1991) reported new disease resistant genes in rice through induced mutation.

Further introgression of resistances genes to the genotypes that showed resistances of infection type < 2 is recommended. Genotype SP-16 and SP-31 which showed moderate susceptibility to stem rust are potential mutant lines for gene staking which could be advanced for future release. Further screening of genotype SP-21 and SP-26 should continue to monitor the effectiveness of their resistance. Inheritance studies should also be done among the resistant mutant lines to elucidate the exact genes and their effects in conditioning the stem rust resistance. The use of simple sequence repeat (SSR) molecular markers in evaluating disease resistance in wheat genotypes proved to be a powerful tool in showing major separations between different wheat genotypes.

The results obtained from this study indicate that stem rust is in constant mutation state in order to infect new wheat genotypes that are an output of breeding and majority of the old commercial varieties are either moderately susceptible or susceptible. The development of new resistant wheat varieties to stem rust is a substantial step towards the improvement of rust resistance in wheat breeding in Kenya and a major step towards fulfilling the food security objective.

Therefore genotypes SP-21 and SP-26 were identified as resistant wheat genotypes that are recommended to national performance trials (NPT) for further evaluations to be released to farmers for growing as new wheat varieties. Their genes should also be characterized and used as donors for introgression of resistance genes to adopted but susceptible Kenyan wheat varieties.

CHAPTER SIX

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussions

The study showed that selection that selection for spike traits of wheat contributed to the major separation of Kenyas wheat genotypes using the 25 agro-morphological characters. The wheat breeders are familiar with the need of the farmer for a genotype that is disease resistant and high yielding while taking into consideration the bakers requirements for genotypes with good baking qualities The polymorphic SSRs markers used were usefully in producing informative bands. The genotypes belonging to the same sub-cluster were genetically similar while those belonging to the different sub-clusters were different from each other. The SSRs used in this study demonstrate the ability of SSRs to produce unique DNA profiles and establish discrete identity and disease resistance patterns.

The results obtained on rust diversity from this study indicate that the rust pathogen is highly variable and most locally grown commercial varieties are susceptible to the pathogen. But also few locally grown genotypes though they were moderately susceptible, showed moderate resistance at adult stage a show that they may contain adult plant resistant (APR) minor genes for instance SP-N. There was a negative correlation between yield and stem rust response which revealed a linear and an inverse relationship between yield and disease parameters. Yield variability and disease pressure existed across the three sites

6.2 General conclusions

Morphological characteristics clustered the mutant lines with their parents majorly on seed weight, number of grains per spike and seed colour/texture. Considerable amounts of genetic diversity were observed between the mutants, parents and commercial checks varieties. There was low genetic distance between the genotypes in each sub-cluster attributed to the high genetic similarity between the mutants and their parents. Observed heterozygosity was higher than expected heterozygosity due to the high genetic variations between the genotypes and within the groupings there were high similarities due to the close relationships and the effects of intense selection in search of the good quality attributes. The effects of genotypes, location and season were noted as this lead to different responses of the genotypes to different environments brought about by their differences in genetic makeup. The disease pressure in each environment influenced yield performance of each genotype.

This study identified wheat mutant lines with resistance to stem rust and this is significant in breeding for stem rust resistance. Mutant lines SP-21 and SP-26 were identified as the genotypes with moderate resistance to stem rust and also high yielding. These mutant lines gave significantly higher yields compared to their parents. Other important sources of resistance identified in this study were SP-16 and SP-31. Disease severity at early stage was highly positively correlated to disease progress. And in subsequent stages severities was negatively correlated to yield irrespective of level of resistance. Important SSR markers for selection of wheat were identified and the useful ones can be used for marker-assisted selection during breeding.

6.3 General recommendations

This study makes the following recommendations;

(i) The resistant mutant lines SP-21 and SP-26 can be recommended to National Performance Trials (NPT) for further evaluation and future release as new wheat varieties with stem rust resistance.

(ii) Resistant mutant lines SP-21 and SP-26 can be used in wheat breeding as sources of resistance genes to wheat stem rust.

(iii) Further screening of genotype SP-21 and SP-26 should continue to monitor the effectiveness of their resistance to stem rust.

(iv) Further introgression of resistances genes to the genotypes that showed resistances of infection type < 2 is recommended.

(v) Genotype SP-16 and SP-31 which showed moderate susceptibility to stem rust are potential mutant lines for gene staking which could be advanced for future release.

(vi) Inheritance studies should also be done among the resistant mutant lines to elucidate the exact genes and their effects in conditioning the stem rust resistance.

(vii) The use of simple sequence repeat (SSR) molecular markers in evaluating disease resistance in wheat genotypes proved to be a powerful tool in showing major separations between different wheat genotypes.

REFERENCES

Agrios, G. N. (1997). Plant Pathology 4th ed. Academic Press, San Diego. U.S.A. Pp 368-375.

Bade, CIA, Carmona AM (2011). Comparison of methods to assess severity of common rust caused by *Puccinia sorghi* in maize. *Trop. Plant Pathol. 36*(4):264-266.

Ben-Ari, Giora; Lavi. (2012). *Plant Biotechnology and Agriculture. Marker-assisted selection in plant breeding (http://www.sciencedirect.com/topic/biochemistry-genetics-and molecular-biology/marker-assisted-selection*). Science Direct. pp. 163-184.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M. and Parr-Dobrzanski, B. (2002). Review: The Strobilurin Fungicides. *Pest Management Science* 58: 649-662.

Borlaug, N. E. (1954). Mexican wheat production and its role in the epidemiology of stem rust in North America. *Phytopathology* 44: 398-404.

Browder, L. E. (1971). Pathogenic specialization in cereal rust fungi, especially *Puccinia recondita* f. sp. *tritici*: Concepts, Methods of Study, and Application. ARS USDA Tech Bull No. 1432.

Bruce, A., McDonald, L. and Celeste, L. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* 40: 349-79.

Collard, B., Jahufer, M., Brouwer, J. and Pang, E. (2005). An Introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica 142*(1-2): 169-96.

Clayton W. and Renvoize S. A. (1986). *Genera Graminum Grasses of the World*, LondonHMSO.p 146-158.

Eagles H. A., Barina H.S. and Ogbonnaya F.C. (2001). Implementation of markers in Australia wheat breeding. *Crop and Pasture Science 52*(12):1349-56.

Das, K. B., Saini, A. Bhagwat, S. G. and Jawali, N. (2006). Development of SCAR markers for identification of stem rust resistant gene *Sr31* in homozygous or heterozygous conditions in bread wheat. *Plant Breed*. 125:544-549.doi: 10.1111/j.1439-0523.2006.01282.x

Dubcovsky J. and Dvorak J. (2007). Genome plasticity a key factor in the success of polyploidy wheat under domestication, Science, vol. 316 (p. 1862-1866)

Dudley, J (1993). Molecular markers in plant improvements: Manipulation of genes affecting quantitative traits. *Crop Science; 33*(4):660-68

FAO, (2010). FAOSTAT. Food and Agriculture Organization of the United Nation, Rome. http://faostat.fao.org. (Accessed 30th June, 2010).

FAO, (2012). Crops and Cropping systems. http://www.fao.org/ag/ca/../06CROP1.PDF. Accessed 18/11/2015.

FAO, (2016). *Agricultural commodities profiles and relevant WTO negotiations issues*. Economic and Social Development Department. Retrieved September 1, 2016, from http://faostat.fao.org/economics/ess/ess-home/en.

FAO, (2017). World Food Situation. Available: http://www.fao.org/worldfoodsituation/csdb/en

Farooq, S., Iqbal, N. and Arif, M. (1996). Detection of Genetic variability in Basmati and non-Basmati rice varieties and their radiation induced mutants through Random Amplified Polymophic DNA (RAPD). Application of DNA based marker mutations for improvement of cereals and other sexually reproduced crop plants proceedings of a final research co-ordination meeting Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna (Austria).

Feldman M. and Kislev M. (2007). Domestication of emmer wheat and evolution of free-threshing tetraploid wheat, Israel J. Plant Science., vol 55 (p. 207-221).

Finney, K. F., Yamazaki, W. T., Youngs V. L. and Rubenthaler, G. L. (1987). Quality of hard, soft and durum wheats. p. 677-748 in E.G.Heyne (ed.). Wheat and Wheat Improvement. 2nd ed. Agron. Monogr. 13. ASA. CSSA and SSSA. Madison. WI, USA.

Friebe, B., Jiang, J., Raupp, W. J., McIntosh, R. A. and Gill, B. S. (1996). Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91: 59–87.

Forster B. and Shu Q. (2012). Plant mutagenesis in crop improvement: Plant mutation breeding and biotechnology. Wallingford: CABI; p.9-20

Gamalat, A. and El-sawi S. (2015). Inheritance of stem rust resistance and some yield components in crosses from five Egyptian wheat cultivars. Egypty J. Plant Breed. 19:71-87.

Gill, B. S., Friebe, B. R., and. White, F. F. (2011). Alien introgressions represent a rich source of genes for crop improvement. Proc. Natl. Acad. Sci. USA 108: 7657–7658.

GOK (2010). Agricultural sector development strategy 2010-2020. Jaetzold R, Schmidt H, Hornetz B, Shiisanya C (2010). Farm Management Handbookk of Kenya. Volume II, Part B: NaturalConditions and Farm Management Information, Central Kenya (Rift Valley and Central Provinces)pp. 379-404

Grain Research and Development Cooperation (GRDC). (2011). Stem rust of wheat fact sheet. *Seasonal conditions drive outbreaks*. Retrieved from http:// www.grdc.comau/rustlinks

Green, G. J., Martens, J. W. and Ribiero, O. (1968). Epidemiology and specialization of wheat and oat stem rusts in Kenya in 1968. *Phytopathology* **60**: 309-314.

Greens, G. J. (1981). Identification of physiological races of *Puccinia graminis* f. sp. t*ritici* in Canada. *Canadian journal of plant pathology* **3:** 33-39.

Haq M. (2009). Development of mutant varieties of crop plants at NIAB and the impact on agricultural production in Pakistan. Induced mutation in the genomic era. Proceedings of an international joint FAO/IAEA symposium. Rome: Food and Agriculture Organization of the United Nations; p. 61-64.

Harder, D. E. and Chong, J. (1984). Structure and physiology of haustoria. In: Bushnell, W.R. Roelfs, A.P. (eds). *The cereal rusts.* **Vol 1.** Origins, specificity, structure, and physiology. Academic Press. Orlando, 333-373 pp.

Hermansen, J. E. (1968). Studies on the spread and survival of cereal rust and mildew diseases in Denmark. Contrib. No. 87, Dept. *Plant Pathology Royal Veterinary Agriculture College Copenhagen.* 206 pp.

Huang S, Sirikhachornkit A, Su X, Faris J, Gill B, and Haselkol R. (2002). Gene encoding plastid acetyl-CoA carboxylase and 3-photophoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionanry history of polyploidy wheat, *Proc. Natl Acad. Sci*. USA, vol 99 (p. 8133-8138)

IAEA. IAEA mutant data base. (2015). Viena International Atomic Energy Agency. Accessed from July 2015

Jahufer, M., Barret. B., Griffiths, A. and Woodfields, D. (2003). DNA fingerprinting and genetic relationships amoung white clover cultivars. Proceedings of New Zealand Grassland Association

Jin, Y. and Singh, R. P. (2006). Resistance in U.S. wheat to recent Eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. *Plant Diseases* **90:**476-480.

Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M., Njau, P., Fetch, T., Pretorius, Z. A. and Yahyaoui, A. (2007). Characterization of Seedling Infection Types and Adult Plant Infection Responses of Monogenic *Sr* Gene Lines to Race TTKS of *Puccinia graminis* f.sp. *tritici. Plant Disease* **91**:1096-1099.

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

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

Johnson, R. (1992). Past, present and future opportunities in breeding for disease resistance, with examples from wheat. *Euphytica* **63**:3-22.

Joshi, K. R., and S. Nayak. (2010). Gene pyramiding. A broad spectrum technique for developing durable stress resistance in crops. Biotechnol. Mol. Biol. 5:51-60

Kenya Institute for Public Policy Research and Analysis (KIPPRA), ‘‘Kenya Economic Report 2013, *Creating Enabling Enviroment for Stimulating Investment for Competitive Sustainable Countries’’* Nairobi, Kenya p74. 2013

Kerber, E. R. and Aung, T. (1999). Leaf rust reistance gene Lr34 associated with nonsuppression of stem rust resistance in wheat cultivar Canthatch. Phytopathology. 89: 518-521.pmid:18944724

Kharkwal M. and Shu Q. (2009). The role of induced mutations in the world food security. Rome: Food and Agriculture Organization of the United Nations; p. 33-38

Khokhar, KM., Gupta R. (2014). Integrated Disease Management. Pop. *Kheti 2*(1):87-91.

Khoury W, Makkouk K (2010). Integrated Plant Diseas Management in developing countries. J. *Plant Pathol. 92*(4):35-42.

Kihara H. (1944). Discovery of the DD-analyser, one of the ancestors of *Trticum Vulgare* (abstr) (in Japanese) *Agric. Hortic*., vol 19 (p. 889-890)

Kinyua, M. G. and Ochieng, D. O. (2005). Crop Production Handbook for Wheat, Oil crops and Horticulture. J.A.W. (Eds.*). Gansdil Printers & Stationers, Nairobi, Kenya.* Pp 1- 19

Kinyua, M. G. Karanja, L. Kimurto, P. Spencer, M. D and Shu,Q. Y. (2008). Development of wheat varieties for marginal areas of Kenya through mutation and doubled haploid techniques. FAO/IAEA International symposium on Induced mutations in plants. Vieenna, Austria. Book of abstracts, pp7.

Knott, D. R. (1989). The Wheat Rusts- Breeding for Resistance. *Springer-Verlag, Berlin Heidelberg*. 201 pp.

Knott, D. R. (1991). What determines the success of mutation breeding?. IAEA, V.1. p. 111-118.

Konzak, C. F. (1987). Mutations and Mutation Breeding. In: E.G. Heyne (ed.), Wheat and Wheat Improvement, Second Edition, 428-443, ASA. CSSA. SSSA. Inc. Press, Madison, WI, USA.

Kõrtok ,Y., Gen, Ü., YaÛbasanlar T. and Ikesen, M. (1988). Tescilli Ekmeklik ve Makarnalõk BuÛday .eßitlerinin .ukurova Koßullarõnda Baßlõca Tarõmsal Karakterleri .zerine Araßtõrmalar..ukurova .niversitesi Ziraat Fak.ltesi Dergisi, 3(3): 98-106.

Kurt, J. L. and Szabo, L. J. (2005). Stem rust of cereals and grasses. *Molecular plant pathology* **6**:99-111.

Lal Ahamed, M., Singh, S. S., Sharma, J. B. and Ram, R. B. (2004). Evaluation of inheritance to leaf rust in wheat using area under disease progress curve. *Hereditas* **141:** 323-327.

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

Leonard, K. J. and Szabo, L. J. (2005). Rust of small grains and grasses caused by *Puccinia graminis*. *Molecular plant pathology* **6**:99-111.

Littlefield, I. J. (1981). Biology of the plant rusts. *Iowa State University. Press. Ames.*

Liu, S., L. X. Yu, R.P. Singhh, Y. Jin, M. E. Sorrells, and J.A. Anderson. (2010). Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes Sr25 and Sr26. Theor. Appl. Genet. 120: 691-697. Doi:10.1007/s00122-009-1186-z

Liu, W., Rouse, M., Friebe, B., Jin, Y. and Gill. B. (2011). Discovery and molecular mapping of a new gene conferring resistance to stem rust, Sr53, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. Chromosome Res. 19: 669–682.

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

Luo M, Yang Z, Kawahara T, Waines J and Dvorak J (2007). The structure of wild and domesticated emmer wheat population, gene flow between them, and the site of emmer domestication, *Theor. Appl. Genet*, vol 144 (p. 947-959

Macharia, K. J. (2009). Yield losses of bread wheat (*Triticum aestivum* L.) cultivars resulting from a new race of stem rust Ug99 (TTKS) (*Puccinia graminis* f.sp *tritici)* in Kenya. *MSc Thesis, Egerton University*, pp 90.

Mago, R., H.S. Bariana, I. S. Dundas, W. Spielmeyer, G. J. Lawrence, A.J.Pryor and Ellis. (2005). Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasmm. Theor. Appl. Genet. 111:496-504. Doi: 10.1007/s00122-005-2039-z

Mahagayu C. Kamwaga J., Ndiema C. Kamundia W. and Gamba P. (2007). Wheat productivity constraints associated in the eastern parts of Kenya Timau Division. Afr. Crop Science. Soc. 8:1211-1214.

Maluszynski, M. Szarejko, I. Bhatia, C. R. Nichterlein, K. Lagoda, P. J. (2009). Methodologies for generating variability Part 4: Mutation techniques. Pp 159-194, Plant Breeding and farmer participation. Ceccarelli, S. Guimaraes, P.E and Weltzien, E. (eds). FAO 2009.

Manova, V. Geogieva, M. Borisov,B. Gecheff, K. Stoilova, B. and Stoilov. L. (2008). Genomic and gene-specific induction and repair of DNA damage in barley. FAO/IAEA International symposium on Induced mutations in plants. Vieenna, Austria. Book of abstracts, pp34.

McGrath, M. T. and Pennypacker, S. P. (1991). Reduction in the rate and duration of grain growth in wheat due to stem rust and leaf rust. *Phytopathology* **81**:778-787.

McIntosh, R. A. (1976). Genetics of wheat and wheat rusts since Farrer: Farrer Memorial Oration 1976. J. Aust. Inst. *Agriculture Science.* **42**:203-216.

McIntosh, R. A. (1988). The role of specific genes in breeding for durable 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.

Mclntosh, 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., Dubcovsky, J., Rogers, J., Morris, C. (2010). Catalogue of gene symbols for wheat. MacGene 2010. National Institute of Genetics, Mishima, Shizuoka, Japan. Available: http://www.shigen.nig. ac.jp/wheat/komugi/genes/download.jsp. (Accessed November 17, 2011).

McIntosh, R. A., Dubcovsky, J., Rogers, J., Morris, C., Appealss, R., X. C., & Azul, B. (2014). *Catalogue of gene symbols for wheat: 2013-2014.*

MOA (Ministry of Agriculture). (2013). Food security assessment reports. Department of crops management. MOA/CRP/FIELD/01/20.

Monroy L, Mulinge W, Witwer M (2013). Analysis of incentives and disincentives for wheat in Kenya. Technical notes series, MAFAP, FAO, Rome

Michelmore, R. W. Paran, I and Kesseli, R. V. (1991). Identification of markers linked to disease- resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natii Acad Sci. 888: 9828-9832. Pmid:1682921

Nakai, H., Nakamura, K., Kuwahara, S and Saito, M. (1990). A new gene, developed through mutagenesis, for resistance of rice to bacterial leaf blight (Xanthomonas compestris pv. Orysae). Journal of Agricultural Science 114 (2), 219-224.

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 Dis. 93:317.

Nelson, R. R., Wilcoxson R. D., Christeseen J. J., (1955). Heterokaryosis as a basis for variation in *Puccinia graminis tritici*. *Phytopathology* **45**:639-643.

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. *Plant Breeding and Crop Science* **Vol. 1(2)**. pp. 022-027.

Njau, P. N., Jin, Y., Huerta-Espino, J., Singh, R. and Keller, B. (2010). Identification and Evaluation of Sources of Resistance to Stem Rust race Ug99 in Wheat. *Plant Disease, 94* (4), 413-419. http://dx.doi.org/10.1094/PDIS-94-4-0413

Ooro, P. A., Bor, P. K., Amadi, D. O. K., Tenywa, J.S., Joubert, G. D., and Nampala, M. P. (2009). Evaluation of wheat genotypes for improved drought tolerance through increased seedling vigour. *9th African Crop Science, Conference Proceeding,* Cape Town, South Africa, 28 September-2 October 2009. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20133232444

Ozkan H, Brandolini A, Pozzi C, Effgen S and Salamini F. (2005). A reconsideration of the domestication geography of tetraploid wheats, Theor. Appl. Genet., vol 110 (p. 1052-1060)

Paterson, A. H. (1996). Making genetic maps. In: Paterson A. H, Genome mapping in plants: R. G. Landes; p. 23-39

Parlevliet, J. E. (1988) Strategies for the utilization of partial resistance for the control of cerealrust. In: Simmonds N.W, Rajaram S, editors. Breeding Strategies for Resistance to the Rusts of Wheat. Mexico: CIMMYT. p. 48-62.

Payne, T.S., Joseph, K., Wanjama and Bedada, G. (2000). Eastern Africa Wheat Pool. In: The world wheat book: *A history of wheat breeding* (Alain & Williams eds). 870-888.

Peterson, R. F., Campbell, A. B. and Hannah, A. E. (1972). A diagrammatic scale for estimating rust intensity of leaves and stems of cereals. *Canadian Journal of Research* **26**: 496-500.

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

Pretorius, Z. A., Bender, C. M., and Visser, B. (2010). First Report of a *Puccinia graminis* f. sp. *tritici* Race Virulent to the *Sr24* and *Sr31* Wheat Stem Rust Resistance Genes in South Africa. *Plant Disease* **94** (6): 784.

Pretorius, Z. A., Szabo L. J., and Visser, B. (2012). "First Report of a new TTKSF race of wheat stem rust (*Puccinia graminis* f. sp. *Tritici*) in South Africa and Zimbabwe" *Plant Diseases; 96*: 590.

Ravi S., David H., Huerta-Espno j., Jin Y., Bhavani S., Hurrera-foessel S., Singh P. and Govindan V (2011). The emergence of Ug99 races of stem rust fungus is a threat to the world wheat production. Annu. Rev. Phytopathol. 49:465-481.

Roelfs, A. P. (1978). Estimated losses caused by rust in small grain cereals in the United States-1918-1976. *Miscellaneous Publication. U.S. Department Agriculture.* **1363:**1-85.

Roelfs, A. P. (1984). Race specificity and methods of study. Pp 132-164 *In The Cereal Rusts.* **Vol 1** Origins, Specificity, Structure, and Physiology. W. R Bushell and A.P Roelfs, eds, Academic Press, Orlando, FL. 546 pp.

Roelfs, A. P. (1985). Wheat and rye stem rust. *In* T*he Cereal Rusts*. **Vol. 2** (Roelfs, A.P. and Bushnell, W. R., eds):pp.3-37

Roelfs, A. P., Marteans J. W. (1988). "An international system of nomenclature for *Puccinia graminis* f. sp. *Tritici*." Phytopathology: 78:526-33.

Roelfs, A. P., Singh, R. P. and Saari, E. E. (1992). Rust diseases of wheat: concepts and methods of disease management. *CIMMYT, Mexico, D.F*. 81 pages.

Roychowdhury R. and Tah J. (2013). Mutagenesis – a potential approach for crop improvement: new approaches and modern techniques. New York(NY): Springer; p. 149-187

Sakamura T. (1918). Kurze Mitteilung uber die Chromosomenzahlen und die Verwandtschaftsverhaltnisse der *Triticum*- Arten. Bot Mag (Tokyo) 32: 151-154.

Sakün, M. A., Yildirim, A. and Kmen, S. G. (2005). Gaziosmanpaßa University, Faculty of Agriculture, Department of Field Crops, 60240, Tokat – TURKEY.

Sebesta, E .E., Woods, E. A., Porter, D. R., Webster, J. A. and Smith, E. L. (1995). Registration of Amigo wheat germplasm resistant to greenbug. *Crop Science.* **35:** 293.

Sharma, R., Alexey M., Hans B., Beyhans A., Mesut R. and Sanjaya R. (2012). Yield stability analysis of wheat genotypes targeted to semi-arid environments in the international winter wheat improvement program. Int. J. Plant Breed. 171:53-64.

Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security, Springer 5(3):291-317.

Simmonds, N. W. and Rajaram, S. (1988). Breeding Strategies for Resistance to the Rusts of wheat. *CIMMYT: Mexico, D.F.* 151pp.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M., Wanyera, R., Njau, P., Ward, R. W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB Reviews: Perspective in Agriculture, Veterinary Science and Natural Resources* **54:**1-13.

Singh, R .P., Kinyua, M. G., Wanyera, R., Njau, P., Jin, Y., Huerta-Espino, J. (2007). Spread of a highly virulent race of *Puccinia graminis* f.sp *tritici* in eastern Africa: *Challenges and opportunities* **12:**51-57

Singh, R .P., Kinyua, M. G., Wanyera, R., Njau, P., Jin, Y., Huerta-Espino, J. (2008). Will stem rust destroy the world wheat crop? Advv Agron. 2008: 98: 271-309

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin Y., Bhavani. S. (2011). *The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu. Rev.* Phytopathol. 49: 465–481.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin Y., Bhavani. S. (2015). Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. *Phytopathology*, *105*(7), 872-884. http://dx.doi.org/10.1094/PHYTO-01-15-0030-FI

Sourdille P. Singh S, Caalen T. Brown-Guedira GL, Gay G, Qi L, et al Microsatellite-based deletion bin system for the establishment of genetic-physical map relationship in wheat (*Triticum aestivum* L.). Funct Integr Genomics. 2004: 4: 12-25. Pmid: 15004738

Stakman, E. C., Stewart, D. M. and Loegering, W. (1962). Identification of Physiologic Races of *puccinia graminis* var *tritici* U.S. Dept. of Agric. *Agricultural Research Service E-617.*

Staples, R. C. and Macko, V. (1984). Germination of urediospores and differentiation of infection structures. In: Bushnell W. R. Roelfs A. P. (eds). *The cereal rusts.* **Vol I.** Origins, specificity, structure, and physiology. *Academic Press.* Orlando, pp 255-289.

Taura, S., Ogawa, T., Yoshimura, A., Ikeda, R. and Omura, T. (1991). Identification of a recessive resistance gene ininduced mutant line XM5 of rice bacterial blight. Japan J. Breed 41: 427-432

Teng, P. S., Blackie, M. J. and Close, R. C. (1977). A simulation analysis of crop yield loss due to rust disease. *Agricultural Systems* **2**: 189-198.

Thorpe, H. C. (1958). Wheat breeding in Kenya. In*: 1st International wheat genetics symposium, Winnipeg, Canada.* 268 pp.

Todorovska., E., N. Christov, S. Slavov, P. Christova, and D. vassilev. (2009). Biotic stress resistance in wheat breeding and genomic selection implications. Biotechnol. Biotechnol. Eq. 23:1417-1426. Doi.2478/v10133-009-0006-6

Tsunewaki K. (2009). Plasmon analysis in the Triticum-Aegilopscomplex, Breed. Science., vol 59 (p. 455-470)

USAID (2010). Staple foods value chain analysis. Country Report. Kenya.

USDA (2017). "Kenya Corn, Wheat and Rice Grains and Feed Annual Report."

Van der plank, J. E. (1968). Disease Resistance in Plants. *New York: Academic.* 216 pp.

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.

Wanyera, R. (2008). Status and impact of Ug 99 in Kenya. In: Proceedings of International Conference on Wheat Stem Rust Ug99- A Threat to Food Security; *Indian Agricultural Research Institute, New Delhi, India* pp 9-11.

Wanyera, R., Macharia, J. K., Kilonzo, S. M. and Kamundia, W. (2009). Foliar fungicides to control wheat stem rust race TTKS (Ug99) in Kenya. *Plant Disease.* **93:** 929-932.

Welty, R. E. and Barker, R. E. (1992). Latent period responses to stem rust in tall sescue incubated at 4 temperatures. *Crop Science*.**32**:589-592.

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

Zadoks, J. C. and Bouwman, J. J. (1985). Epidemiology in Europe. Pp. 329-369 in A.P. Roelfs and W.R. Bushnell, eds. *The Cereal Rust* ***Vol.II;*** Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando.

Zhang, W., E. Olson, C. Saintenac, M. Rouse, Z. Abate, Y. Jin, E. Akhunov, M. Pumphrey, and J. Dubcovsky. (2010). Genetic maps of stem rust resistance gene Sr35 in diploid and hexaploid wheat. Crop Sci 50:2464-2474. Doi:10.2135/cropssci2010.04.0202

APPENDICES

Appendix I: Yield of parents and their respective mutants

Genotype	N	Mean	Std Dev	Sum	Minimum	Maximum
SP-N	20	2.920	0.20175	49.25	2.33	3.45
SP-NM		2.607	1.00648			
SP-K	20	1.914	0.51111	49.76	1.03	2.55
SP-KM	20	2.113	0.11458	29.87	0.27	4.16

Appendix II: Relative contribution (partial and model R^2),F value, and probability in predicting wheat grain yield by the stepwise procedure analysis for SP-N Parent

Step	Variable entered	Variable removed	Partial R^2	Model R^2	Parameter estimate	P value ER	SE	P value M
1	AL	-	0.2822	0.2822	5.57376	0.2445	4.55386	0.0159
2	GS	-	0.0934	0.3756	0.50165	0.0341	0.20985	0.1293
3	GY	-	0.1082	0.4838	-0.83316	0.0308	0.34058	0.0858
4	S	-	0.0440	0.5278	-0.83212	0.0890	0.44973	0.2554
5	M	-	0.0700	0.5978	-10.5715	0.1129	6.17997	0.1410
6	EE	-	0.0514	0.6492	-1.88601	0.1324	1.16819	0.1907
7	SD	-	0.0389	0.6881	-0.03080	0.9018	0.24429	0.2445

Appendix III: Relative contribution (partial and model R^2), F value, and probability in predicting wheat grain yield by the stepwise procedure analysis for SP-N Mutants

Step	Variable entered	Variable removed	Partial R^2	Model R^2	Parameter estimate	P alue ER	SE	P value M
1	GY	-	0.8330	0.8330	17.65928	0.0495	7.9063	<.0001
2	M	-	0.0390	0.8720	1.22140	0.0002	0.2152	0.0361
3	GS	-	0.0491	0.9211	0.94867	0.0029	0.2421	0.0061
4	**S**	-	0.0145	0.9356	-0.29088	0.4081	0.3368	0.0862
5	SL	-	0.0099	0.9455	1.08641	0.0890	0.5768	0.1329
6	H	-	0.0067	0.9522	0.81082	0.1344	0.4977	0.1997
7	AL	-	0.0036	0.9558	-2.89605	0.1003	1.5993	0.3408
8	GT	-	0.0092	0.9650	-0.85318	0.0892	0.4533	0.1177
9	L	-	0.0029	0.9679	0.34951	0.3650	0.3683	0.3650

Appendix IV: Relative contribution (partial and model R^2), F value, and probability in predicting wheat grain yield by the stepwise procedure analysis for SP-K Parent

Step	Variabl entered	Variable removed	Partial R^2	Model R^2	Paramet estimate	P value ER	SE	Pvalue M
1	S	-	0.3237	0.3237	0.75106	0.0299	0.30142	0.0088
2	FLA	-	0.1364	0.4601	1.03743	0.0087	0.32582	0.0537
3	GY	-	0.1424	0.6025	4.28253	0.1752	2.95546	0.0292
4	L	-	0.0735	0.6761	0.26494	0.7001	0.67015	0.0848
5	GT	-	0.0265	0.7026	-0.39968	0.3570	0.41572	0.2825
6	M	-	0.0195	0.7221	0.16101	0.2364	0.12855	0.3571
7	H	-	0.0126	0.7347	0.28444	0.4245	0.34298	0.4643
8	EE	-	0.0135	0.7483	-0.33428	0.4579	0.43450	0.4579

Appendix V: Relative contribution (partial and model R^2), F value, and probability in predicting wheat grain yield by the stepwise procedure analysis for SP-K Mutants

Step	Variable entered	Variable removed	Partial R^2	Model R^2	Parameter estimate	P value ER	SE	P value M
1	GY	-	0.9452	0.9452	18.28023	0.0008	3.84922	<.0001
2	FLA	-	0.0139	0.9590	-0.06751	0.5908	0.12154	0.0283
3	M	-	0.0158	0.9748	0.16502	0.0097	0.05179	0.0061
4	AL	-	0.0042	0.9790	7.72849	0.0014	1.77250	0.1034
5	H	-	0.0053	0.9843	0.77330	0.0110	0.24832	0.0477
6	L	-	0.0056	0.9899	0.55849	0.0235	0.20928	0.0187
7	SL	-	0.0007	0.9906	0.35484	0.1383	0.22029	0.3543
8	EE	-	0.0013	0.9919	0.06184	0.2645	0.05231	0.2164
9	GS	-	0.0007	0.9926	-0.10499	0.3430	0.10547	0.3430

Appendix VI: Photo of a resistant wheat variety SP-26, (AC) and the susceptible variety SP-P, (BD)

Source: Author, 2015

Appendix VII: SSR markers linked to rust resistant genes

	Name of genes	Linked marker	Nucleotide sequence	Reference	Expected band size (bp)	Gene Action
1	*Lr34*	CsLV34	F 5′-GTTGGTTAAGACTGGTGATGG-3′ R 5′-TGCTTCCTATTGCTGAATAGT-3′	Lagudah*et al.*, 2006	150	
2	*Lr46*	Barc80	F 5′-CGAATTAGCATCTGCATCTGTTT GAG-3′ R 5′-GGTCAACCAACTACTGCACAAC-3′	Somers *et al.*, 2004	105	
3	Sr22	***WMC633***	F 5'- ACA CCA GCG GGG ATA TTT GTT AC -3' R 5'- GTG CAC AAG ACA TGA GGT GGA TT -3'	Gerechter*et al.*, 1971	100, 117, 250	
4	*Sr 26*	***Sr26#43***	F5'- AAT CGT CCA CAT TGG CTT CT -3' R R 5'- CGC AAC AAA ATC ATG CAC TA -3'	Knott*et al.*, 1961	207	Major gene
5	Sr28	***wPt -7004***	F 5'- CTC CCA CCA AAA CAG CCT AC -3' R 5'- AGA TGC GAA TGG GCA GTT AG -3'	Jin*et al.*, 2007	214-, 217-, and 220-bp	Additive
6	Sr 2	gwn533	F 5'- CAA GGG TTG CTA GGA TTG GAA AAC -3Â' R 5'- AGA TAA CTC TTA TGA TCT TAC ATT TTT CTG -3Â'	Spielmeyer et al., 2013	120, 155, 172	Additive
7	Sr25	BF145935	F 5'- CTT CAC CTC CAA GGA GTT CCA C -3' R 5'- GCG TAC CTG ATC ACC ACC TTG AAG G -3'	Liu et al., 2010	198, 207	Additive
8		Gb	F 5'- CAT CCT TGG GGA CCT C-3' R 5'- CCA GCT CGC ATA CAT CCA -3'	Liu et al., 2010	150	Additive
9	Sr 33	*Xbarc152*	F 5'- CTT CCT AAA ATC GGG CAA CCG CTT GTT G -3' R 5'- GCG TAA TGA TGG GAG TGG CTA TAG GGC AGT T -3'	Periyannan et al., 2013		Additive
10	Sr 31	csSr32#1	F 5'- GGT TTG GTG GCA ACT CAG GT -3' R 5'- CAT AAG CCA AAG AGG CAC CA -3'	Mago et al., 2013	184	Additive

Appendix VIII: Anova for disease severity scores of the seventeen wheat genotypes.

Source.	D.F	Sum of squares.	Mean square	FValue	pr> F
Rep.	2	2.66666	1.3333	0.069	0.953
Variety	16	9148.8549	571.803	18.76	<.001
Error	32	1.764	1.470		
Totals	50	9153.2855			

Appendix IX: Anova for AUDPC values of the seventeen wheat genotypes.

Source.	D.F	Sum of squares.	Mean square	FValue	pr> F
Rep.	2	604.02	302.01	0.10	0.901
Variety	16	937082.672	58567.667	16.36	<.001
Error	32	2.537	2.132		
Totals	50	937689.229			

Analysis of variance (ANOVA) computation showed that there was significant variation in amoung varieties ($P<0.001$) but no significant variation between replicates ($P>0.1$)

Appendix X: Mean squares from ANOVA for quantitative traits of seventeen wheat genotypes in 2012

Source.	**D.F**	**S**	**PH**	**GS**	**SL**	**SD**	**GT**	**GY**	**SW**	**M**
Rep.	2	25.26	1.527	1.13	0.414	0.001	56.89	3257.3	10.82	8.2
Variety	16	1.23	1.743	1.37	10.53**	0.85**	467.3**	2790.1**	46.68**	95.8
Error	32	1.16	2.64	1.09	0.98	0.17	45.8	810.3	2.425	110.1
Totals	50	38.05	5.92	3.59	11.92	1.55	570.3	6,857.7	59.93	214.2

, * significant at P<0.05, 0.001, respectively

Wheat genotype 1,000 seed weight= SW, Number of tillers = GT, maturity periods (days) = M, spike length = SL, plant height = H, grain yields per spike = GY, 8. Spikelet's per spike = S, 9. Seed diameter = SD, and Grains per spike = GS

Appendix XI: Mean squares from ANOVA for quantitative traits of seventeen wheat genotypes in 2013.

Source.	D.F	S	PH	GS	SL	SD	GT	GY	SW	M
Rep.	2	3.42	1.31	0.834	2.035	0.03	895	8757	14.52	145.6
Variety	16	4.53.	1.65*	1.45*	596**	0.33	578**	3524**	76.32**	50.1**
Error	32	2.46	1.05	0.53	0.775	0.17	135	886	4.225	23.5
Totals	50	10.4	4.01	2.814	8.4	0.53	1608	13167	95.06	219.2

, * significant at P<0.05, 0.001, respectively

Wheat genotype 1,000 seed weight= SW, Number of tillers = GT, maturity periods (days) = M, spike length = SL, plant height = H, grain yields per spike = GY, 8. Spikelet's per spike = S, 9. Seed diameter = SD, and Grains per spike = GS

Appendix XII: Pearson's correlation coefficient for the disease parameters among the seventeen wheat genotypes

Source.	ACI.	AUDPC	FDS.
ACI	1		
AUDPC	0.9353***	1	
FDS	0.9856 ***	0.899***	1

*** Significant relationship between the variables at P<0.05, 0.001, respectively

ACI = average coefficient of infection, AUDPC = area under disease progressive curve, FDS = Final disease severity

Appendix XIII: Pearson's correlation coefficient for the different agronomic traits amoung the seventeen wheat genotypes across the seasons

	SW	SD	GT	M	S	GS	PH	SL	GY
SW	1								
SD	0.7724	1							
GT	- 0.6789***	-0.5577	1						
M	-0.6388**	-0.5109	0.2570	1					
S	0.2969	0.4271*	0.0007	0.2567	1				
GS	0.7672***	0.4122	0.7317**	0.3640	0.8902***	1			
PH	0.2733	0.3812***	0.0747	0.0989	0.3517	-0.1248	1		
SL	-0.1506	0.3217	-0.5392	0.4291	0.6083	0.2801	-0.1165	1	
GY	0.9683***	0.7406***	-0.5616***	-0.5332***	0.8802	0.7242***	0.2026	0.7188	1

***= Highly significant at P≤0.01; **= Significant at P≤0.05; *= Significant at P≤0.1

Appendix XIV: Growth stages of small grains

CODE	Description	CODE	Description
0	**GERMINATION**	37	Flag leaf just visible
00	Dry seed	38	Flag leaf ligule just visible
01	Start of imbibitions	**4**	**BOOTING**
03	Imbibition complete	41	Flag leaf sheath extending
05	Radicle emerged from seed	43	Boots just visible swollen
07	Coleoptile emerged from seed	45	Boots swollen
09	Leaf just at coleoptiles tip	47	Flag leaf sheath opening
1	**SEEDLING GROWTH**	49	First awns visible
10	1st leaf through coleoptiles	**5**	**EAR EMERGENCE**
11	1st leaf unfolded	51	1st spikelet of ear emerged
12	2 leaves unfolded	53	One-fourth of ear emerged
13	3 leaves unfolded	55	One-half of ear emerged
14	4 leaves unfolded	57	Three-fourths of ear emerged
15	5 leaves unfolded	59	Emergence of ear completed
16	6 leaves unfolded	**6**	**FLOWERING**
17	7 leaves unfolded	61	Beginning of flowering
18	8 leaves unfolded	65	Flowering half-way complete
19	9 leaves unfolded	69	Flowering complete
2	**TILLERING**	**7**	**MILK DEVELOPMENT**
20	Main shoot only	71	Seed water ripe
21	Main shoot and 1 tiller	73	Early milk

22	Main shoot and 2 tillers	75	Medium milk (An increase in the solids of the liquid of the endosperm is notable when crushing the seed between fingers)
23	Main shoot and 3 tillers	77	Late milk
24	Main shoot and 4 tillers	**8**	**DOUGH DEVELOPMENT**
25	Main shoot and 5 tillers	83	Early dough
26	Main shoot and 6 tillers	85	Soft dough (Fingernail impression not held)
27	Main shoot and 7 tillers	87	Hard dough (Fingernail impression not held; head losing chlorophyll)
28	Main shoot and 8 tillers	**9**	**RIPENING**
29	Main shoot and 9 tillers	91	Seed hard (difficult to divide by thumbnail)
3	**STEM ELONGATION**	92	Seed hard (can no longer be dented by thumbnail)
30	Pseudo stem erection	93	Seed loosening in daytime
31	1st node detectable	94	Over-ripe; straw dead/ collapsing
32	2nd node detectable	95	Seed dormant
33	3rd node detectable	96	Visible seed giving 50% germination
34	4th node detectable	97	Seed not dormant
35	5th node detectable	98	Secondary dormancy induced
36	6th node detectable	99	Secondary dormancy lost

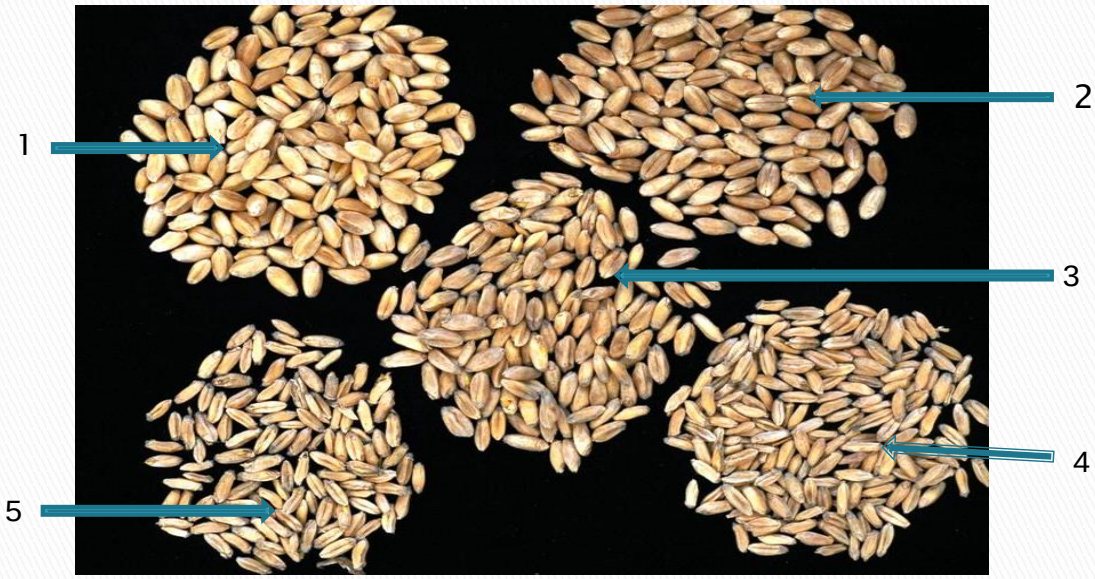
Appendix XV: field pictures

Field planting (source author 2015)

Resistant mutant lines bulking (source author 2015)

Field inspection (Source Author 2015)

Field infection of stem rust (Source Author 2015)



Kernel shrivelling (1-5 scale)

1=20%, 2=40%, 3=60%, 4=80% and 5=100%

Green house planting (source author 2015)