SCREENING OF SELECTED WHEAT (*Triticum aestivum* L.) LINES FOR RESISTANCE AND STEM RUST (*Puccinia graminis* f.sp. *tritici*) RACE IDENTIFICATION IN NAKURU COUNTY, KENYA

MILLICENT ACHIENG

A Thesis submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Crop Protection of Egerton University

JULY 2025

DECLARATION AND RECOMMENDATION

Declaration

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

Signature: Date: 1st July 2025

Millicent Achieng

KM122/09095/20

Recommendation

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

Signature: Date: 3rd July 2025

Dr. Miriam Karwitha Charimbu, PhD

Department of Crops, Horticulture and Soils

Egerton University

Signature: Date: 2nd July 2025

Dr. Alex Machio Kange, PhD

Department of Agriculture and Natural Resources

Bomet University College

A Constituent College of Moi University

DEDICATION

I dedicate this work to the almighty God and my family for their unwavering and relentless support throughout the study duration.

ACKNOWLEDGEMENTS

I acknowledge the financial and technical support from Kenya Agricultural and Livestock Research Organization. I appreciate the financial support and germplasm provided by the International Maize and Wheat Improvement Centre (CIMMYT) through the Delivering Genetic Gain in Wheat (DGGW). The institutional support extended to me by Egerton University in hosting my academic study and thesis research is greatly acknowledged. I sincerely thank my academic supervisors Dr. Miriam Karwitha Charimbu

and Dr. Alex Machio Kange for their professional guidance, critique, support, advice, and mentorship given for the entire time at egerton University. I also acknowledge the whole teaching and non-teaching staff in the department of Crops, Horticulture and Soils for making this dream come true. I extend my gratitude to the KALRO Njoro Centre Director Dr. Godwin Macharia, Head of Pathology Section, Dr. Zennah C. Kosgey, and other staff at the centre, Mr. Bernard Otukho, Peter Githinji and Bernard Chirchir for their relentless support during research. I also appreciate my colleagues Sammy Madahana, Grace Wanyoike and Jeniffer Chelimo Kemboi for their assistance and suggestions during my research period.

ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the major cereals in Kenya, yet its production remains below national demand due to the stem rust disease. The evolution of Ug99 race evident with the emergence of aggressive races such as TTKTT has rendered more cultivars susceptible. The studies aimed at identifying spring wheat lines with adult and seedling plant resistance to wheat stem rust and also identify wheat stem rust races in Njoro and Mau-Summit in Nakuru County, Kenya. The field experiment was conducted at the Kenya Agriculture and Livestock Research Organization (KALRO), Njoro disease screening field in a 12×12 partially balanced lattice design. Area Under Disease Progress Curve (AUDPC), Coefficient of infection (CI), and the final disease severity (FDS) were used as a measure of

adult plant resistance (APR). 54.9 % of genotypes with AUDPC of ≤200, 52.8 % with CI of ≤20, and 60.42 % with FDS of ≤30 was resistant. Effects due to season, genotype, and genotype-by-season interaction were significant (p≤0.001) for plant height, 1000-kernel weight (TKW), biomass, and AUDPC. Mean grain yield and TKW ranged from 0.8 t ha^{-1} to 9.05 t ha^{-1} and 20.03 g to 46.42 g, respectively. Genotypes 6022, 6095, 6096, 6107, 6134, 6136, 6137, 6138, and 6139 were found to possess APR and be high-yielding. For the seedling experiment, the lines were inoculated with stem rust race TTKTT at two-leaf stage. The infection type was scored 14 days post inoculation. One hundred and four genotypes were resistant with a score of ≤2. The lines identified for APR had a score of '0', hence can progress to national performance trials. Bulk stem rust spores collected from Njoro and Mau-Summit were purified to obtain single pustules, which were then inoculated onto a 20 North American stem rust differential set at 7 days. The infection types were determined 14 days post-inoculation. Isolates from both sites had stem rust race TTKTT, a *Ug99* variant virulent to genes *Sr24*, *Sr31,* and *SrTmp* present in most Kenyan wheat cultivars. This calls for the incorporation of new genes like *Sr22*, *Sr26*, *Sr36*, *Sr47*, *Sr57* and *SrND643* that were identified as resistant in both SPR and APR into the already adapted Kenyan wheat cultivars.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF ABBREVIATIONS & ACRONYMS

asl Above sea level

APR Adult plant resistance

AUDPC	Area under disease progress curve
CAN	Calcium ammonium nitrate
CDL	Cereal disease laboratory
CIMMYT	International Maize and Wheat Improvement Centre
DAP	Di-ammonium phosphate
FAO	Food and Agriculture Organization of the United Nations
16th SRRSN	16th Stem Rust Resistance Screening Nursery
IT	Infection type
KALRO	Kenya Agriculture and Livestock Research Organizations
MR	Moderately resistant
MS	Moderately susceptible
MSS	Moderately susceptible to susceptible
ppm	Parts per million
Pgt	*Puccinia graminis* f.sp. *tritici*
R	Resistant
SAS	Statistical Analysis Software
SPR	Seedling plant resistance
Sr	Stem rust
TKW	Thousand kernel weight
Ug99	Stem rust race identified in Uganda in 1999
Yr	Yellow rust
APR	Adult plant resistant
DPI	Days post inoculation
GLM	General Linear Model
PM	Physiological maturity
MC	Moisture content
CI	Coefficient of infection
FDS	Final Disease Severity

CHAPTER ONE
INTRODUCTION

1.1 Background information

Rusts caused by *Puccinia spp*. are obligate bio trophic-fungi that affect bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum*), barley (*Hordeum vulgare* L.) and triticale (*XTriticosecale Witmark*) with yield losses reaching up to 100 % in unadapted varieties (Aime *et al*., 2017). Rust infection on wheat has been a concern worldwide with high epidemics observed in Africa and Asia due its adaptability in various climate, emergence of different races and long-distance migration (Jin *et al*., 2010; Singh *et al*., 2004). The major disease of wheat, stem rust, is caused by *Puccinia graminis* f.sp. *tritici (Pgt*) causes losses estimated at 5.5 million tonnes/year (Beddow *et al*., 2015). Stripe and leaf rusts are prevalent in Africa, America, Asia and Europe while stem rust is predominant in Africa, Australia and America (Bhardwaj *et al*., 2019). While stem rust is a affect wheat globally, the situation is dire in East Africa as *Pgt* causes major yield losses due to presence of aggressive virulent *Ug99* strain (Worku *et al*., 2016).

Despite ongoing breeding efforts and usage of fungicide, the *Pgt* remains a persistent threat due to ever changing virulence. Tessema *et al*. (2024) revealed *Pgt* has increased virulence in most of the resistant wheat cultivars, mostly occurring in Kenya. The disease causes epidemics attributed to constant emergence of races following the release of different resistant genes (Fetch *et al*., 2021; Pinto & Hurd, 1970). The *Ug99* strain has caused havoc since its emergence in Uganda and in addition to the already identified 12 races; two new variants were identified in Kenya in 2019 and 2020, $TTKTT_{+}$ with additional virulence to *Sr24* and $Sr8_{155}B_{1}$, TKTTF and TTHTT indicating that *Ug99* strain is spreading at a high rate (Wanyera & Wamalwa, 2022). The demand for wheat product is increasing with Kenya depending on imports from Russia, Ukraine and Argentina among others accounting for 84 % of total wheat consumed in Kenya (KNBS, 2025). Kenya only produces 309,500 T of locally consumed wheat against a consumption of 900, 000 tonnes per year (KNBS, 2025).

This highlights a crucial need for evaluating resistance under local conditions and updating knowledge on prevailing stem rust races as the disease pose food security threat in Kenya (Bhavani *et al*, 2019). Erratic weather patterns and *Pgt* virulence causes farmers to depend on expensive fungicides which ultimately leads to high production cost (Wanyera &

Wamalwa, 2022). This forces most scale farmers to abandon the crop causing a huge deficit in production (Langyintuo, 2020).

To enhance genetic resistant against rust, exchange of germplasm and growing of resistant cultivars with durable resistance is necessary to protect the crop against the various rust races (Shamanin *et al*., 2020). Resistant genes can either be from wild or cultivated wheat relatives allowing for expansion of genetic diversity thereby conferring durable resistance (Leonova *et al*., 2020). Twenty-six stem rust resistant genes have been picked out and incorporated from wild wheat relatives to bread wheat (Shamanin *et al*., 2020). Genes *Sr2*, *Sr13* and *Sr14* sourced from *T. turgidum* and, *Sr22* and *Sr35* sourced from *T. monoccocum* are effective against *Ug99* (Singh *et al*., 2014). Deployment of resistant genes for rust has proved effective in controlling rust infection however, a number of *Pgt* races and its variants are virulent to currently deployed resistant genes including *Sr21*, *Sr24*, *Sr31*, *Sr36*, *SrTmp* and $Sr8_{155}B_1$ (cymmit.org, 2021; Olivera *et al*., 2015). Mengesha (2020), noted that the application of fungicides on resistant wheat varieties has been effective in controlling rust epiphytotic although they often become susceptible to virulent races. Increase in pathogenicity of the pathogen due to presence of a favourable climate or increase in susceptibility of either primary or secondary host due to changes in agronomic aspects (Priyamvada *et al*., 2011). This necessitates constant surveys on rusts to a keep track on prevailing rust races (Bhardwaj *et al*., 2019; Chen *et al*., 2021).

Majority of the breeding programmes rely on limited wheat germplasm and currently, global grown wheat represents just 10 % of wheat diversity (Savadi *et al*., 2018). According to Beddow *et al*. (2015), Wheat yield potential has to be increased and avert epidemics as witnessed with race TKTTF that caused localized epiphytotic in Ethiopia (Olivera *et al*., 2015). Surveillance, continuous screening and generation of new wheat lines possessing both seedling and adult plant resistance is necessary to elucidate on the distribution and incidence of rust races (Bhavani *et al*., 2019). For these reasons, CIMMYT develops international nurseries each year with an aim of acquiring information about adaptability of wheat varieties, their yield potential and finally, disease and pest resistance. The disease screening nursery is mostly screened in different key stem rust hotspot areas to aid in monitoring the durability of resistance genes over time.

Despite much effort has been directed in stem rust resistance breeding, the Ug99 variants are breaking the resistance in most of the deployed genes in Kenyan cultivars. Most studies in Kenya focuses on either adult plant resistance (APR) or seedling plant resistance (SPR) separately, and very few have evaluated both APR and SPR within the same set of

lined under both field and greenhouse condions. Furthermore, there is limited recent surveillance of prevailing Pgt race diversity in Joro and Mau-Summit as they are a major Pgt hotspot and wheat growing region of Kenya. This creates a critical gap in developing integrated, location-adapted management strategies based on up-to-date virulence profiles and resistance durability.

To address these gaps, CYMMIT releases a set of wheat lines each year for screening. The 16th stem rust resistance screening nursery (SRRSN) is a collection of different advanced wheat lines derived from various nurseries released for multi-environment trials. The present study aimed to identify wheat lines in the 16th SRRSN with bot adult and seedling plant resistance and also to identify the prevailing stem rust races in Joro and Mau-Summit.

1.2 Statement of the problem

Stem rust in wheat has been a problem since the pre and post domestication of wheat crop and is projected to reduce wheat production by an average of 40 % and 50 % in Asia and Africa by the year 2050 respectively. The 2025 Kenya National Bureau of Statistics shows a 15 % decline in both growth and yield of wheat due to abiotic and biotic stresses leading to high production cost. Kenya's wheat production is 2.2 million tonnes (T) indicating a deficit of 1.89 million T thus only meeting 14 % of the country's wheat demand. Fungus, *Pgt* is the leading biotic-related threat to wheat production globally with losses ranging between 50 - 90 % depending on disease severity and susceptibility of a cultivar. Huge losses experienced with stem rusts is because it infects different parts of wheat which include the column, leaf peduncle and spike responsible for photosynthesizing metabolites important for wheat growth and production. Stem rust infection destroys phloem and xylem responsible for transport of photosynthates and water. Currently, *Pgt* virulence is increasing on resistant wheat cultivars due to recombination that takes place during sexual reproduction thereby producing new recombinants. The emergence of new races reduces the number of effective genes currently used in wheat breeding programmes. Currently, the widely used cropping system of wheat in Kenya is intensive monoculture in production areas of Nakuru, Narok, Uasin-Gishu and Laikipia Counties. This increases the risk of disease epiphytotic due to absence of other flora and fauna to limit the spread and sporulation of rust spores. This means larger amounts of fungicides have to be applied to limit the sporulation of the spores. The downside of intensive fungicide application is that the pathogen may develop resistant mutants in order to remain in the population. This in turn leads to development of new aggressive strains that pose big challenge to the current wheat breeding programme due to diverse nature of the pathogens

and migration of rust spores. In addition, fungicide usage in controlling rust disease has significant effects to health and the environment. This necessitates a constant rust screening in hotspot areas with utmost heterogeneity to aid in the identification and release of new resistant lines.

1.3 Objectives

1.3.1 General objective

To contribute towards improved wheat production through the identification of stem rust-resistant wheat lines and rust races.

1.3.2 Specific objectives

i. To identify wheat lines in the 16th stem rust resistance screening nursery (SRRSN) with adult plant resistance (APR) to wheat stem rust under field conditions.
ii. To identify wheat lines in the 16th SRRSN with seedling plant resistance (SPR) to wheat stem rust under greenhouse conditions.
iii. To identify wheat stem rust races in Njoro and Mau-Summit areas in Nakuru County, Kenya

1.4 Hypotheses

i. There is no significant difference in APR of 16th SRRSN lines to stem rust under field experiments.
ii. There is no significant difference in SPR of 16th SRRSN lines to stem rust under greenhouse conditions.
iii. There is no significant difference in stem rust races in Njoro and Mau- Summit areas in Nakuru County, Kenya

1.5 Justification

In order to meet the sustainable development goals (SDG) and vision 2030 agenda on ending all forms of hunger, poverty and protecting the planet, wheat production must be increased as it is among the top cereal crops cultivated and consumed by at least 33 % of the global population and livestock (Abbas *et al.,* 2009). Global warming plays a critical role with the occurrence of new aggressive pest and diseases in new areas as observed with emergence of new rust races in East Africa (Bhavani *et al*., 2019; Chen, 2005; Shiferaw *et al*., 2013; Wamalwa *et al*., 2022). New virulent races have caused low wheat production forcing about 84 % importation of the commodity to meet local demand (KNBS, 2025).

Constant loss of resistance in most wheat varieties is due to increased aggressiveness of *Puccinia spp.* coupled with intensive monoculture and migration of rust spores. To achieve sustainable wheat production, surveillance and constant screening of new wheat lines that are vaersatile to the prevailing weather conditions is mandatory to aid in predicting wheat rust epiphytotic as witnessed with *Pgt* digalu race that caused huge losses of wheat in Ethiopia in 2014 (Olivera *et al*., 2015). Furthermore, identification of other 5 new stem rust variants (TTKTK, TTKTT, TTHSK, TTHST, PTKTK) in Kenya, three of which exhibit virulence on *SrTmp* gene consequently calls for more rigid measures to combat rust diseases (Bhavani *et al*., 2019). Reduction of disease occurrence in Kenya through release of new resistant cultivars possessing multiple adult plant resistant genes will reduce overdependence on fungicides and thus contribute to vision 2030 and SDGs through promoting sustainable agriculture and eco-friendly practices.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bread wheat: Global and regional importance

Wheat (*Triticum aestivum* L.*)* is universally cultivated for its cereal kernel which is among the 'top three' staple food grain cultivated and taken by 33% of the world's inhabitants and livestock (Abbas *et al.,* 2009; Shewry *et al.,* 2009). In 2023, its global production was 788.5 million

In Africa, Ethiopia, South Africa, Sudan, Kenya, Tanzania, Nigeria, Zimbabwe and Zambia are the leading cultivators of wheat (FAO, 2018). Ethiopia is the top wheat producer at 3.6 million metric tons followed by South Africa, Sudan then Kenya at around 309,500 T (Tadesse *et al*., 2019). In Kenya, it is also the second largest grown grain after maize in the North rift (Eldoret, Tranzoia and Uasin Gishu counties), the South Rift (Narok and Transmara regions), the Central Rift (Nakuru) and Mount Kenya (Laikipia and Nyandarua counties) (Kamwaga *et al*., 2016; Wamalwa *et al*., 2022). According to Kenya National Bureau of Statistics, 2025, the country produced an average of 309, 500 T of wheat in 2023

against a consumption demand of 2.2 million tonnes, resulting in a deficit of 1.89 million tonnes. This highlights the need for sustainable local wheat improvement strategies.

2.2 Anatomy and physiology of wheat plant

Wheat is a seasonal plant belonging to the triticale family. It is a plant that utilizes the calvin benson cycle and mainly found in temperate regions. It consist of a coleoptile, leaves, tillers, stem, head, a seminal root, and nodal roots, which emerge during tillering (Kirby, 2002). A mature plant ranges from 0.7 to 1.2 m tall and consist of main stem that emerges from the seed and the tillers that emerges from buds (Evers *et al*., 2005).The tillers arise from the main stem and depends on soil fertility and prevailing environmental factors (Friend, 1965). Comprising nodes, internodes, leaves and a tiller bud in the leaf axil the stem functions as the main transport and storage system linking the roots and the reproductive organs (Evers *et al*., 2005; Kirby, 2002; Oyewole, 2016). The spike comprises a rachis bearing one spikelet at every node, each subtended by glumes, and typically housing 3 or more florets. Wheat plant is self-pollinated and anthesis is dependent on the prevailing environmental factors (Acevedo *et al*., 2006; Friend, 1965). The leaf consists of a blade and leaf sheath. The sheath is folded to protect the developing shoot (Setter & Carlton, 2000). The leaves are produced on opposite sides of the stem and are arranged in an all even numbers leaves are on the same side of the stem (Setter & Carlton, 2000). Roots consist of two types, seminal roots that appear after seed germination providing support to the plant immediately after germination and adventitious roots which appears after emergence of the fourth leaf and tillering has started (Kirby, 2002).

Wheat physiology is defined by organ differentiation and is divided into different stages. Growth stage 1 begins during germination to emergence and occurs when it receives a water content of 35 to 40 % by weight and an optimum temperature of between 12 °C to 25 °C (Acevedo *et al*., 2002). Germination is characterised by emergence of radicle and coleoptile which protects the first leaves during emergence. After production of the 1st three primary roots, coleoptile elongation pushes the growing point towards the soil surface. At this stage, the wheat plant has 6 primary roots and 3 leaves that support the entire stem (Acevedo *et al*., 2006). Tillers emerge from the axils and not all tillers are productive due to influence by environmental and genetic factors (Gallagher & Biscoe, 1978). During stem elongation, internodes elongate, moving the nodes to produce a long stem (Feekes, 1941). Visibly enlarged heads marks the beginning of booting, and ends with emergence of awns from the flag leaf opening the leaf sheath. Just before flowering, the heads emerge from the stem with

flowering beginning in the central part moving towards the basal and apical parts in around 4 days (Peterson, 1965). Milk stage begins during early kernel formation and the endosperm accumulates starch and nutrients followed by dough formation where the kernel has fully developed. Ripening follows and moisture content decreases with the kernel having a hard texture and green stems forming straw (Feeks 1941).

2.3 Biotic constraints to wheat production

The challenges in wheat production are mainly as a result of biotic influences and abiotic conditions (Afzal *et al*., 2015). Major abiotic constraints include post-harvest losses especially during rainy harvesting seasons, low soil fertility, high cost of fertilisers, high seed prices and shortage of improved seeds (Carew *et al*., 2017). Biotic constraints include fungal, bacterial and viral diseases like rusts (*Puccinia spp*.), smuts (*Ustilago spp*.), blights (*Pseudomonas)*, Fusarium and *Sclerotium spp*., powdery mildew caused by *Blumeria* and *Erysiphe spp*. and wheat mosaic viruses (Simon *et al*., 2021). Wheat rusts (stripe, leaf and stem rusts) caused by *puccinia spp.* is the leading cause for yield losses and is continuously threatening world's wheat supply (Roelfs *et al*., 1992).

2.4 Stem rust disease

Stem rust is a fungus in the phylum *Basidiomycota,* class *Urediniomycetes,* in the order *Uredinales,* family *Puccinaceae*, and enus *Puccinia* with at least 15 genera and 4000 species (Aime *et al*., 2017). It is epiphytoti in areas experiencing warm and moist atmospheric conditions (Prank *et al*., 2019). The yield lose due to *Pgt* can range between 50 % - 90 % based on disease severity and susceptibility of the cultivar (Huerta-Espino *et al*., 2020; Leonard & Szabo, 2005). It affects wheat, rye, barley, and goat grass (Saari & Prescortt, 1985; Wegulo & Byamukama, 2012). Since 'sounding the alarm' announcement was made and *Ug99* variant declared a threat to world's wheat production in 2005, 12 new variants present in Kenya. This reverses the wheat breeding efforts, posing a significant threat in East Africa region (Wanyera *et al*., 2006).

2.4.1 Symptoms of stem rust disease

Stem rust infections in wheat occur on stems, awns, and leaf sheaths, but may sometimes occur on blades and glumes of the leaf. The initial symptom is a miniature chlorotic fleck that occurs several days following infection that later develops to pustule that formed due busting of the plant's epidermis due pressure of urediniospores produced (Leonard & Szabo, 2005). The symptoms are usually visible on the entire upper parts of a

wheat plant. It affects the stem, lower and upper leaf surfaces and sometimes on seeds and head with a characteristic orange-red to dark-red oval shaped pustule (Wegulo & Byamukama, 2012). Urediniospores erupt through the epidermis from uredial pustules with a characteristic dusty appearance that drastically changes to black as teliospore production progresses (Schumann & Leonard, 2000). These uredial pustules are either diamond shaped or linear and may expand up to 10 mm long. As the plant ages, urediniospore production ceases as layers of black teliospore covers the surface of the pustules causing a black appearance on the stem late in the season (Leonard & Szabo, 2005).

2.4.2 Epidemiology of stem rust

Establishment of *Pgt* infection requires a minimum temperature of 2 ˚C, optimum temperature of 15 ˚C and a 30 ˚C for maximum temperature while sporulation requires a minimum temperature of 5 ˚C, optimum of 30 ˚C and a maximum temperature 40 ˚C respectively (Hogg *et al*., 1969; Singh *et al*., 2002). Infections can be more severe in warm humid climates, higher altitudes, an in either late sown or late maturing wheat cultivars (Bhardwaj *et al*., 2019). Inoculum source can be from barberry or overwintering plants since *Pgt* can overwinter on volunteer crops and non-crop grass hosts as mycelium or uredinia (USDA, 2017). This is characterized be heavy infection on the lower surface of the stem as compared to younger leaves. Urediniospores are produced with 1-2 weeks of infection implying that there can be several inoculums produced within a single wheat growing season. With favourable environmental conditions, disease epidemics can occur quickly as at least 100,000 urediniospores can be produced by a single uredinium resulting in over 50 % yield losses (Schumann & Leonard, 2000).

The *Pgt* causes losses in by absorbing nutrients from the host tissue that would otherwise be used by the plant for grain filling. Transpiration is affected as pustules rupture through the epidermal wall affecting metabolism leading to desiccation and prone to other pathogens. Aftermath of vascular tissues interference is shrivelled grains and lodging due to weaker stem making mechanical harvesting difficult (Schumann & Leonard, 2000).

2.4.3 Life cycle of *Puccinia graminis*

The *P. graminis* is a heteroecism pathogen thus requires a primary and secondary hosts with primary host being haploid common wheat and *Berbers vulgaris* being secondary host (Schumann & Leonard, 2000). Near the end of the growing cycle, *Pgt* produces two-celled teliospores with two haloid nuclei which is a resting spore stage and remains dormant

in the stalk. Teliospore cells produce a basidium after undergoing meiosis producing quadruple haploid nuclei that is partitioned from each other by three transverse septa (Leornard & Szabo, 2005). A sterigma forms from the basidium thus facilitating movement of the haploid nuclei to the basidiospore (Roelfs, 1985). Mature basidiospore are transportedby air currents to infect the secondaery host mainly barberry producing a flask-shaped pycnia on upper leaf surface. Pycniospores are generated within the pycnium and is exuded in the form of pycnial nectar disseminated by either insects or rain drops (Leornard & Szabo, 2005).

Pycniospores serves as the male gametes possessing single haploid nucleus while the hypha at the tip of pycnia serves as the female gamete. When in contact, they fuse and a haploid aecium is formed below the pycnium that ruptures the lower epidermal wall. . This can then infect wheat producing a thick layer of hyphae beneath the epidermis (Smith *et al*., 2009). Sporophores germinate producing clusters of unicellular dikaryotic urediniospore rupturing the epidermis and generatring a uredinium pustule. During late season, urediniospore production ceases and teliospore production begins (Leornard & Szabo, 2005).

2.5 Stem rust management

2.5.1 Cultural control

In crop cultivation, cultural practices are among the oldest and common ways employed in the management of stem rust epidemics by separating host and the pathogen in time and space (Moricca & Ragazzi, 2008). In most cases, crop rotation is the most effective way in eliminating rusts that overwinter on infected leaf debris in between the seasons by reducing the inoculum in the fields. Furthermore, avoiding scouting in the wheat fields during the rainy seasons may minimize the spread of spores to healthy plants (Tu, 1986). Rogueing is recommended so that seeds from the infected plants are not harvested to further spread the disease in future (Chen & Kang, 2017).

In irrigated fields, a lower humidity reduces the spread of urediniospores by limiting fungal dissemination. Overhead irrigation favours epidemic development by adding free water on the foliage and promoting a dense foliage thereby creating dew favouring rust establishment (Roelfs, 1986). Drip irrigation is the best as it does not create a favourable humidity for development of fungal disease on plant parts (Goldhamer *et al*., 2002). Wheat rust can also be managed by planting early in a clean field as rusts overwinter on debris. This reduces inoculum source and reduces infection severity (Berger, 1977).

Eradication is a preventive measure used to prevent occurrence of epidemics by interrupting life cycle and eliminating food source, mostly used in cases where rust disease has not well established (Davis, 2001). Stem rusts being biotrophic organisms, eradicating either primary host or secondary host over large geographical areas before it spread to larger area would greatly reduce dissemination of spores. In 1918, barberry elimination in the United States reduced stem rust epiphytotic in wheat field (Roelfs, 1982). This also proved successful in Puerto-Rico in 1903 when coffee rust agent *Hemileia vastatrix* was accidentally introduced in the island through eradicating coffee plants for a year (Wellman *et al*., 1981). Similarly, coffee rust in Papua New Guinea was successfully contained in 1965 by destroying infected fields before the disease was well established (Shaw, 1970).

2.5.2 Biological control

Biological is a method which uses living organism in controlling other pathogen for instance phytopathogens, weeds, insects and mites (Flint *et al.*, 1998). They reduce negative effects of pathogens by establishing antagonistic relationships, improvement of plant health, prevent phytopathogens and enhance nutrient and water uptake by plant (Vinale *et al.*, 2014). This method exploits parasitism which is an antagonistic symbiosis between a rust pathogen and another organism that can either be, Mycoparasitism which is a symbiosis relationship occurring between two fungi or hyper parasitism, which is a relationship between a beneficial fungi and rust pathogen (Moricca & Ragazzi, 2008).

In a study by El-Sharkawy *et al*. (2018), *T. harzianum* HL1 and *T. viride* HL5 was shown to reduce disease incidence, increase total phenol content, increase peroxidase and polyphenol oxidase enzymes responsible for increasing defence mechanism in wheat plant. Plant extract of carnation, ginger and cinnamon reduced stem rust severity by between 2.3- 8.6 % (El-Gamal *et al*., 2022). Si *et al*. (2022), demonstrated the potential of *Simplicillium lanosoniveum* in control of stem rust by inhibiting production and germination of urediniospores through structural damage.

2.5.3 Chemical control

Discovery of plant diseases began at around 17th century with the advent of microscope. Prevost in 1807 worked with bunt of wheat (*Tilletia caries*) and discovered copper sulphate was effective in controlling the disease (Prevost, 1807). In 1862, addition of

lime to copper sulphate was adopted to reduce phytotoxic effects of the latter on grains with usage of synthetic fungicides beginning at around 1930s (Rusell, 2005). In 1952, agricultural act was enacted that deal with operator safety following release of various chemicals into the market (Rusell, 2005). Disease control was necessary to avert 35% of crop yields losses experienced caused by wee, insects and pathogen attack thus a dependency on fungicide (Rusell, 2005).

Stem rusts when identified early and intervention taken before they reach economic threshold, yield loses can be averted by use of an effective fungicide (Wanyera *et al*., 2016). In the early years of wheat cultivation, inorganic chemicals such as Sulphur dust was used and considered effective in the control of rust (Forsyth & Peturson, 1958). Though, with the emergence of stem rust races 56 and 15B in the United States, there was an interest in the revival of controlling cereal rusts with fungicides (Peturson, 1958).

Fungicides can inhibit sterol synthesis, mitochondria electron transport or inhibit nucleic acid, enzymes and proteins (Yang *et al*., 2011). Fungicides used in controlling rust are classified based on their action pathway. Non-penetrating fungicides such as mancozeb is a preventive contact fungicide. It hinders enzyme activity in fungi by impairing biochemical processes occurring in the cytoplasm and mitochondria (Gallo & Lawryk, 1991). Bordeaux is also a preventive fungicide consisting of a mixture of copper (II) sulphate ($CuSO_4$) and quicklime (CaO) that inactivate fungal spore thus preventing germination. Moreover, sulphur, Nickel and cupric salts demonstrated antifungal action against rusts due to their toxicity (Mishra *et al*., 1974; Rathmell & Skidmore, 1982). Systemic fungicides such as triazoles act by inhibiting succinase dehydrogenase (Huang *et al*., 2019).

2.5.4 Genetic resistance

To minimize losses caused by rusts, resistant varieties with a wide genetic base in conjunction with other rust control measures need to be adopted to manage epidemics caused by acquisition of virulence in rust pathogen (Hovmoller *et al*., 2008; Wellings, 2007). Breeding for resistance which basically involves incorporation of new genes and screening of diverse cultivars for rust resistance is most effective in controlling stem rust coupled with other environmentally friendly practices (Goutam *et al*., 2015). Pyramiding of minor genes and major genes provide the most durable resistance in cultivars (Fetch *et al*., 2021; Mundt, 2018). To curb the ever-emerging rust races, an integration of genome editing, genomic selection, marker-assisted breeding and phenotypic resistance screening is necessary (Mapuranga *et al*., 2022). This has been made possible through sequencing of diploid,

hexaploid, tetraploid and wild wheat relatives leading to discovery and characterization of new resistance genes (Ramirez-Gonzalez *et al*., 2018). Up to date, 60 *Sr* genes have been identified, sourced from bread wheat and others introgressed from wheat-related species (Karelov *et al*., 2022).

Race-specific genes are major genes expressed at the seedling stage and provide qualitative resistance (Boyd *et al*., 2013). This resistance follows Flor's gene for gene theory proposed by Flor explains that each resistance gene in the host plant is paired with a specific complementary gene that confers avirulence in the pathogen (Flor, 1971). Observable response for qualitative resistance is hypersensitive reaction type and causes cell death in the affected area thus limiting spread of the pathogen (Flor, 1971). Widespread use of R-genes singly in multiple varieties leads to faster breakdown of resistance in a phenomenon called boom bust cycles (Johnson, 1961). Virulent *Ug99* races have rendered most cultivars possessing R-genes ineffective, attributable to their negative linkage drag and unadapted backgrounds (Bhavani *et al*., 2019). Some of the catalogued race specific genes include *SrCad*, *SrTmp*, *Sr5* and *Sr42* located on chromosome 6DS, *Sr6* located on chromosome 2D, *Sr7*(*7a*, *7b*) located on chromosome 4AL, *Sr8*(*8a*,*8b*) located on chromosome 6AS, *Sr9*(*9a*, *9b*,*9e*, *9f*, *9h*), *Sr28* and *Sr16* located on chromosome 2BL, *Sr10*, *Sr19* and *Sr20* located on chromosome 2B, *Sr15* located on chromosome 7AL, *Sr18* located on chromosome 1DL, *Sr28* located on chromosome 2BS, *Sr29* located on chromosome 6D, *Sr30* located on chromosome 5DL, *Sr41* located on chromosome 4D, Sr48 located on chromosome 2AL, *Sr49* on chromosome 5BL and *Sr54* on chromosome 2BL (Karelov *et al*., 2022; Spanic *et al*., 2015).

Race-nonspecific adult plant resistance genes are controlled by minor genes (*Sr2*, *Sr55*, *Sr58*, *Sr57* and *Sr56*) each with additive effect conferring durable resistance to *Pgt* than major gene-based resistance (Laido *et al*., 2015; Singh *et al*., 2015). It provides quantitative resistance exhibiting incomplete resistance mainly expressed at adult plant stage and effective against a wide range of pathogen. Distinct feature of APR genes is increased latency period, low spore production and reduced infection points (Bhavani *et al*., 2019). High level of resistance can be attained by combination of at least 5 APR genes each with additive affect (Knott, 1982; Knott, 1988). Notably, *Sr2* complex genes provide the most important APR resistance against *Pgt* including the virulent *Ug99* strain, with a characteristic pseudo-black chaff marker and confer average levels of APR under high disease pressure (Hare & McIntosh, 1979; McIntosh, 1988).

2.6 Stem rust research and race evolution in Kenya

Wheat farming in Kenya dates back to twentieth century in Njoro region with severe cases of stem rust and stripe rust recorded (Pinto & Hurd, 1970). In 1925, the first resistant wheat variety 'Kenya Governor' was released but became susceptible to *Pgt* prompting the beginning of stem rust resistance studies (Guthrie 1966). Two *Pgt* races, avirulent race 17 and virulent race 21 were found by McDonald by using stackman differentials (Stakman *et al*., 1962). In subsequent years, races 21, 34, and 116 became virulent to *Sr5* and *Sr6* resistant genes. In 1938, a new cultivar 'Kenya 117A' was developed and later, sister lines with *Sr7a, Sr9b*, and *Sr10* genes were released leading to rapid breakdown of resistance and emergence of 12 other new races including race 24, 189, 295, 296, 297 by 1961 (Stakman *et al*., 1962). Researchers began to introgression resistant lines into Kenyan cultivars in 1955, using CI 12632 (*Sr36*), Frontana-Kenya 58-Newthatch and Mida-McMurachy-Exchange (Dixon, 1960).

In the years 1955 to 1965, 62 Kenyan cultivars were released however, a year later, many genes from *Triticum timopheevii* were ineffective against race EA4 that was frequent in the area (Green *et al*., 1970). This called for more research that saw release of other Kenyan wheat cultivars with crosses from CYMMIT cultivar 'Kavko' possessing *Sr31* gene to improve rust resistance. This comprised of Kenya Paa', Kenya Kwale and Kenya Duma released in 1982, 1987 and 1993 respectively (Fetch *et al*., 2021). In 1999, resistance in Kenyan cultivars declined swiftly due to emergence of a new virulence race that was later named *Ug99*, including those that contained *Sr31* gene. This led to virulence characterization of *Sr31* carriers with samples from Uganda that led to occurrence of unusually higher disease scores ranging from '3' to '4' (Pretorious *et al*., 2000). Confirmation of the pathogen's virulence for *Sr31* led to naming of *Ug99* according to country of origin and the year the sample was collected. Further test on differential lines revealed virulence on *Sr5-6, Sr7b, Sr8a*, *Sr8b*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr11*, *Sr15*, *Sr17*, *Sr30-31*, and *Sr38* and avirulence for *Agi*, *Em, Sr21*, *Sr22*, *Sr24-27*, *Sr29*, *Sr32-36*, *Sr39*, *Sr40* and *Sr42-43*.

In 2005, 'sounding the alarm' announcement was made and *Ug99* declared a menace to wheat production globally leading to the formation of Borlaug Global Rust Initiative in 2008. As of 2019, *Pgt Ug99* race has 12 variants present in Kenya: *TTKST*, *TTKSK*, *PTKSK*, *PTKST*, *TTKTT*$_{+}$, *PTKTK*, *TTHST*, *TTKTK*, *TTHST*, *TTTSK*, *TTHTT* and *TTHSK* (Fetch *et al*., 2014; Wanyera and Wamalwa, 2022). Other races are causing epidemics globally for instance, Germany, Sicily, Italy and United Kingdom (Bhattacharya, 2017; Meyer *et al*., 2017).

2.6.1 Race diversity of stem rust

Puccinia graminis genotypic and phenotypic global monitoring has revealed several races apart from the *Ug99* (Olivera *et al*., 2017). Kenya has the highest number of races revealed through various phenotypic and genotypic analyses (Bhavani *et al*., 2019). Major threat is the TTKSK race group that has caused havoc in many wheat growing regions. Its variants include TTKSF (virulent on *Sr31*, *Sr21*), TTKST (virulent on *Sr21*), TTTST (virulent on *Sr31*), TTTSK (virulent on *Sr31*, *Sr21*, *Sr36*), TTKSP (virulent on *Sr21*, *Sr24*), PTKST (virulent on *Sr31*, *Sr21*), TTKSF$_+$ (virulent on *Sr9h*, *Sr21*), TTKTT (virulent on *Sr31*, *Sr21* and *SrTmp*), TTHSK (virulent on *Sr31*, *Sr21*), PTKTK (virulent on *Sr31*), TTHST (virulent on *Sr31*, *Sr21* and *Sr24*) and TTKTK (virulent on *Sr31*, *Sr21* and *SrTmp*) (Bhavani *et al*., 2019). Races in other groups include TKTTF that caused epidemics in Ethiopia during the 2013-2014 wheat growing season (Olivera *et al*., 2017). The TTRTF race caused epidemics in 2016 in Sicily while race RRTTF caused losses in Ecuador (Barnes *et al*., 2016; Bhattacharya *et al*., 2017).

2.6.2 Challenges and strategies in stem rust research

Puccinia graminis is a major concern due to its faster spread and emergence of more virulent races. Various epiphytotic have been recorded worldwide, and this poses a setback to stem rust resistance breeding (Bhattacharya *et al*., 2017; Olivera *et al*., 2017). Furthermore, the existence of genetic vulnerability in most grown wheat cultivars encourages spread and build-up of the pathogen (Bhavani *et al*., 2019). Vulnerability occurs when the pathogen overcomes host resistance and renders a cultivar susceptible to a specific race. This can occur when a cultivar carrying single race-specific genes undergoes faster breakdown of resistance in a phenomenon called boom-bust cycles (Johnson, 1961).

A wider genetic base for stem rust resistant cultivars is essential in keeping up with the ever-emerging races by providing immediate option for replacement in the event of a cultivar susceptibility. This can be achieved through identification and characterization of new genes and their subsequent incorporation into cultivars through marker assisted breeding (Goutam *et al*., 2015). Active surveillance in key hotspot areas can also help in forecasting the distribution stem rust races.

2.7 Research gap

Despite most studies conducting for stem rust resistance, they always focus on either seedling plant resistance (SPR) or adult plant resistance (SPR) in isolation. Few studies always focus on the same set of genotypes during APR and SPR. In addition, there is inadequate surveillance data on the virulence diversity of rust races in major hotspot region like Mau-Summit and Njoro. The limited data on stem rust race distribution and resistance profiles of wheat lines present a gap in designing location-specific durable resistance. This study addressed the gap by evaluating both SPR and APR and also characterizing the prevailing stem rust races. However, validation of durability of the identified resistance is needed under multi-environmental trials over seasons.

CHAPTER THREE

EVALUATING SPRING WHEAT (*Triticum aestivum* L.) LINES FOR ADULT PLANT RESISTANCE AGAINST STEM RUST AND THE EFFECTS ON YIELD

ABSTRACT

Stem rust remain a threats in wheat production with the causative pathogen *Puccinia graminis f. sp tritici* (*Pgt*). Adult plant resistance (APR) is one of the most effective methods of controlling this disease in wheat. This study aimed to identify the 144 diverse spring wheat lines named in the 16th stem rust resistance screening nursery (SRRSN) with adult plant resistance (APR) against stem rust under natural conditions. The lines were assessed for APR against stem rust and its effects on plant height, number of seeds per spike, thousand kernel weight, spike length, biomass, and yield at Kenya Agricultural Research Organization (KALRO), Food Crops Research Centre, Njoro over the course of two seasons in a 12×12 partially balanced incomplete block design. The effects due to season, genotype, genotype-by-season interaction were significant ($p \leq 0.001$) for plant height, 1000-kernel weight (TKW), biomass and area under disease progress curve (AUDPC). Mean grain yield and TKW ranged from 0.8 t ha^{-1} to 9.05 t ha^{-1} and 20.03 g to 46.42 g, respectively. Cacuke and 6096 genotypes recorded the lowest and highest yields of 0.8 t ha^{-1} and 9.05 t ha^{-1}, respectively. There was a positive correlation between TKW and grain yield, except for Area Under Disease Progress Curve (AUDPC). Regression analysis showed a decrease in grain yield, TKW and biomass due to stem rust by 16.13 %, 17.92 % and 4.60 %, respectively. The highest yielding and resistant genotypes were 6022, 6095, 6096, 6101, 6134, 6136, 6138 and 6139. These genotypes can be exploited and provide a good genetic germplasm for Kenyan wheat varieties.

3.1 Introduction

Wheat (*Titicum aestivum* L.) is among the primary staple foods globally alongside rice (*Oryza sativa*) and maize (*Zea mays* L.) occupying roughly 217 million ha each year (Einstein *et al*., 2022). The production is distributed in areas of Africa, Asia, Europe, Oceania and the United States with annual production at 750 million tonnes (Bhavani *et al*., 2019; FAO 2020). Africa's wheat production is insufficient and its import is increasing by 9% each year due to high population growth and diminishing agricultural lands due to urbanization (Noort *et al*., 2022). This deficit forces huge imports to fill the production versus consumption gap. For instance, Kenya imported 33,000 metric tons in 2021 from leading wheat producing continents such as Russia and Australia (KNBS, 2021). Wheat crop is affected by various diseases but with current control measures, yield losses is 5% less compared to 18% losses without the use of resistant varieties coupled with other integrated pest management measures such as crop rotation and chemical control (Oerke, 2005).

Rust disease caused by *Puccinia spp* is a threat to global wheat supply (Huerta-Espino *et al*., 2020). Yield losses caused by rusts can range between US$ 4.3-5.0 billion (Figuera *et al*., 2018). Stem rust caused by *Puccinia graminis f. sp tritici* (*Pgt*), stripe rust caused by *Puccinia striiformis* f.sp. *tritici* (*Pst*) and leaf rust caused by *Puccinia triticina* are the three common rusts that affect wheat (Berlin *et al*., 2013; Singh *et al*., 2008). Stem rust is a major threat in wheat production with epidemics observed over the past seven decades spanning different areas of the world (Bhattachaya, 2017; USDA-ARS, 2020). In addition to the areas of Canada, Australia, Sicily, Ethiopia and the United States, Kenya has experienced wheat rust epiphytotics since early 1900s that has resulted in reduction of wheat imports (Pinto & Hurd, 1970). *Pgt* aggressiveness through production of new race variants is attributed to the recombination that takes place at the sexual phase of its life cycle (Terefe *et al*., 2016). Stem rust is common in areas experiencing warm and moist weather conditions where they are capable of producing new variants that are capaable of overcoming host resistance thereby causing yield losses (Gitachew *et al*., 2019; Prank *et al*., 2019). The *Pgt* causes yield losses ranging between 50 % to 90 % depending on disease severity and cultivar susceptibility (Huerta-Espino *et al*., 2020; Leonard & Szabo, 2005). Survey indicated a local production of 309, 500 T against a local consumption of 2.2 million T, indicating a 1.9 million T deficit thus forcing an importation of 1.99 million T to meet the local demand (Kenya Economic Survey Report, 2024). The *Pgt* infection reduces yield by affecting kernel filling phase, kernel quality, development and overall survival of the plant (Bhavani *et al*., 2022).

To overcome rapid resistance breakdown, introgression and pyramiding of 5 or more genes that confer APR to stem rust is mandatory to attain high resistance level (Singh *et al*., 2015). The most commonly used *Sr2* gene derived from cultivar Hope is an APR slow rusting gene which reduces the rate of disease progression (Singh *et al*., 2008). It has been used in CIMMYT-Mexican spring wheat and possesses a unique phenotypic expression of pseudo-black chaff synptoms expressed on glumes and below the nodes of the wheat crop (Borlaug *et al*., 1949).

3.2 Materials and methods

3.2.1 Description of the study area

In this study, rust spores were collected from KALRO-Njoro and Mau-Summit wheat fields in Nakuru County, Kenya. The screening for resistance in wheat cultivars was conducted at KALRO, Food Crops Research Centre, Njoro (0° 20′ 47'' S, 35° 56′ 1.7'' E). The KALRO research centre, Njoro is situated at an elevation of 2120 meters above sea level (m. a.s.l.) at Njoro sub-county, Nakuru County in central - Rift Valley region of Kenya. It receives an annual rainfall of 939.3 mm with a minimum and maximum mean temperature of 9˚C and 24˚C, respectively. The research centre located within the agro-ecological zone (AEZ) III and the soils are predominantly *Mollic Andosols*, well-drained, and dark reddish brown with humic top soils (ioVision, FAO-derived dataset, 2024; ISRIC, 2023).). The area is suitable for growing wheat, barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.). Since the inception in the early 20th century of wheat growing in the early 20th century in Kenya, Njoro has been considered a hotspot for stem rust (Pinto & Hud, 1970). The Mau-Summit wheat field site (0° 09′ 41'' S, 35° 41′ 12.0'' E) is at an elevation of 2538 m.a.s.l. in Nakuru County, Kenya. It receives an average annual rainfall of 998 mm with minimum and maximum temperatures of 8 °C and 21 °C, respectively. The site lies in the AEZ of upper highland III, and the soils are predominantly andosols (BioVision, FAO-derived dataset, 2024; ISRIC, 2023). These sites have been chosen because their environmental conditions are representative of wheat growing areas in Kenya.

3.2.2 Wheat lines and rust differentials

The 16th SRRSN (Stem Rust Resistance Screening Nursery) (appendix A) CIMMYT lines were utilized for this study. It consists of a single nursery set that has 144 diverse spring bread wheat (*Triticum aestivum* L.) germplasm resilint to all mega-environments with white/red grain color. The lines are derived from various parents and assembled based on

their disease and agronomic performance. The 20 stem rust differentials (Table 5.3) were obtained from the United States Department of Agriculture-Cereal Disease Laboratory (CDL), Minnesota, USA.

3.3 Experiment 1: Evaluating 16th SRRSN lines for adult plant resistance

3.3.1 Experimental procedure

KALRO Njoro wheat screening field was used for this study. Land was ploughed using a disc plough and harrowed to a fine tilth in readiness for sowing wheat. Wheat lines were sown for evaluation in a 12×12 partially balanced lattice design with three replicates in one location for the two seasons (Gomez & Gomez, 1984). The 16th SRRSN screening plots consisted of two rows of 0.7 m with an inter-row spacing of 0.2 m separated by a 0.3 m alleyway. The plots were surrounded with a continuous double row stem rust spreader in an equal proportion mixture of highly susceptible genotypes around each replicate and perpendicular to each entry at an interval of one block. An artificial epidemic was created by inoculation of the spreader rows with aqueous solution of stem rust spores. A hypodermic syringe was used to facilitate inoculation of spreader rows at the booting stage. Sowing was done by an experimental drill at a uniform seed rate of 125kg ha^{-1} in wheat main season in July 2022 and off-season in January 2023 at KALRO Njoro. The seeds were planted at a depth of 5 cm in double rows spaced 0.2 m apart.

Di-ammonium phosphate ($(NH_4)_2(HPO_4)$) (DAP, 18% N, 46% P_2O_5, 0% K_2O) fertilizer was applied at a rate of 150 kg ha^{-1} at sowing supplying 23.4 Kg N ha $^{-1}$ as both NH_4^+ and NO_3^- and 59.8 Kg P_2O_5 as either of $H_2PO_4^-$ or HPO_4^{2-}. Calcium ammonium nitrate (CAN 23% N, 0% P_2O_5, 0% K_2O) was be applied for top dressing at tillering at a rate of 200 kg ha^{-1} to provide 52 kg N ha^{-1} to each plot at tillering stage (GS20) (Plate 3.1) (Zadoks *et al.*,1974). Broadleaf weed was controlled by use of Buctril® MC, a selective post emergence herbicide at a rate of 1.5 L ha-1. Russian wheat aphids (*Duraphis noxia*) was controlled by Thunder® (Beta-cyfluthrin 0.45 Kg l^{-1} + Imidacloprid 0.1 Kg l^{-1}) at 300 ml ha^{-1} and Prove® (Emamectin Benzoate) at 0.5 l ha^{-1} at tillering stage.

Plate 3.1: Wheat nursery at tillering stage before onset of stem rust infection (KALRO, Njoro, 2022 main season)

3.3.2 Rust severity

The stem rust disease infection on wheat was evaluated three times at a 7 days' interval. Stem rust scoring began when the susceptible checks attained 50 % infection. The disease was scored on the stem and leaf sheath when the crop attained the following growth stages; GS55 (heading), GS65 (50% flowering) and GS77 (late milk) (Zadoks *et al.*, 1974). It was visually assessed based on modified Cobb's Scale of 0 to 100% as follows: 0 = Immune, 1 - 5% = resistant, 6 - 30% = resistant to moderately resistant, 31 - 60% = moderately susceptible, 61 - 80% = moderately susceptible-susceptible and 81 - 100% = highly susceptible (Ali *et al*., 2020). Based on the percent of the surface covered with pustules, necrosis and chlorosis present host response to the infection was classified into the following categories: Immune = no visible infection, R=resistant, MR = moderately resistant, MS = Moderately susceptible (S =susceptible MSS = moderately susceptible to susceptible and MRMS = infection response that overlap the MR and MS categories (Ali *et al*., 2020). Disease severity was done per plot as opposed to single plant tagging as it is sown in drills. Furthermore, one seed produces several tillers making it hard to tag one plant.

Coefficient of infection (CI) of ≤ 20 and 21 - 50, 51- 70 and ≥ 70 represented high, moderate low and very low levels of resistance against stem rust disease. CI was derived by multiplying the final disease severity with a constant (I = 0.0, R = 0.1, RMR = 0.2, MR = 0.3, MRMS = 0.5, MS = 0.7, MSS = 0.9 and S = 1.0) (Knott, 2012).

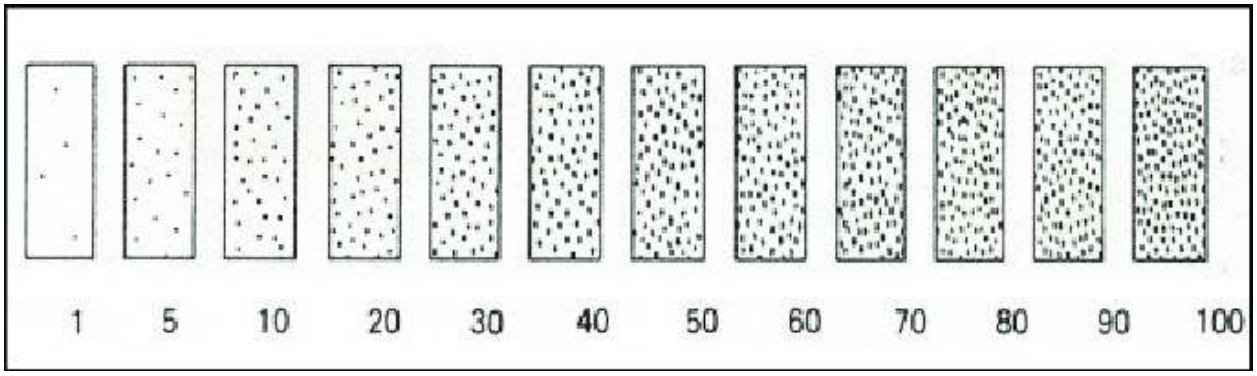

Figure 3.1: Visual representation of the modified Cobb scale used for assessing wheat rust severity.

The diagram illustrates severity levels ranging from 0 to 100% alongside typical infection types: Immune (0), Resistant (1-5 R), Moderately Resistant (6 – 30 MR), Moderately Susceptible (31 – 60 MS), Moderately Susceptible to Susceptible (61 - 80 MSS), Susceptible (81 - 100 S)

3.3.3 Yield and yield components

The days to 50 % heading was determined when 50 % of spikes completely emerged from the boot. Plant height (cm) was measured at physiological maturity (PM) from the base to the tip of the spikes of five plants selected randomly per plot using a metre ruler. Spike length (cm) was recorded in cm, and the number of kernels per spikelet was counted from five plants per plot for each wheat line. The number of seeds per spike was determined by harvesting five tillers per plot, threshing them separately, then counting the number of grains on each spikelet and finding the average. The entire experimental plots were harvested at PM by cutting the crops at the base, then measured the biomass per plot separately using a digital balance (Mettler PC 4400 DeltaRange®), threshed using an electronic threshing machine and winnowed. Grain yield was determined by weighing the kernels in each plot after drying to moisture content (MC) of 12 % using a digital balance (Mettler PC 4400 DeltaRange®)). A thousand kernel weight (TKW) from each plot was counted using a seed counter (Contador Pfeuffer, S/No. 14100009) and weighed.

3.3.4 Data analysis

To determine disease severity, the scores were converted into Area under the Disease Progress Curve (AUDPC) (equation 1) and estimated as described by Campbell and Madden (1990). The AUDPC values were square root transformed using AUDPCTrans method in

SAS software to obtain a normal frequency. Yellow rust occurred earlier than stem rust, and its severity was recorded.

$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{(i+1)} - ti)$……Equation 1

Where; n is the total number of observations, t is time of each reading in days, y_i is cumulative disease severity expressed as a proportion at the i^{th} observation, y_{i+1} is disease severity on the assessment date $(i+1)$, t_i is the time (days after planting) at the i^{th} observation and t_{i+1} is the second assessment date of the two consecutive assessments.

The data for disease (AUDPC), plant height, spike length and TKW collected for each season were first tested for normality to check for outliers using *Shapiro-Wilk* test (Shapiro & Wilk, 1965) (equation 2) and Levenes test of homogeneity to asses homogeneity of data (Levene, 1960)

$W = \frac{(\Sigma_{i=1}^{n} a_i X_{(i)})^2}{\Sigma_i^n (x_i - \bar{x})^2}$ ……Equation 2

Where X_i are the ordered random sample values, a_i are constants generated from the covariance, variance, and means of the sample (n) from a normally distributed sample. Tukey's studentized range was used for mean separation to determine the best-performing wheat variety.

The following SAS procedure for normality was used to analyse data:

```
Title ‘wheat stem rust ';
Data wheat;
Input var season rep block genotype height yield tkw spikelength audpcsr audpcyr;
Cards;
;
proc univariate normal plot;
proc sort; by. Var Plant height spikelength TKW;
proc univariate plot; by Var Plant height spikelength TKW
run;
```

The data were then subjected to the PROC GLM procedure in Statistical Analysis System version 9.2 (SAS, 2002) with a probability level of ≤ 0.05. Coefficient of variation, mean, and R^2 values were calculated from the AUDPC and yield parameters using the linear model below (equation 3), with fixed effect being replicate and genotype, while random effect being effect due to blocks and season.

$$Y_{ijklm} = \mu + S_i + R_{j(i)} + B_{k(ji)} + G_l + GS_{li} + \varepsilon_{ijklm} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 3}$$

Where; Y_{ijkl} is the observation of the experimental units; μ is overall mean; S_i is effect due to i^{th} season; $R_{j(i)}$ is effect of the j^{th} replicate nested in the i^{th} season; $B_{k(ji)}$ is effect of the k^{th} block in the j^{th} replicate in the i^{th} season; G_l is effect due to l^{th} genotype in the i^{th} season; GS_{li} is effect of interaction due to j^{th} genotype and i^{th} season and $\mathcal{E}_{ijklm}$ is random error component.

The following SAS procedure was used to perform a combined analysis of variance for field data:

```
Title “Stem rust”;
Data Wheat;
Input Season Rep Block Genotype Maturity Height Yield TKW Spikelength AUDPCSR
AUDPCYR;
Cards;
;
Proc Print;
Proc Glm;
Class Season Rep Block Genotype;
Model Maturity Height Yield TKW Spikelength
AUDPCSR = Season Rep (Season) Block (Rep*Season) Genotype Genotype*Season/p;
Test H=Genotype E=Genotype*Season;
Test H=Season E=Rep (Season);
Test H=Rep (Season) E=Block (Rep*Season);
Random Season Genotype*Season Block (Rep*Season);
Means Season Genotype Genotype*Season /Tukey;
Run;
```

Where the analysis of variance was significant, mean separation was performed using the Tukey honestly significant test at 5% level of significance (Tukey, 1949) given by the following formula in equation 4.

$$R = q[\alpha, p, fe] \times \sqrt{\frac{MSE}{r}} \quad \text{.....Equation 4}$$

Where; P is number of treatments mean, *fe* is degree freedom of error, α is level of significance, *MSE* is mean square error and *r* is number of replicates.

Pearson’s correlation coefficient analysis was used to determine the relationship between stem rust and yellow rust severities, biomass, TKW and yield (Cohen & Aiken, 2014), using proc corr procedure in SAS version 9.2 (SAS Institute, Cary, NC, 2002) using equation 5.

$$r = \frac{n(\sum x_i y_i) - (\sum x_i)(\sum y_i)}{\sqrt{[n\sum x^2 (\sum x_i)^2]\,[n\sum y_i - (\sum y_i)^2]}} \quad \text{.....Equation 5}$$

Where; r is Pearson's correlation coefficient, n is the number of samples, x is the dependable variable and y is the independent variable.

Stepwise multiple regression was conducted following *SAS PROC. REG* forward selection to determine the effect of *Sr* and *Yr* on grain yield, 1000-kernel weight and biomass using the following equation 6.

$Y_i = \beta_0 + \beta_1 x_{1(i)} + \beta_2 x_2 + \varepsilon_i$ …….....Equation 6

Where, Y_i is expected value of the dependent variable for a given set of independent variables x_1 and x_2; β_0 is the expected value of the dependent variable at $x_1, x_2 = 0$; β_1 and β_2 is the partial regression coefficient for every unit increase or decrease in the dependent variable. $x_1$and x_2 respectively and ε_i is the residual component. Yield, TKW, and biomass were considered as dependent variables while stem rust x_1 and yellow rust x_2 were considered as independent variables.

3.4 Results

3.4.1 Analysis of variance for yield and agronomic traits

At significance level ($P \leq 0.0001$), the effect due to season was significant for plant height (PH), days to heading (DH), biomass, 1000-kernel weight (TKW), AUDPC_Sr and AUDPC_Yr traits except seed per spike (S P^{-1}). Conversely, the effect due to genotype was significant for PH, spike length (SL), S P^{-1}, yield, TKW, AUDPC_Sr and AUDPC_Yr. Genotype by season interaction was only significant for TKW and AUDPC_Yr at ($P \leq 0.0001$). At a significance level ($P \leq 0.05$), the season effect was significant for SL and S $P^{-1,}$ while genotype by season interaction showed significance for S P^{-1}, biomass, and yield. Only biomass was significant at ($P \leq 0.01$) for the effect due to genotype (Table 3.1).

Main season 2022 had the highest mean for SL, DH, and days to maturity (DM), TKW, AUDPC_Sr and AUDPC_Yr by 1.89 %, 3.27 %, 0.87 %, 9.45 %, 53.61%, and 11.21%, respectively. On the other hand, off-season 2023 had a higher mean for PH, S P^{-1}, and biomass by 11.3 %, 2.58 %, and 41.28 %, respectively (Table 3.2). Yield was not significantly affected by seasons and had a mean of 5.4.

3.4.2 16th SRRSN wheat lines yield and agronomic performance over the seasons

Grain yield, biomass, and TKW varied across the genotypes and across seasons, with the overall highest yield being 9.05 t ha^{-1} for genotypes 6096 and the lowest being 0.08 t ha^{-1} in susceptible check, Cacuke. The PBW 343, a moderately resistant genotype, had 2.53 t ha^{-1}

while Robin had a yield of 3.45 t ha^{-1}. The yield between the seasons was not significantly different, with the main season having a yield of 1.1 % higher despite having higher AUDPC values. Genotypes with lower yields showed a lower plant biomass ranging from 30-65 t ha^{-1}. The genotypes with higher yield on the other hand however had biomass of between 66-141 t ha^{-1}. Genotype 6096 had the highest biomass and yield, with notably longer days to maturity of 128. Other genotypes with higher biomass were 6107, 6134, 6136, and 6097 with 118.93 t ha^{-1}, 116.25 t ha^{-1}, 127.2 t ha^{-1} and 124.76 t ha^{-1}, respectively. The TKW ranged from 19 - 44 grams (g) with higher TKW ranging between 40-44 g, and possessed moderate plant height and days of maturity of between 81-83 cm and 121-124 days, respectively. Genotypes 6026, 6032, 6035, 6038, 6052, 6054, 6055, 6080, 6081, 6101, 6108, 6122, and 6136 were the best-performing lines in terms of TKW (Appendix D). These genotypes also had a higher number of S P^{-1} and, similarly, a higher biomass compared to the shorter genotypes. The susceptible check recorded the lowest TKW of 19.3 g (Table 3.4).

Plants were generally taller in the off-season (OS) 2023 than main season (MS) 2022, although with a generally shorter spike length. The highest plant height recorded was 115 cm in genotype 6082, and the lowest height of 79.1 cm being genotype 6028. The majority of the shorter genotypes (79-84 cm) recorded early heading days than taller genotypes (95-115 cm), ranging from 62-66 and 69-72 days, respectively. The taller genotypes took a longer time to reach maturity as compared to the shorter genotypes. The majority of the tall genotypes took between 124-131 days as compared to the shorter genotypes that took between 116-122 days. The earliest heading date was observed in a generally shorter genotype, 6008 at 62 days, as compared to a taller genotype, 6126, heading at 77 days and matured at 119 and 131 days, respectively (Appendix D).

3.4.3 Evaluation of 16th SRRSN wheat lines for stem rust resistance

The lines exhibited varying resistance levels to stem rust, although yellow rust infection occurred naturally much earlier than stem rust onset and disease severity. While susceptible control Robin and Cacuke scored up to 80 % and 40 % respectively, genotypes 6049, 6079, and 6081 were completely immune to yellow rust infection in both seasons (Appendix D). No genotype was immune to stem rust in both seasons. Stem rust and yellow infection were both high in 2022 MS as compared to 2023 OS (Appendix B).

Stem rust severity for susceptible check Robin was 100 % in both seasons, while Cacuke had 90 % in MS and 80 % in OS (Appendix C). Among the tested wheat lines, 42

and 49 genotypes had a moderate type of reaction against stem rust in MS and OS, respectively (Appendix B). Only 1 genotype had a resistant (R) type of reaction during MS, while OS had 3 genotypes with an R type reaction. Other noted disease reactions were moderately susceptible (MS), moderately susceptible to susceptible (MSS), resistant to moderately resistant (RMR), and susceptible (S), with 38 and 36, 26 and 36, 11 and 3, 2 and 3 in MS and OS, respectively.

The AUDPC_Sr ranged from 23-1003 and 3.5-1038 during the 2022 main season (MS) and 2023 off-season (OS), respectively. 2023 OS had the lowest mean AUDPC of 201.36 compared to 2022 MS, which had an AUDPC estimate of 226.84, indicating high disease pressure in 2022 MS.

Table 3.1: Analysis of variance over two seasons for plant height, spike length, number of seeds per spike, days to heading, days to maturity, biomass, yield, 1000-kernel weight, and area under disease progress curve

Source of variation	Df	Plant height (cm)	Spike length (cm)	seeds per spike	Days to heading	Days to maturity	Biomass (t ha^{-1})	Grain yield (t ha^{-1})	1000-kernel weight (g)	AU
Season (S)	1	24152.54***	6.87*	307.53*	1091.49***	256.2	435.27***	3.13	13.99***	380
Replicate (R)/S	4	2171.05***	1.61	178.64*	199.85***	622.74*	148.96***	1.4	0.061**	93.9
Block/ (R×S)	66	125.55***	1.65	75.88	19.09	188.14	3.1***	4.82***	0.23***	37.1
Genotype (G)	143	102.30***	2.87***	105.49***	32.89	197.43	5.01***	15.17***	0.87***	168
G×S	143	70.41	1.58	80.36*	13.86	186.03	2.35***	2.67**	0.12***	17.5
Error	505	58.59	1.35	61.91	14.12843	185.97	1.28	1.93	0.012	4.68
CV (%)		8.69	12.37	16.98	5.57	10.98	12.6	25.71	1.65	20.2
R^2		0.69	0.53	0.53	0.58	0.43	0.79	0.749	0.97	0.93

Significant at (p≤0.05) is indicated by *, (p≤0.01) is indicated by **, (p≤0.001) is indicated by ***, CV= Coefficient of variation and df=degrees of freedom, Significant at (p≤0.05) is indicated by *, (p≤0.01) is indicated by **, (p≤0.001) is indicated by ***, CV= Coefficient of variation and df=degrees of freedom, AUDPC_Yr =Area Under Disease Progress Curve for yellow rust (Yr) AUDPC_Sr =stem rust (Sr)CI_Sr = coefficient of infection for stem rust

Table 3.2: Mean separation for yield, disease and agronomic parameters across two seasons

Season	Plant height (cm)	Spike length (cm)	Seeds per spike	Days to heading	Days to maturity	Biomass (t ha^{-1})	Grain Yield (t ha^{-1})	TKW (g)	AUDPC_Yr	AUDPC_Sr	CI
2022 MS	82.8 b	9.46 a	45.75b	68.58 a	124.71a	60.04 b	5.34a	35.47a	199.31a	226.84a	17.37a

2023 OS	93.38a	9.28 b	46.96a	66.33 b	123.62b	87.48 a	5.46a	32.12b	92.45b	201.41b	30.64a
MEAN	88.09	9.37	46.35	67.456	124.17	73.93	5.4	33.8	145.88	214.12	24.01
TUKEY	1.02	0.16	1.07	0.50	1.82	2.77	0.19	0.19	7.73	11.17	27.98
$MSD_{(0.05}$											

Means followed by different letter down the column are significantly different at P≤0.05, MSD means square difference, TKW 1000-kernel weight, AUDPC Area Under Disease Progress Curve for yellow rust (Yr) and stem rust (Sr),

Table 3.3: Range of yield, agronomic traits, and AUDPC for 16th SRRSN wheat lines evaluated for two seasons at KALRO Njoro

	Plant height (cm)	**Mean**	**Spike length (cm)**	**Mean**	**Seeds spike^{-1}**	**Mean**	**Days to heading**	**Mean**	**Days to maturity**	**Mean**
2022 MS	70.53-126.73	83.3	7.67-14.53	9.46	25.67-61.67	44.75	63-81.67	68.62	114-130.33	123.87
2023 OS	81.13-149.2	93.34	7-14	9.28	31.4-66	46.86	62-74	67.25	113-133.67	123.88

	Biomass (t ha^{-1})	Mean	Grain Yield (t ha^{-1})	Mean	TKW (g)	Mean	CI	Mean	AUDPC_Sr	Mean
2022 MS	23.29-126.67	59.94	0.75-8.66	5.43	18.1-46.42	35.43	0-100	22.27	23.33-1000.3	226.84
2023 OS	20.33-170.36	87.48	0.85-10.48	5.373	17.4-41.14	32.06	1.5-90	22.22	3.5-1038.33	201.36

TKW = 1000-kernel weight, CI = coefficient of infection

Table 3.4: Mean values for yield, agronomic traits, and disease variables for resistant genotypes and three controls evaluated for stem rust resistance for two seasons at KALRO Njoro

Genotype	Plant height (cm)	Days to heading	Days to Maturity	Spike Length (cm)	Seeds per spike	Biomass (t ha^{-1})	Yield (t ha^{-1})	1000-kernel Weight (g)	AU_S
6015	80.87	66.00	124.67	9.97	51.13	75.77	5.95	35.13	54.
6022	88.77	66.00	123.83	8.90	41.07	66.88	8.21	38.53	47.
6040	88.77	64.33	122.17	10.30	51.37	68.63	5.05	34.79	12
6041	90.50	66.00	123.33	10.03	47.13	60.36	6.71	37.20	81.
6074	84.83	65.00	119.83	8.90	55.70	71.31	6.09	37.55	90.
6095	94.57	70.33	127.67	10.27	52.80	86.90	7.89	39.68	72.
6096	97.23	69.17	128.83	10.27	52.00	141.73	9.05	36.60	72.
6107	90.53	73.00	129.33	9.50	48.03	118.93	7.58	35.44	59.
6134	91.60	69.67	124.33	9.80	51.73	116.25	7.29	38.23	13.
6136	94.37	66.67	124.83	10.13	48.13	127.20	7.59	40.21	32.
6137	86.47	67.33	126.50	9.67	46.03	94.88	7.61	36.03	35.

6138	86.60	68.17	126.83	9.83	46.60	73.66	7.23	38.54	41.
6139	88.93	71.00	126.00	9.87	52.33	89.14	8.46	38.13	40.
PBW 343[a]	83.13	69.83	122.00	8.60	40.33	51.67	2.53	22.95	52
Robin[b]	83.13	67.83	125.67	10.20	44.17	30.50	3.45	22.64	10
Cacuke[c]	85.87	67.00	122.00	9.73	34.97	42.50	0.80	19.29	76

AUDPC = area under disease progress curve, CI = coefficient of infection, FDS = final disease severity, OS = off season, MS = main season [a] control for stem rust-moderately resistant, [b] control for stem rust-susceptible variety, [c] control for stem rust-susceptible variety

Coefficient of infection had a mean of 22.27 and 22.21 for MS and OS, respectively (Table 3.3), while disease severity was in the range 1-100. Genotypes that showed resistance in both seasons were 6015, 6022, 6040, 6041, 6074, 6095, 6096, 6107, 6134, 6136, 6137, 6138, and 6139. Their AUDPC, CI, and Final disease severity range were 23 - 151, 0 - 6, and 5 - 20 for the main season and 3.5 - 93, 2 - 8, and 1 - 15 for the off-season, respectively (Table 3.3). Stem rust severity of 15 % to 50 % was the most common in both seasons, with a response of M to MSS (Figure 3.2a and 3.2b). Other host plant reactions (HPR) of resistant (R), moderately resistant (MR), and resistant to moderately resistant (RMR) were also observed in best best-performing genotypes. In the MS, 10 and 3 genotypes displayed an MR and RMR type of reaction, respectively, while in the OS, 7, 3, and 3 genotypes displayed an MR, R, and RMR type of reaction, respectively. Genotypes 6015, 6134, 6137, 6138, and 6139 had the lowest HPR (Appendix B).

3.4.4 Correlation and regression analysis

Plant height was positively correlated with spike length (0.07*), seeds spike^{-1} (0.09**), and biomass (0.11*). It was, however, negatively correlated with AUDPC_Sr and AUDPC_Yr at -0.17*** and -0.24*** respectively. Days to heading was positively correlated to spike length (0.1**), biomass (0.42***), and yield (0.18***) while days to maturity was negatively correlated to biomass (-0.28**) and yield (-0.14**). Spike length was positively correlated seeds spike^{-1} (0.14**), yield (0.1**) and TKW (0.08*). It was however negatively correlated to AUDPC_Yr (-0.07*). Seeds spike^{-1} was positively correlated to biomass (0.11**), yield (0.2**) and TKW (0.08*). It was however negatively correlated to AUDPC_Sr and AUDPC_Yr are -0.13** and -0.24 respectively. Furthermore, biomass was positively correlated to yield (0.54***) and TKW (0.12**) while yield was positively correlated to TKW (0.58***), AUDPC_Sr (-0.41***) and AUDPC_Yr (-0.4***). TKW was negatively correlated to AUDPC_Sr (-0.42**) while AUDPC_Sr had a positive correlation to AUDPC_Yr (0.23**) (Table 3.6).

Stepwise regression analysis revealed a reduction in grain yield, TKW and biomass by 16.13 %, 17.92 % and 4.6 % due to stem rust effect respectively. Similarly, yellow rust caused significant reduction of grain yield, TKW and biomass by 9.48 %, 7.71 % and 0.2 % respectively (Table 3.5).

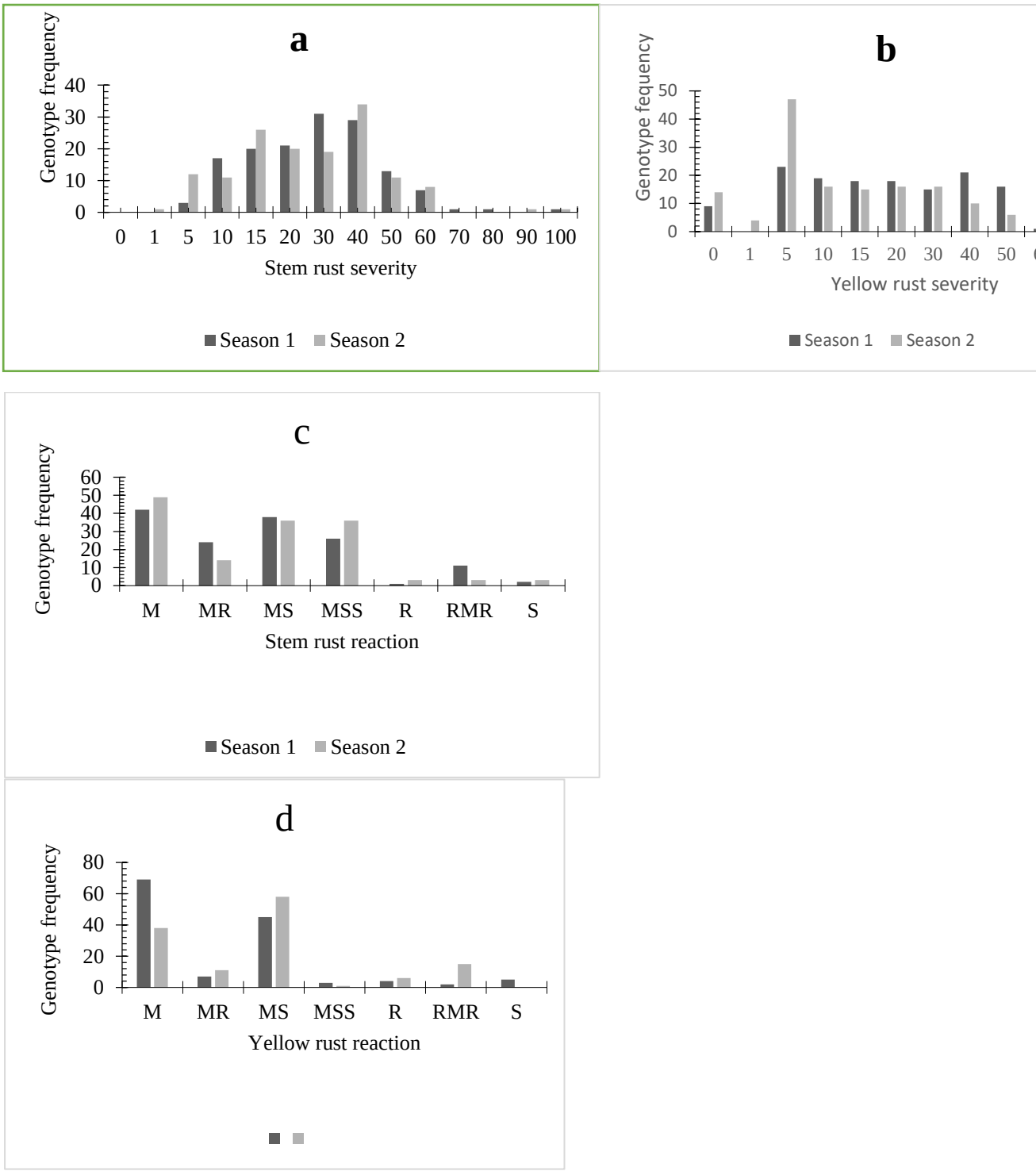

Figure 3.2: Distribution of stem rust and yellow rust severity, and response for 16th SRRSN wheat lines evaluated at KALRO Njoro over two seasons

Table 3.5: Stepwise multiple regression analysis on effect of stem and yellow rust on grain yield, thousand kernel weight and biomass

Dependent variable	Independent variable	Intercept	Parameter estimate	Standard error	Partial R^2	Model R^2	C(P)	Model
Grain yield	AUDPCYr	6.87908	-0.00406	0.0003 9543	0.0948	0.2561	3	$6.88-0.0042_{(AUDPC\_Sr)}-0.0041_{(AUDPC\_Yr)}$
	AUDPCSr		-0.00417	0.0003 9582	0.1613	0.1613	106.342	
TKW	AUDPCYr	37.91546	-0.01254	0.0010 9	0.0771	0.26	3	$37.92-0.015_{(AUDPC\_Sr)}-0.012_{(AUDPC\_Yr)}$
	AUDPCSr		-0.01497	0.0011 1	0.1792	0.18	86.69	
Biomass	AUDPCYr	85.71	-0.07	0.007	0.145	0.14	16.21	$85.71-0.03_{(AUDPC\_Sr)}-0.07_{(AUDPC\_Yr)}$
	AUDPCSr		-0.03	0.007	0.016	0.16	3	

TKW=1000-kernel weight, AUDPC=Area Under Disease Progress Curve for yellow rust (Yr) and stem rust (Sr), R^2=coefficient of determination, C(P)=Mallow's complexity parameter

Table 3.6: Pearson's correlation coefficient on yield, agronomic traits, and AUDPC for stem rust and yellow rust

Traits	Plant	Days to	Days	Spike	Seeds	Biom	Yield	TKW	AUDP

	height	heading	to maturity	length	per spike	ass			C_Sr
Heading	0.02								
Maturity	0.03	-0.06							
Spike length	0.07*	0.1**	0.051						
Seeds spike^{-1}	0.093**	0.03	0.05	0.14***					
Biomass	0.5***	0.08*	-0.08*	0.06	0.15***				
Yield	0.11	0.18***	-0.14***	0.1**	0.2***	0.22***			
TKW	-0.06	0.03	-0.01	0.08*	0.08*	0.03	0.58***		
AUDPC_Sr	-0.17***	-0.04	-0.03	-0.01	-0.13**	-0.22***	-0.41***	-0.42***	
AUDPC_Yr	-0.24***	0.02	-0.03	-0.07*	-0.24***	-0.38	-0.4***	-0.37	0.23***

AUDPC=Area under disease progress curve, TKW=1000-kernel weight. *, ** and *** = significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

3.5 Discussion

The tested genotypes showed variation in disease response, evidenced by AUDPC values and the coefficient of infection. This implies a presence of genetic variation about season and location (Singh *et al*., 2011). AUDPC values and final disease severity for stem rust and yellow rust were significantly higher in the main season, highlighting the impact of environmental variation on disease expression. The resistant genotypes 6015, 6022, 6040, 6041, 6074, 6095, 6096, 6107, 6134, 6136, 6137, 6139, and 6139 possessed adult plant resistance (APR) genes as indicated in their pedigree. The lines displayed an R and MR type of reactions and had low AUDPC and CI values. Genotypes 6041 and 6074 had gene *Lr34* and co-segregate with the stem rust gene *Sr57*. Genotype 6040 had a characteristic pseudo-black chaff (PBC), indicating the presence of the Sr2 gene. Genotypes 6095 and 6096 are sister lines and have genes *Sr47+Sr26* and a characteristic PBC, suggesting the presence of the APR gene *Sr2*. Genotype 6107 had gene Sr22 and PBC. Sister lines 6134, 6136, 6137, 6138, and 6139 possessed *Sr50+SrND643*. This is in line with studies by Singh *et al*. 2015 that showed that pyramiding adult plant resistance genes confer high levels of resistance. The other 20 genotypes that had a moderate type of reaction (MRMS) in both seasons had a severity range of 5%-40 %. A number of them had a pyramid of genes, for instance, 6094, 6131, and 6132 had genes *Sr50+SrND643*, 6094 and 6098 had *Sr50+SrND643*, *Sr35+SrND643*, respectively. The low and moderate response to stem rust indicates the effectiveness of the APR genes in reducing stem rust infection.

Stem rust gene *Sr2* is an APR and a widely deployed gene among CIMMYT wheat lines. It is a pleiotropic gene and to *Yr30* and *Lr27* genes that confers resistance against yellow rust and stem rust, respectively. *Sr2* is a recessive gene that confers modest resistance levels of when bulked with other minor stem rust resistance genes (Knott, 1982). It has a characteristic pseudo-black chaff phenotype and confers modest levels of APR under high disease pressure (Hare & McIntosh, 1979). This gene is located on chromosome arm 3BSA and was sourced from *Triticum turgidum* (McFadden, 1930). The PBC expression always appears in glumes and stem nodes, although its expression is affected by genotype-environment interaction (McFadden, 1939). A total of 31 genotypes expressed the PBC characteristic, and their disease severity ranged between 5 % and -100 %. This gene slows disease progression as compared to other genotypes without this gene, which displayed a moderately susceptible/resistant reaction. These observations are consistent with Gordon *et*

al. 2021 who found that genotypes with the *Sr2* gene often exhibit a moderately susceptible response in stem rust field conditions.

A number of genotypes carried the pleiotropic APR gene *Lr34*/*Sr57* in combination with a new gene *SrHuw234*. The genotypes displayed a low to moderate AUDPC value of between 49-352, and CI values ranging 2-43, respectively. In this study, genotypes 6041, 6065, 6073, 6074, 6111, 6112, 6113, and 6114 had a moderate resistance against stem rust. The *Lr34*/*Sr57* gene carries a leaf tip necrosis phenotype and is easily identified (Bhavani *et al*., 2019). This agrees with a study by Randhawa *et al*. (2018), who identified this gene as an APR gene against stem rust.

Stem rust can cause epidemics when an early infection occurs and is dispersed over large areas. This can be worsened by favorable weather conditions like high temperature, wind, and rainfall (Roelfs *et al*., 1992). In the main season 2022, there was a well-distributed rainfall totaling 629 mm and favorable weather compared to the off-season 2023 with a rainfall of 316 mm. The minimum and maximum temperatures were 9.22 - 22.11 and 8.98 - 25.53 in 2022 and 2023, respectively (Appendix F). This is evident with the higher stem rust infection experienced during the main season. The disease affects the phloem and limits translocation of nutrients to the sink, thereby affecting nutrient movement to the head. This results in shriveled grain, poor grain fill or no grain at all when a heavy infestation causes brittle stems that lodge (Roelfs *et al*., 1992).

Grain yield, biomass, and TKW were all significant for the main effects, genotype, and genotype by season interaction. This implies that the seasons greatly influence these agronomic traits and corresponds with findings reported by Pennachi *et al*. (2018) on the influence of seasons in wheat growth and production. Genetic background and seasonal variation, including temperature, rainfall, and disease pressure, affect the overall performance of the wheat plant. In the main season, there was a significantly higher disease pressure and favorable weather for the development of the disease.

Thousand-kernel weight and yield are greatly influenced by an interaction between the genes and environmental conditions (Gonzalez *et al*., 2014). Under high disease pressure, genes conferring these traits are masked, hence a low record of these parameters (Kumar & Kumar, 2014). Correlation analysis between stem rust and yellow rust indicated the possibility of these rusts occurring in one host and has been proven to reduce yields (Schumann & Leonard, 2000). An increase in the intensity of stem rust had a negative correlation to TKW (r=-0.42), yield (r=-0.41), plant height (r=-0.17), and seeds spike^{-1} (r=-0.13) (Table 3.6). This suggests that an increase in stem rust severity interferes with the

metabolic processes that ultimately lead to a low record on these parameters (Chen *et al*., 2015). Macharia and Wanyera (2012), reported similar findings that an increase in stem rust severity and AUDPC led to a decrease in yield and TKW.

Main season 2022 had generally shorter plants with a mean of 83.3 cm and a mean AUDPC estimate of 226.84. They produced more tillers compared to taller plants and matured a bit earlier. This is supported by Singh *et al*. (2015), who reported that shorter plants produce more tillers and spikelets. These plants efficiently use sunlight for the production of assimilates and rarely lodge (Gonzalez *et al*., 2014). On the contrary, taller plants had a higher yield and biomass as compared to shorter plants and had late maturity. The tall nature gives it a sunlight advantage as it can intercept sunlight better as compared to shorter plants (Kirby 2002).

3.6 Conclusion

Screening of genotypes with different APR genes is crucial in establishing the vulnerability of available wheat lines against the prevailing rust races. Pyramiding of APR genes has proven as a successful tool in managing stem rust. In this study, genotypes 6022, 6095, 6096, 6107, 6134, 6136, 6137, 6138, and 6139 showed resistance against stem rust and were also high yielding. Some had a combination of *Sr47+Sr26*, *Sr22*, *Sr50+SrND643*. These lines were identified as resistant and can be utilized as breeding germplasm. Once the genes are identified through genotyping and cloned, they can be pyramided through marker-assisted selection into an adapted cultivar to provide durable resistance in wheat.

CHAPTER FOUR

IDENTIFICATION OF WHEAT (*Triticum aestivum* L.) LINES WITH SEEDLING PLANT RESISTANCE TO WHEAT STEM RUST RACE TTKTT

ABSTRACT

Puccinia graminis f. sp. tritici is one of the devastating pathogen in wheat due to the emergence of aggressive rust races rendering most of the commonly used resistance genes susceptible at the seedling stages. The aim of this study was to identify wheat lines in the 16th SRRSN with seedling plant resistance (SPR) to wheat stem rust under greenhouse conditions. A set of 144 wheat lines, including four controls, was used for the seedling test in the greenhouse. They were planted in plastic pots and inoculated after 7 days with stem rust isolate TTKTT at the Kenya Agricultural and Livestock Research Organization (KALRO), Food Crops Research Centre, Njoro. The seedlings were sprayed with prepared spores and disease scored 14 days post inoculation. (DPI). Among the wheat lines, 100 were resistant to stem rust race TTKTT, while 43 of the wheat lines were susceptible with IT≤2+ and IT≥2+, respectively. A 45.5 %, 23 %, and 31 % of the lines were resistant, moderately resistant, and susceptible with infection type (IT) '0' to '1', '2-' to '2+', and '3-' to '3+', respectively. The majority of the resistant to moderately resistant lines possessed seedling resistance genes *Sr22*, *Sr26*, *Sr35*, *Sr47*, *Sr50*, and *SrND643*. The most effective seedling resistant genes against TTKTT, based on this study, were *Sr26*, *Sr36, Sr47,* and *Sr50;* hence can be used in various breeding programmes aimed at improving resistance to virulent stem rust races.

4.1 Introduction

Wheat is a staple foods consumed by at least 30 % of global population (Abbas *et al.*, 2009). It is a good source of fiber, vitamins, minerals, and 20 % protein, making it an important source of cereal protein compared to maize and beans (Kumar *et al*., 2011). Its production must be increased if the Vision 2030 agenda on ending all forms of hunger and malnutrition has to be met. Stem rust disease in wheat is caused by the pathogen *puccinia graminis f. sp tritici* (*Pgt*) is the leading biotic factor and losses can reach up to 90 % (Park, 2015). The most efficient method in managing this disease is the adoption of resistant lines possessing both seedling (Race specific) and adult plant resistance (Race non-specific) genes coupled with other management measures (Huerta-Espino *et al*., 2020; Mapuranga *et al*., 2022). However, the durability of these genes often becomes less effective with time due to the appearance of more aggressive strains (Bhattacharya, 2017). This leads to the

development of disease epidemics under optimal environmental conditions and can cause total yield losses (Bhattacharyra, 2017).

Stem rust resistance genes are classified as either seedling stage resistance (SPR) or adult plant resistance (APR) genes (Figueroa *et al*., 2020; Hueta-Espino *et al*., 2011). However, APR has been proved to confer higher level of resistance against *Pgt* as it involves minor genes combined and provides modest levels of resistance. This implies that they might be susceptible at the seedling stage but become resistant at the later stages (Hueta-Espino *et al*., 2011). The purpose of conducting plant resistance in a controlled greenhouse environment is to reduce effects posed by environmental conditions, the difference in growth stages, attack by other diseases, and the disease pressure caused by the spreader lines. Furthermore, Infection types produced in the greenhouse are always more uniform compared to the ones produced in the field (Riaz & Hickey, 2017).

Race-specific genes represent key genetic factors primarily active during the seedling stage, contributing to qualitative resistance (Boyd *et al*., 2013). This form of resistance follows gene-for-gene theory as postulated by flor, wherein each resistance gene within the host plant corresponds with a complementary gene in the pathogen, determining avirulence (Flor, 1971). This form of resistance is characterized by a hypersensitive reaction through triggering localized cell death, completely prohibiting the spread of the pathogen (Flor, 1971). Overreliance on single R-genes across various cultivars accelerates resistance breakdown in a phenomenon called the boom-bust cycle (Johnson, 1961). Many R-genes possess negative linkage drag due to unadapted backgrounds, making most wheat lines susceptible to the virulent *Ug99* races (Bhavani *et al*., 2019).

Screening for resistance in new wheat lines is important in identifying new resistance genes that can be introduced into already adapted varieties with undesirable traits. Therefore, the objective of this experiment was to identify wheat lines in the 16th SRRSN resistant to wheat stem rust under greenhouse conditions.

4.2 Materials and methods

4.2.1 Sample collection and storage

Infected leaf sheaths bearing moderately susceptible to susceptible pustules were cut from infected wheat fields in areas of Njoro (0° 20′ 47’’ S, 35° 56′ 1.7’’ E) during the main growing season (July-October, 2022) in Kenya. They were cut into pieces 5 cm long and transported to the laboratory. The stem rust spores were collected into gelatin capsules (size 00) and stored at -4 °C until further use.

4.2.2 Inoculation of collected samples

Susceptible variety (Cacuke) was planted in pots and filled with vermiculite of 125 cm^3. About 5 granules of Di-ammonium Phosphate (18:46:0) were added to the vermiculite then seeds sown at a depth of about 2 cm. They were then watered and placed on trays in the at room temperature. Watering was done depending on the media condition ensuring the seedlings had adequate water.

The seedlings were placed on a table and inoculated with spores on completely expanded leaves at 9 days. The spores were then suspended in distilled water (H_2O) and two drops of light mineral oil Soltrol® 100 Isoparaffin (Chevron Philips Chemical Company) was added then mixed by gently inverting the capsule. The inocula was sieved and drained into a dispenser and adjusted to a concentration of 1×10^6 spores/ml (Jin *et al*., 2008). Using a calibrated hand sprayer, each plant received approximately 0.6 ml of the prepared inocula on the upper and lower leaf surfaces. Inoculated seedlings were then air dried for a period of 1 hour and thereafter dampened with water using an atomizer. The seedlings were then transferred in a dark dew chamber for 24 hours at 20 $^{\circ}$ C. They were later transferred to a greenhouse bench at 25 $^{\circ}$C.

4.2.3 Single pustule isolation and bulking of spores

Fourteen days after the development of the disease, a single pustule bearing a distinct feature (size/shape) was collected onto a gelatinous capsule, well labelled and sealed. After each collection, the vacuum pump and inoculator were wiped using cotton wool soaked in 70 % alcohol. The spores were then inoculated and incubated separately on another set of Cacuke until a bulk inoculum was achieved, enough to inoculate the stem rust differentials as described in experimental procedure in inoculation of collected samples above.

4.2.4 Inoculation of wheat lines

One hundred and forty-four wheat lines alongside 4 checks, together with 20 stem rust differentials, were planted separately in two sets as described in 4.2.2. They were inoculated and incubated separately at growth stage 1 (Feekes scale), and the experiment was repeated to reduce biases in the results.

4.3 Data collection

The infection type was determined 14 days after inoculation, based on a 0 - 4 scale as described by Stakman *et al*. (1962). The data was visual with low infection type (IT) (0, 1-, 1, 1+, 2-, 2 and 2+) implied that the tested isolate was avirulent to the resistant host while high infection type (3 and 4) indicated virulence to the susceptible host. The infection types were scored as 0 = immune, 0; = very resistant, 1 = resistant, 2 = moderately resistant, 3 = moderately susceptible and 4 = susceptible (Table 6.1). The symbols ("+" and "-") described the sizes of the pustules whereby; "+" represented larger pustules and "-" represented smaller pustules for infection types 1, 2 and 3 as described by Roelfs & Martens, (1988) and Stakman *et al*, (1962) (Fig. 4.3). Those with IT<2 was considered as resistant, those with IT≥3 was considered susceptible (Plate 4.1). A forward slash indicated the occurrence of different symptoms on the same stem or leaf, while a comma (,) indicated more than one IT.

Stem rust pathogen typing was conducted using the North American Nomenclature for *puccinia graminis f. sp tritici* (Roelfs & Martens, 1988). The differential was grouped into five differential sets, and races identified based on their infection types on differential set.

Table 4.3: Stem rust seedling infection type and symptoms scale

Infection type	Host plant reaction	Symptoms
0	Immune	No uredinia or presence of hypersensitive necrotic flecks
;	Very resistant	Few faint flecks with the absence of uredinia
1	Resistant	Minute uredinia are often bodered by necrotic regions
2	Moderately resistant	Minute to medium sized uredinia bordered by necrosis
3	Moderately susceptible	Medium-sized uredinia without necrosis
4	Susceptible	Large sized uredinia without necrosis
X	Mesotetic/heterogeneous	A range of disease response types on one leaf, ranging from resistant to susceptible.

Seedling infection type scale according to Stakman *et al*. (1962).

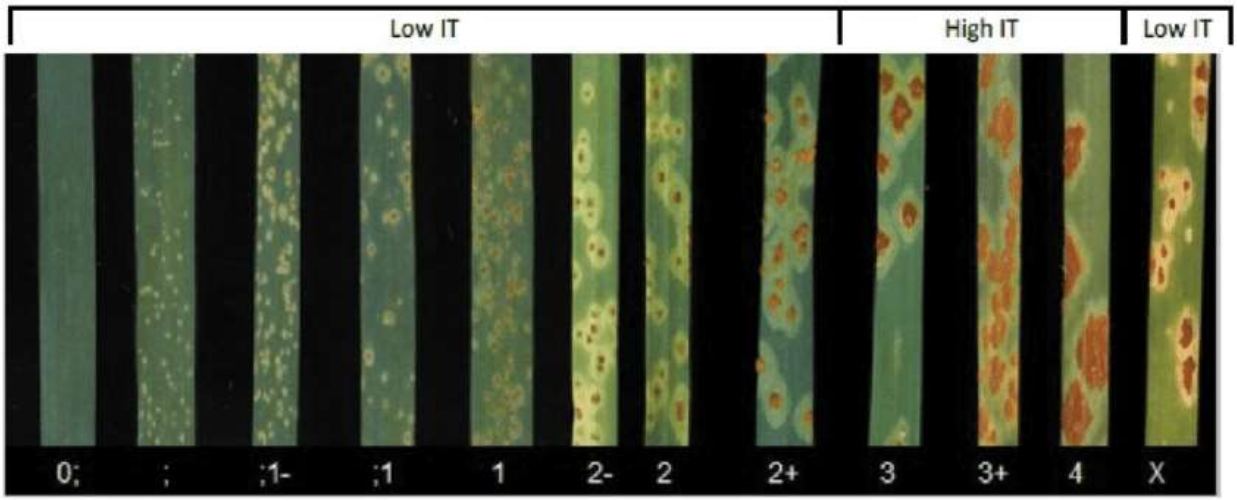

Figure 4.1: Infection types 14 days' post inoculation with *Puccinia graminis* f. sp *tritici* (*Stakman* et al. *1962*)

Plate 4.1: Susceptible infection type on leaf 14 days' post inoculation (March, 2023).

4.4 Results and discussions

This study did not find infection types (IT) 'X' and '4', as 3+ was the highest score in susceptible lines, as indicated in Figure 4.2. The checks PBW 343, Cacuke, and Kenya Robin exhibited 0, 3+, and 3+, respectively. About 72.7 % (104) of the lines exhibited resistance to stem rust race TTKTT, while 27.3 % (39) of the lines were susceptible with IT≤2+ and IT≥3, respectively. The highest IT observed was '3+', '3', and '3-' at 8 %, 20 %, and 0.7 %

respectively. The lowest IT observed was ‘0’, ‘;’, ‘1’, ‘2’ and their derivatives at 18 %, 4.9 %, 23.2 % and 23.9 % respectively (Figure 4.2).

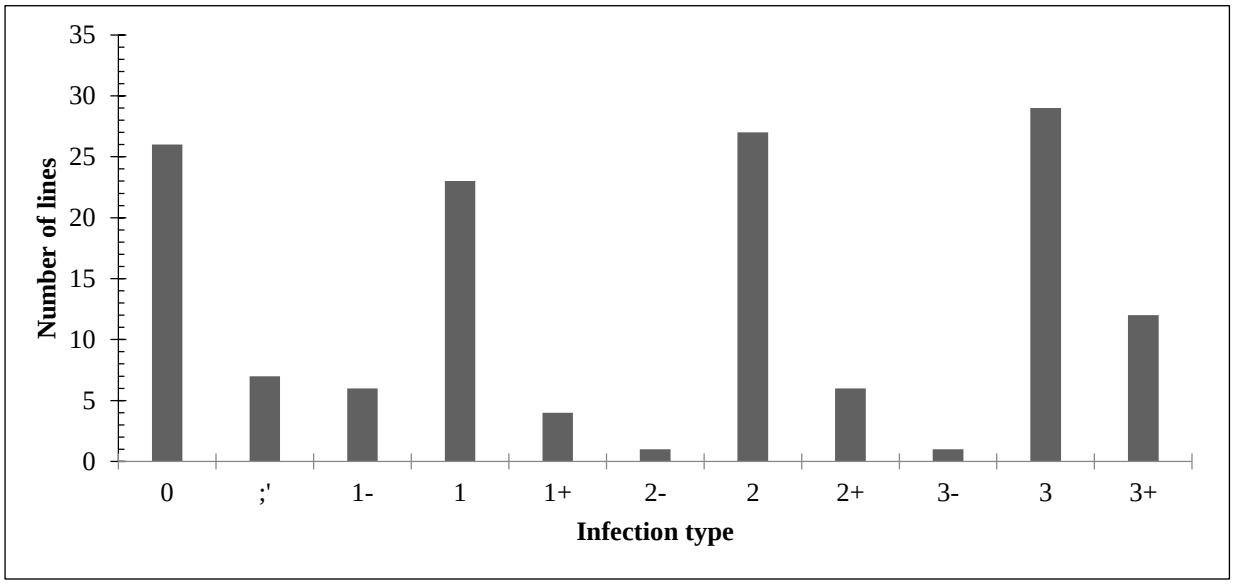

Figure 4.2: Frequencies of infection types for 144 diverse wheat lines including 3 controls evaluated against stem rust race TTKTT in the greenhouse at seedling stage

Infection type varied among the wheat lines, ranging from low (≤2+) to high (≥2+), indicating that the lines possessed different seedling resistance genes against race TTKTT (Lin *et al*., 2021; Njau *et al*., 2019). The high resistance observed among the lines indicated that they had various resistance genes to which race TTKTT was avirulent (Flor, 1971; Jin *et al*., 2008). Most of the resistant lines (IT≤2+) showed a disease severity of between 5 % - 60 % in the field, suggesting that the lines possessed either seedling resistance or adult plant resistance genes (Appendix B) (Fetch *et al*., 2021; Figueroa *et al*., 2020). Resistance at the seedling stage among the lines was exhibited by the presence of hypersensitive regions either with or without chlorosis (Flor, 1971).

Several genotypes had low infection types at the seedling and low severity at the adult plant stage across both seasons, suggesting near resistance or strong resistance. Notably, line 6015, 6022, 6029, 6046, 6081, 6123, 6133, and 6135 displayed RMR reaction across the two seasons and infection types, such as 0 and 1 during the seedling stage. They also recorded a disease severity score below 10 %, demonstrating a hypersensitive reaction and minimal pathogen establishment. Their consistent performance suggests the presence of durable resistance under different environmental conditions. Quite a number of the genotypes (95) fell into the intermediate category MR-MS, exhibiting moderate resistance/susceptibility with

severity scores ranging from 15 %- 40 % and varied slightly between seasons. At the seedling stage, they showed infection types of 2-, 2, or 2+. The changes in disease severity between the seasons suggested a potential genotype-by-environment interaction. The genotypes classified as susceptible displayed large uredinia with little or no chlorosis and had high severity scores above 60%. Notably, lines with high seedling and adult plant stage susceptibility, including, Cacuke, Robin, 6005, 6120, 6125, 6126, and 6127, had uniform S infection type and susceptible reaction (3) at the seedling stage. These lines likely lack genes conferring resistance and are vulnerable to the prevalent stem rust races in the trial environment. This makes them unsuitable for breeding programmes targeting both the seedling and adult plant stages.

The *SrND643* is a stem rust gene that confers moderate resistance to *Ug99* races at both seedling and adult plant stages (Singh *et al*., 2011). Basnet *et al*. (2015) established the location of this gene at the distal region of chromosome arm 4AL, and gene associated with high protein content, *gpc-B1*. This study found that gene *SrND64*3 incorporated singly in a genotype produced 8 and 3 lines bearing resistant and susceptible IT, respectively to race TTKTT. Low IT lines included 6016, 6017, 6021, 6028, 6032, 6057, 6108, 6109, 6142, while high IT included 6024, 6030, and 6050. In combination with *Sr50*, the lines 6094, 6131, 6132, 6133, 6134, 6135, 6136, 6137, 6138, and 6139 produced a low IT, with the majority being '0'. The stem rust gene *Sr50* was incorporated from rye (cultivar Imperial) through translocation from the short arm (p-arm) of chromosome 1R into wheat chromosome 1D (Mago *et al*., 2015). This gene encodes an pathogen effector protein, *AvrSr50*, produced during the uredinial stages of *Pgt* and is released into the plant cells during pathogen infection (Chen *et al*., 2017).

The *Sr22* is a gene effective to TTKSK race groups and was incorporated into wheat through interspecific hybridization from *Triticum monococcum ssp. Boeoticum* (Kerber & Dyck, 1973; Singh *et al*., 2008). Despite its resistance to most virulent races, it is linked to genes negatively affecting yield and other traits (Olson *et al*., 2010). A study by Hatta *et al*. 2022 rreported resistance of this gene to the virulent Ug99. Correspondingly, the wheat lines 6023, 6029, 6036, 6107, and 6130 possessing the *Sr22* and other undescribed genes were resistant to TTKTT at the seedling stage. Furthermore, a combination of this gene with *SrND643* conferred resistance in line 6123. However, in line 6007, this gene was susceptible to the TTKTT race, suggesting that the gene might have been used singly, rendering it susceptible to this race.

Gene *Sr47+Sr26* exhibited low IT in sister lines 6095 and 6096. The gene *Sr47* was introduced to the wheat background from *Aegilops speltoides* through induced homologous recombination (Faris *et al*., 2008). On the other hand, *Sr26* was derived from *Agropyron elongatum* demonstrates activity against most of stem rust races (Mago *et al*., 2011). The *Sr26* gene in 6038 and 6110 equally produced a low IT as demonstrated in a study conducted by Madahana *et al*. (2021), where they found gene *Sr26* to be effective against stem rust race TTKTT. A combination of these two genes proved effective at the seedling stage against TTKTT as evidenced in sister lines 6095 and 6096.

Another notably resistant gene among the studied lines is the *Sr35* gene, which was introduced to hexaploid wheat from *Triticum monococcum* and is effective against TTKSK races and their variants (Jin *et al*., 2008). It is located on the long arm of chromosome 3 Am and codes for) proteins responsible for detection of various pathogens (Saintenac *et al*., 2013; https://maswheat.ucdavis.edu/protocols/Sr35). Consequently, sister lines 6097, 6098, 6099, 6100, and 6140 produced a resistant infection type when tested against TTKTT singly or in combination with the *SrND643* gene. A combination of this gene and *Sr22* produced an IT '0' which is consistent with Hatta *et al*., who reported the effectiveness of *Sr22* and *Sr35* against the TTKSK group in barley (*Hordeum vulgare*). Identification of genes conditioning these resistances can be of importance in formulating research strategies, though it was not possible in this experiment (Park *et al*., 2011).

4.5 Conclusion

The majority of the lines in the 16th SRSSN (98) are resistant at the seedling stage, and exploiting their genetic makeup can be of help in stem rust resistance breeding. The genes *SrND643, Sr22, HUW234+LR34, Sr50, Sr47, Sr26, Sr36, and Sr35* are effective against TTKTT and are vital in stem rust resistance. Genotyping the lines with specific gene markers could help unfold the resistance genes in the line, thus providing a wider genetic base for breeding targeting seedling stage resistance

CHAPTER FIVE

IDENTIFICATION OF WHEAT (*Triticum aestivum* L.) STEM RUST RACES (*Puccinia graminis f. sp tritici)* IN NJORO AND MAU-SUMMIT AREAS IN NAKURU COUNTY, KENYA

ABSTRACT

Puccinia graminis f. sp tritici (*Pgt*) exists in Kenya with different physiological races virulent to various stem rust genes. The aim of this study was to identify wheat stem rust races in Njoro and Mau-Narok wheat production areas in Nakuru County, Kenya. A total of 6 infected wheat stem rust samples were collected from Njoro and Mau-Summit during the main wheat growing season of October 2022 and were used for race identification. The rust spores from Njoro and Mau-Summit were bulked separately and inoculated onto a susceptible check Cacuke, for single pustule isolation and spore multiplication. The spores were separately inoculated onto the 20 North American stem rust differential sets and an additional 51 CIMMYT stem rust differential lines. The differentials were evaluated 14 days post inoculation (DPI) to determine the races present. The isolates from Njoro and Mau-Summit produced infectious pustules, race TTKTT, virulent to 95 % of the North American stem rust differential lines. The changing virulence of the *Pgt* pathogen would render most Kenyan wheat cultivars susceptible, as the majority contains genes *Sr24*, *Sr31,* and *SrTmp*. This calls for rigid measures and research aimed at characterizing additional resistance genes against *Pgt* that can be readily incorporated into wheat varieties to provide durable resistance.

5.1 Introduction

Stem rust is a devastating disease in wheat growing regions in the world (Bhattacharya, 2017). According to Pinto and Hurd, (1970), it has been a challenge globally with negative consequences on wheat production since early 1900s. The evolving nature of the pathogen, mostly characterized by new races, underscores the importance of continuous surveillance and monitoring efforts (Bhavani *et al*., 2022). The technique of deoxy-nucleic acid (DNA) sequencing and genotyping has enabled researchers to swiftly detect and categorize stem rust races, thus aiding in the timely deployment of resistant cultivars (Babu *et al*., 2011). Molecular methods, field surveys and disease monitoring networks have proved essential in tracking stem rust epiphytotic (Singh *et al*., 2015). This research aims to highlight the importance of ongoing vigilance in safeguarding Kenyan wheat production from the menace of stem rust (Fetch *et al*., 2021).

The *Pgt* has various races spanning different geographical regions, named in accordance with the North American Scientific Nomenclature for stem rust. The virulent group is the *Ug99* (TTKSK clade) first noted in Uganda in 1999 (Pretorious *et al*., 2010). It has since gained significant attention due to its virulence on widely deployed resistance genes *Sr24, Sr31, Sr36,* and *SrTmp* (Jin *et al*., 2009; Patpour *et al*., 2016). It migrated over Eastern Africa to Zimbabwe, South Africa, and to the Middle East (Singh *et al*., 2011; Nazari *et al*., 2009). This race poses a serious threat to wheat production because 90 % of grown wheat is susceptible to this race (Singh *et al*., 2011). So far, this race contains 15 variants with twelve variants already detected in Kenya and are rapidly expanding across other countries in Africa, virulent to genes *Sr31* and *Sr24* (Pretorius *et al*., 2010). Another notable group is clade IV, the TKTTF and its variants. It is virulent to the resistance gene *SrTmp,* evidenced by the 2013 and 2014 localized epidemics in Ethiopia on cv. Digalu (Olivera *et al*., 2015). This variant was also behind stem rust outbreaks in Germany in 2014 and the United Kingdom in 2013 (Firpo *et al*., 2017; Meyer *et al*., 2017; Patpour *et al*., 2022). Clade III races typified by race TTRTF, which was 1st identified in Georgia in 2014 and responsible for the stem rust outbreak in Sicily in 2016 (Bhattacharyya, 2017). This race was reported in Egypt during the 2015 - 2016 wheat growing seasons and is now spreading in East Africa (Esmail & Szabo, 2018; Patpour *et al*., 2020; Tesfaye *et al*., 2019).

Globally, there have been various efforts made in breeding for resistance, with different genes possessing both seedling and adult plant resistance being incorporated into different wheat lines (Pretorious *et al*., 2000; Singh *et al*., 2011). In Kenya, the *Sr31* gene was deployed in cultivars 'Kenya Paa' and 'Duma' in 1982 and 1983, respectively (Fetch *et al*., 2021). Virulence to these genes was detected in Uganda in 1998 by the race TTKSK (Fetch *et al*., 2021). Since sounding the alarm in 2005, characterization of the virulence dynamics of stem rust population in Kenya has been ongoing and is being integrated into the Global Cereal Rust Monitoring System (Park *et al*., 2011). According to Jin *et al*. (2008), *Sr24* and *Sr36* virulence was detected in 2005 and 2006, respectively. This implied that only a few adapted germplasms with genes *Sr13a*, *Sr25*, and *SrTmp* maintained resistance to the prevailing races (Fetch *et al*., 2021). The gene *SrTmp* was unfortunately deployed singly in cultivar Kenya Robin, which led to virulence to races TTKTT and TTKTK in 2013 (Bhavani *et al*., 2019). The remaining gene *Sr25* has been effective against the *Ug99* race, but the 2019 isolates were virulent to the above gene (Bhavani *et al*., 2019). This indicates that *Ug99* is still mutating, as continued virulence is exhibited in previously resistant wheat cultivars. Due to the dispersible nature and continuous evolution of *Pgt*, monitoring of the prevailing races

is necessary to detect potential new virulence that may render important stem rust resistance genes ineffective (Singh *et al.,* 2015).

Njoro and Mau-Summit regions are key wheat-growing areas in Nakuru County, Kenya. They are key hotspot areas for *Ug99* races, giving an overview of the state of prevailing *Pgt* races in Kenya and neighboring countries. Therefore, the objective of this study was to identify and characterize the existing *Pgt* races in Njoro and Mau-Summit regions. This would ensure successful tracking of *Pgt* races and their distribution in Kenya to prevent disease epiphytotic that arise spontaneously, as witnessed in 2014 with the Digalu race TKTTF that caused localized epiphytotic in wheat growing areas in Ethiopia (Olivera *et al*., 2015).

5.2 Materials and methods

5.2.1 Sample collection, isolation and multiplication of single pustules

The field evaluation was done during 2022 main season in two major wheat growing areas KALRO Njoro (0° 20′ 47'' S, 35° 56′ 1.7'' E) and Mau-Summit (0° 40′ 60" S, 35° 57′ 0" E). The KALRO research centre, Njoro is at an elevation of 2120 metres above sea level (m. a.s.l.) in Njoro sub-county, Nakuru County, Kenya. It receives an annual rainfall of 939.3 mm with a minimum and maximum mean temperature of 9˚C and 24˚C, respectively. The research centre lies in the agro-ecological zone (AEZ) III and the soils are predominantly Mollic Andosols, well drained, dark reddish brown with humic top soils (BioVision, FAO-derived dataset, 2024; ISRIC, 2023) The area is suitable for growing wheat, barley and maize. Since the inception of wheat growing in Kenya, Njoro has been considered a hotspot for stem rusts. The Mau-Summit wheat field site is at an elevation of 2538 m. a.s.l in Molo sub-county, Nakuru County, Kenya. It receives an average annual rainfall of 998 mm with minimum and maximum temperatures of 8 °C and 21 °C, respectively. The site lies in AEZ of upper highland III and the soils are predominantly andosols (BioVision, FAO-derived dataset, 2024; ISRIC, 2023). These locations were selected because of their suitable environmental conditions for disease development and their high wheat production potential. The disease severity was determined according to Modified Cobb's scale (Figure 3.1). Using a pair of scissors, infected wheat stem sheath and leaf bearing moderately susceptible to susceptible pustules were cut at achieve pieces of 5 - 10 cm in length from infected wheat field in KALRO, Njoro area and Mau-Summit during the main wheat growing season (July - October, 2022) in Kenya then inserted onto labeled glassine bags, placed onto a cooler box and transported to the KALRO Njoro laboratory for race identification. Urediniospores were

collected into gelatin capsule (size 00) and stored at - 4 º C until further use. Infected wheat stem sheath and leaf bearing moderately susceptible to susceptible pustules were cut into pieces of between 5 - 10 cm in length using a pair of scissors from infected wheat field in KALRO, Njoro area and Mau-Summit during the main wheat growing season (July - October, 2022) in Kenya then inserted onto labeled glassine bags, placed onto a cooler box and transported to the KALRO laboratory for race identification. Urediniospores were collected into gelatin capsule (size 00) and stored at - 4 until further use. The collected samples were labeled with the date of collection, zone and variety.

5.2.2 Inoculation of collected samples

Susceptible wheat variety (Cacuke) seeds were planted in pots filled with vermiculite of 125 cm^3 (6 cm × 6 cm × 6 cm) mixed with 5 g of di-ammonium phosphate $(NH_4)_2(HPO_4)$ (18 %N: 46 % P_2O_5: 0 % K_2O) at a depth of 2 cm then watered to field capacity. The pots were placed on plastic trays in the growth chamber at room temperature (25 ºC). Watering was done depending on the media condition ensuring the seedlings had adequate soil moisture.

The seedlings were placed on a rotating table and inoculated separately with spores on fully expanded leaves at growth stage 1 (Feekes scale) with the spores collected from Njoro and Mau-Summit. The spores were suspended in distilled water and mixed with two drops of light mineral oil Soltrol® 100 Isoparaffin (Chevron Philips Chemical Company) then gently inversed. The inoculum was sieved and drained into a dispenser and adjusted to a concentration of 1×10^6 spores/ml (Jin *et al*., 2008). Using a calibrated hand sprayer, the inoculum was sprayed on the upper and lower leaf surfaces (Plate 5.2).

Inoculated seedlings were then air dried for a period of 1 hour until the Soltrol had evaporated. Thereafter, they were moistened with fine droplets of water using a hand sprayer. The seedlings were then transferred in a dark dew chamber for a period of 24 hours at 20 $^{\circ}$C followed. They were later transferred into a greenhouse bench at 25 $^{\circ}$C and relative humidity (RH) of about 60 %.

5.2.3 Single pustule isolation and bulking of spores

Fourteen days after development of disease, single pustule bearing distinct feature (size/shape) was collected onto a gelatinous capsule well labeled and sealed. After each collection, the vacuum pump and inoculator were wiped using cotton wool soaked in 70 % alcohol ($C_2H_2O_4$). Single pustules from each site were collected separately onto gelatinous capsule. The single pustules spores collected from each site were inoculated then incubated separately on another set of Cacuke to achieve bulk inoculum as described in 5.2.2. The bulk inoculum was then used in inoculating the differential set.

5.2.4 Inoculation of wheat stem rust differential lines

Five seeds of each of the fifty-one wheat stem rust differentials from CIMMYT and 20 differentials from United States Department of Agriculture Cereal Disease Laboratory with known resistance genes and susceptible variety were grown in pots (measuring 6 cm × 6 cm × 6 cm) in two sets as described in 5.2.2. They were inoculated and incubated separately at growth stage 1 (Feekes scale) and the experiment was repeated to reduce biasness in the results The spores were suspended in distilled water and mixed with two drops of light mineral oil Soltrol® 100 Isoparaffin (Chevron Philips Chemical Company) then gently inversed and inoculated onto a 7-day old seedling of the differentials. The inoculated seedlings were moistened with distilled water and placed in an incubation chamber at 18 – 22 °C and about 60 % RH. Inoculated seedlings were placed in separate glass compartments and greenhouse temperature adjusted to 25 °C (Jin *et al*, 2008)**.**

5.3 Determination of stem rust races

The infection types were determined 14 days' post-inoculation (DPI), based on a 0 - 4 scale described by Stakman *et al*. (1962). The infection type (IT) 0, ;, 1-, 1, 1+, 2-, 2 and 2+ were rated low implying the tested isolate was avirulent (Avr) to the resistant host while 3 and 4 were rated high implying the pathogen was virulent. The infection types were scored as 0 = immune, 0; = very resistant, 1 = resistant, 2 = moderately resistant, 3 = moderately susceptible and 4 = susceptible. The symbols ("+" and "-") were used to describe the sizes of the pustules whereby; "+" represented larger pustules and "-" represented smaller pustules for infection types 1, 2 and 3 as described by Roelfs & Martens, (1988) and Stakman *et al*. (1962) (Fig. 4.1). Those with IT<3 were considered as resistant, those with IT≥3 were

considered susceptible. A forward slash indicated occurrence of different symptoms on the same stem or leaf while a comma (,) indicated more than one IT.

The race identification and designation were conducted using the North American Nomenclature system for *Puccinia graminis f. sp tritici* (Roelfs & Martens, 1988). The differentials were grouped into five subsets and races identified based on their infection types on differential host as illustrated by race TTKTT (Table 1) Seedling infection type scale was done according to Stakman *et al*. (1962) (Table 4.1). Each isolate was assigned five letter of designation code (Jin *et al*., 2008).

Figure 5.1: Stem rust spores collected during soft dough stage with a severity of 70 MSS at KALRO Njoro (A) and (B) Stem rust differential planting

5.4 Results

The isolates collected from Ks Kanga in Mau-Summit and Cacuke in Njoro exhibited virulence on 95 % stem rust resistance genes of the differential lines and were identified race TTKTT as illustrated on Table 5.2.

Table 5.2: The North American differential sets of wheat stem rust resistance genes

Gene sets	**Sten rust resistant gene**			
Set 1	*Sr5*	*Sr21*	*Sr9e*	*Sr7b*
Set 2	*Sr11*	*Sr6*	*Sr8a*	*Sr9g*
Set 3	*Sr36*	*Sr9b*	*Sr30*	*Sr17*
Set 3	*Sr9a*	*Sr9d*	*Sr10*	*SrTmp*
Set 5	*Sr24*	*Sr31*	*Sr38*	*SrMcN*
Pgt Code	**Infection type as observed on differential set**			
B	L	L	L	L
C	L	L	L	H
D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

L – Low / Resistant infection type, H = High / Susceptible infection type Source (Jin *et al*., 2008)

T- No genes in set 1 are effective

T- No genes in set 2 are effective

K - Only gene *Sr36* is effective in set 3

T- No genes in set 4 are effective

T-No genes in set 5 are effective

Plate 5.2: A week seedling on a rotating table being inoculated with purified spores and stem rust scoring 14 days post inoculation (B)

5.5 Discussions

The isolates collected from Ks Kanga in Mau-Summit and Cacuke in Njoro exhibited had race TTKTT. It is a *Ug99* variant with additional virulence to genes *Sr24* and *SrTmp* (Singh *et al*., 2015). The *SrTmp* is an important gene because it was sourced from *T. aestivum* and does not possess the negative linkage drag and can be incorporated readily onto wheat lines (McVey & Hamilton, 1985). The *Pgt* race TTKTT was first detected in Kenya in 2014, Ethiopia in 2018, and Iraq in 2019 (Hei *et al*., 2020; Nazari *et al*., 2021; Patpour *et al*., 2016).

It has acquired virulence to the stem rust gene *SrTmp* found in the Kenyan cultivar (Cv) Robin released in 2011 and the Ethiopian Cv. Digalu (Bhavani *et al*., 2019). Consequently, Gutu *et al*. (2022), identified race TTKTT in samples that were collected during the 2019/2020 cropping season in Ethiopia and exhibited virulence to all 19 genes in the 20-differential set for stem rust genes except *Sr36* (Table 3). This race has the most unique virulence combination compared to other races in TTKSK race group (Gutu *et al*., 2022).

Table 5.3: Virulence characterization of race TTKTT

Ineffective/virulence stem rust genes	Effective/avirulence genes
Sr5, Sr6, Sr8a, Sr9a, Sr9b, Sr9e, Sr9g, Sr9d, Sr10, Sr11, Sr23, Sr24, Sr25, Sr28, Sr29, Sr30, Sr31, Sr37, SrTmp	*Sr36* *Other genes* *Sr1, Sr13, Sr21, Sr22, Sr26, Sr27, Sr35, SrEntrelargo,* and *SrRAGI*

The TTKTT race is distinctly concerning owing to its virulence on genes *Sr24, Sr31,* and *SrTmp* the most commonly used resistance genes in East African wheat cultivars (Hei *et al*., 2021; Wanyera & Wamalwa, 2022). The continued presence of this race since its identification in themed 2010s suggests a well-adapted pathogen population capable of thriving under local environmental conditions. Hei *et al,* (2020) also found that race TTKTT was virulent on 95 % genes, leading to a similar conclusion where only gene *Sr36* exhibited avirulence on the differential set. Gordon et al. (2021) also collected spores from Njoro field between 2014 and 2017 belonging to TTKTT race group only avirulent to stem rust-resistant gene *Sr36* indicating these results are in line with previous studies.

Although only one region was used in this study, other races exist in major wheat-growing areas of Kenya, including Ug99 and non-Ug99 lineages. Races can vary significantly across different geographic areas due to variations in weather patterns, altitude, and environmental conditions. Extensive research is critical to understand the basis of wheat stem rust resistance in Kenyan breeding lines. Current varieties are susceptible, necessitating efforts to incorporate new resistance genes that confer adult plant resistance (APR). APR genes such as *Sr2, Sr55,* and *Sr58* provide additive effects against pathogens and are preferable since they do not exhibit boom-bust cycles like race-specific genes (Bhavani *et al*., 2019).

Table 5.4: Infection types of 20 USDA-CDL stem rust differentials from Njoro and Mau-Summit sites in Nakuru County, Kenya

		Njoro site		Mau-Summit site	
Entry	Gene	Set 1	Set 2	Set 1	Set 2
1	*Sr5*	3-	3-	3	3+
2	*Sr6*	3	3	3	3
3	*Sr7B*	3	3+	4	3
4	*Sr8A*	3	3	3-	3+
5	*Sr9A*	3	3	3	3
6	*Sr9B*	3+	3+	3+	4
7	*Sr9E*	3	3	4	3+
8	*Sr9G*	3, 1	3	3	3+
9	*Sr9D*	3	3	3	3+
10	*Sr10*	4	3	3	3
11	*Sr11*	3	3	3	3+
12	*Sr17,SR13*	3+	3+	3	3+
13	*Sr21*	; 3-	; 1	2	3
14	*Sr24*	3+	3	4	3
15	*Sr30*	3	3	4	4
16	*Sr31*	3	3	3+	3
17	*Sr36*	0	0	0	0
18	*SrTMP*	3	3	3	3
19	*Sr38*	3	3	3	3
20	*SrMCN*	3,1	3	4	3
	Cacuke	3+	4	4	3

L- Low/Resistant infection type 0, ; 1, 2 and combination of these values, H - High/Susceptible infection 3 and 4 and combination of these values

Table 5.5: Table showing infection types of 51 CIMMYT stem rust differentials for Njoro and Mau-Summit areas

Entry	Stem rust (Sr) Gene	Njoro Set 1	Set 2	Mau-Summit Set 1	Set 2	Entry	Stem rust (Sr) Gene	Njor Se
1	*Sr5*	3+	0	3	3	26	*Sr24*	2+
2	*Sr6*	3	3	3	3	27	*Sr25*	3
3	*Sr7A, Sr10*	4	3	4	3+	28	*Sr26*	; 1
4	*Sr7B*	3	3+	4	3	29	*Sr27*	0
5	*Sr8A*	1	2	3+	3	30	*Sr28*	0
6	*Sr8B*	3	3	4	3	31	*Sr29*	2
7	*Sr9A*	3	3	3,2	3-	32	*Sr30*	3
8	*Sr9B*	0	0	;1	;1	33	*Sr31*	3
9	*Sr9D*	3	;3	3	3-	34	*Sr32*	0,
10	*Sr9E*	3+	3	3+	3	35	*Sr33, Sr5*	0
11	*Sr5, Sr9F*	3	3	3	3-	36	*Sr34*	; 1
12	*Sr9G*	3	3	3	2/3	37	*Sr35*	0
13	*Sr10*	3	2	3,2	3-	38	*Sr36*	; 1
14	*Sr11*	3+	3+	3	3+	39	*Sr37*	3
15	*Sr12*	3	3	3	4	40	*Sr10*	3
16	*Sr13*	; 1-	;	;1+	;1	41	*Ap9d*	3
17	*Sr14*	3	3+	3	3	42	*SrGt*	3,
18	*Sr15*	3	3	4	4	43	*SRPL*	3-
19	*Sr16*	3	0	3	3+	44	*SR WLD*	
20	*Sr17*	3+	3+	3	3+	45	*SRH*	3
21	*Sr19*	2	; 1	2	2-	46	*ENTRELARGO*	0,
22	*Sr20*	3+	3	3+	3	47	*SRAGI*	0
23	*Sr21*	; 1	3	;1	;2-	48	*SR7B,SR18,SR19,SR20*	
24	*Sr22*	; 1-	; 1-	;2	;2-	49	*Sr18*	3-
25	*Sr23*	3+	3+	3+	3+	50	LOCAL	3
						51	*SR 1*	; 1

On the 51-differential set, race TTKTT was avirulent on *Sr1*, *Sr13*, *Sr21*, *Sr22*, *Sr26*, *Sr27*, *Sr35*, *SrEntrelargo*, and *SrRAGI*, highlighting their importance in stem rust resistance breeding (Table 5.3). According to Smith *et al*. (2020), resistance genes *Sr22* and *Sr35* have proved effective against race TTKTT due to their broad-spectrum resistance and durability. In a study by Alemu *et al*. (2023), they identified a *Sr22* carrying variety Oda to be resistant against race TTKTT at the seedling and adult plant stages. Similarly, transgenic work by Hatta *et al*. (2021) has shown that *Sr22* and *Sr35* remain effective when transferred into barley. Resistance genes *Sr26* and *Sr27*, derived from wild wheat relatives, have also proven effective against race TTKTT (Kankwatsa *et al*., 2019). Klindwort *et al*. (2025) also observed Sr13c allele continues to confer moderate to high resistance in durum wheat against this race. This aligns with my findings on the avirulence of these genes against TTKTT. Moreover, a recent review by About-Zeid *et al*. (2023) shows *Sr22*, *Sr26*, and *Sr50* as the most effective seedling resistance genes used globally.

Promoting genetic diversity in breeding germplasm for stem rust resistance, along with characterizing novel genes, can significantly reduce susceptibility in cultivars and provide alternatives for rapid replacement. Marker-assisted breeding helps retain genetic diversity within the breeding germplasm, enabling breeders to make informed choices to maintain a broader genetic base.

5.6 Conclusion

The consistent detection of race TTKTT in Nakuru County, Kenya confirms its status as most virulent race among *Ug99* group that need vigilant monitoring and proactive measures to ensure sustainable wheat production and food security. Ultimately, continued research, international collaboration, and strategic breeding efforts to develop wheat cultivars with durable resistance against evolving races is necessary.

CHAPTER SIX

GENERAL DISCUSSION CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Stem rust disease in wheat can cause a devastating catastrophe under favorable weather conditions coupled with the presence of susceptible cultivars (Wanyera & Wamalwa, 2022). It reduces the quality of the kernel and yield loss can reach up to 100 % in cultivars (Soko *et al*., 2018). This calls for continuous breeding and release of resistant varieties. However, it takes between 4-6 years for a resistant variety to become susceptible owing to the changing virulence pattern of stem rust and occurrence of boom-bust cycles (Pauw & Buchannon, 1975: Beddow *et al*., 2015). The *Ug99* races possess unique virulence combination and are subject to change and render more stem rust genes ineffective in key hotspot areas. This necessitates a continuous global surveillance and monitoring to keep up with the emerging stem rust races (Bhavani *et al*., 2019).

In field screening, some genotypes expressed a reaction of resistant to moderately resistant under natural stem rust infection. This suggests that these genotypes possessed adult plant resistant genes that were effective against the prevailing stem rust races, crucial for a long-term disease management. Genotypes 6022, 6095, 6096, 6107, 6134, 6136, 6137, 6138 and 6139 showed stable APR across the seasons, demonstrating their potential utility in wheat improvement. Under greenhouse conditions, a significant number of the lines showed low infection indicating presence of race-specific resistance. However, some genotypes that performed well in the field were susceptible at seedling suggesting that they only possessed APR genes not normally expressed during seedling stage. Race analysis confirmed the presence of TTKTT in both Njoro and Mau-Summit. This race is known for its virulence on genes Sr31 ans SrTmp. Its continued detection in Nakuru County highlights its dominance and the need to deploy multi-race resistance in breeding strategies.

Since sounding the alarm in 2005 following the emergence *Ug99* races, The International Centre for Maize and Wheat Improvement (CIMMYT) through the Borlaug Global Rust Initiative has been carrying out screening of wheat germplasm in different environments. In Kenya, rust phenotyping platform is stationed at Kenya Agricultural and Livestock Research Organization (KALRO) Njoro. This has facilitated screening and identification of new resistance genes, shuttle breeding between Kenya and Mexico, stem rust surveillance and release of new cultivars in Kenya (Bhavani *et al*., 2019).

Thirteen *Ug99* variants virulent to gene *Sr24*, *Sr31*, *Sr8155B1* and *SrTmp* present in most Kenyan cultivar underscores the importance of a broader genetic base (Newcomb *et al*., 2016; Pretorious *et al*., 2012; Singh *et al*., 2015). This can be accomplished through introgression of new genes from either bread wheat or related species (Karelov *et al*., 2021). The 4 - 5 APR genes have proven effective in the control of stem rust especially the lines with *Sr2* and *Lr34* genes (Singh *et al*., 2011). They provide partial but durable resistance (race non-specific) against stem rust (McIntosh *et al*., 1995). Effective gene stewardship should be observed in release of cultivars with these genes to avoid selection pressure on the genes (Fetch *et al*., 2021). These can be fullfilled through a combination of both race specific and race non-specific resistance genes into one wheat background.

Expression of stem rust is largely dependent on the interaction of wheat line and the environment. Best performing genotypes in terms of yield and other agronomic parameters must be selected under high disease pressure so as to identify the yield potential and adaptability of a particular genotype. Genetic diversity in wheat lines is a crucial factor when selecting for yield related traits. These traits are governed by multiple genes and largely affected by genotype and the environment. At seedling stage, most lines were avirulent to TTKTT race with some susceptible at adult plant stage. The avirulence observed in most CIMMYT wheat lines at seedling and adult plant stage is evidence of major and minor genes respectively. The observed morphological marker pseudo black chaffs on awns, spikes and stems in a number of lines suggest that *Sr2* gene was present conferring high stem rust resistance. Furthermore, there are unidentified *Sr* genes that are conferring resistance in the lines. Different disease severities observed among the lines implied presence of gene diversity among the 16th SRRSN wheat lines (Newcomb *et al*., 2013).

To achieve high yielding and resistant wheat lines, a combination of phenotypic selection, genomic selection, marker assisted breeding and genome wide association study must be exploited to ensure effective gene stewardship (Mago *et al*., 2011).

6.2 Conclusions

i. Thirty-six genotypes possessed APR with disease severity ranging from 1 R to 20 M. Genotypes 6022, 6095, 6096, 6107, 6134, 6136, 6137, 6138 and 6139 have been identified to possess APR against stem rust and are high yielding.

ii. Ninety-eight genotypes are resistant against stem rust race TTKTT at seedling stage with infection type ranging from ';' to '2+', with genotypes possessing genes *Sr22*, *Sr26*, *Sr36*, and *Sr47* having the most effective seedling plant resistance.

iii. Stem rust race TTKTT was predominant in both the regions with virulence to resistance genes *Sr24*, *Sr31*, and *SrTmp*.

6.3 Recommendations

i. Genotypes 6022, 6095, 6096, 6107, 6134, 6136, 6137, 6138 and 6139 can be used in wheat breeding programmes as they are resistant against stem rust and are high yielding. Multi-environmental trials are necessary to confirm their yield stability

ii. Incorporate effective seedling resistance genes like *Sr1*, *Sr13*, *Sr21*, *Sr22*, *Sr26*, *Sr27*, *Sr36*, *Sr35*, *Sr47*, *SrEntrelargo*, and *SrRAGI* in breeding programmes targeting early-stage resistance as they are resistant against stem rust race TTKTT. Further studies should evaluate the durability and stability of APR and SPR genes identified in this study across different agro-ecological zones in Kenya using multi-location and multi-seasonal trials

iii. Continuous surveillance of stem rust races in major wheat-growing areas is essential to detect emerging virulent races, such as *Ug99* derivatives.

6.5 Suggestions for further research

This study focused majorly on identifying stem rust races in Nakuru County coupled with identification of resistance lines. However, a multi-environmental trial is crucial for identifying the lines stability across various environments. Molecular studies are also crucial in identifying genes that conditioned their resistance thus strengthening the understanding of host-pathogen interactions. Continuous surveillance of pathogen distribution is also crucial in maintain durability of the resistance genes. Additionally, farmer engagement through participatory field trials is essential to validate yield performance and enhance adoption of the resistant lines under real production environment.

REFERENCES

Abbas, G., Ali, M. A., Azam, M. & Hussain, I. (2009). Impact of planting methods on wheat grain yield and yield contributing parameters. *Journal of Plant Science.* 19(1), 30-33.

Abou-Zeid, M. A., Mabrouk, O. I., Draz, I. S., Saad-El-Din, H. I., Safhi, F. A., ALshamrani, S. M., & Esmail, S. M. (2023). Stem rust appraisal of local and global wheat

germplasm with a new virulence threat to resistance genes. *Australasian Plant Pathology*, *52*(1), 67-87.

Afzal, F., Chaudhari, S. K., Gul, A., Farooq, A., Ali, H., Nisar, S., & Mujeeb-Kazi, A. (2015). Bread wheat (Triticum aestivum L.) under biotic and abiotic stresses: An overview. *Crop Production and Global Environmental Issues*, 293-317.

Aime, M. C., McTaggart, A. R., Mondo, S. J., & Duplessis, S. (2017). Phylogenetics and phylogenomics of rust fungi. *Advances in Genetics*, *100*, 267-307.

Alemu, S. K., Hora, O. D., Terfasa, F. K., Teshale, M., Hailu, B., Bayissa, K. N., … Degete, A. G. (2023). Identification of stem rust resistance genes in released wheat varieties by linked SSR markers and phenotypic screening. *International Journal of Genetics and Genomics*, *11*(2), 48–59.

Ali, S., Hodson, D. P., Rahman, H., Shah, S. J. A., Imtiaz, M., & Singh, R. P. (2020). Visual assessment and scoring of wheat rusts. *Rust Management Guidebook for South Asia*, 2nd edition, 15–22. International Maize and Wheat Improvement Center (CIMMYT)

Beddow, J. M., Sutherst, R. W., Kriticos, D. J., Duveiller, E., & Chai, Y. (2015). *Puccinia graminis (Wheat Stem Rust)*. In R. M. K. Maier (Ed.), *CABI Compendium* (Version 2023). CAB International.

Berger, R. D. (1977). Application of epidemiological principles to achieve plant disease control. *Annual Review of Phytopathology*, *15*(1), 165-181.

Berlin, A., Samils, B., Djurle, A., Wirsén, H., Szabo, L., & Yuen, J. (2013). Disease development and genotypic diversity of Puccinia graminis f. sp. avenae in Swedish oat fields. *Plant Pathology*, *62*(1), 32-40.

Bhardwaj, S. C., Gangwar, O. P., Prasad, P., Kumar, S., Khan, H., & Gupta, N. (2019). Physiologic specialization and shift in Puccinia triticina pathotypes on wheat in Indian subcontinent during 2013–2016. *Indian Phytopathology*, *72*(1), 23-34.

Bhattacharya, S. (2017). Deadly new wheat disease threatens Europe's crops. *Nature*, *542*(7640).

Bhavani, S., Hodson, D. P., Huerta-Espino, J., Randhawa, M. S., & Singh, R. P. (2019). Progress in breeding for resistance to Ug99 and other races of the stem rust fungus in CIMMYT wheat germplasm. *Frontiers in Plant Science*, *10*, 1397

Bhavani, S., Singh, R. P., Hodson, D. P., Huerta-Espino, J., & Randhawa, M. S. (2022). Wheat rusts: current status, prospects of genetic control and integrated approaches to

enhance resistance durability. In *Wheat Improvement: Food Security in a Changing Climate* (pp. 125-141).

BioVision/Food and Agriculture Organization derived dataset. (2024). *Andosols characteristics in steep volcanic highlands*. In BioVision Agro-ecological Soil Classification (Vol. update).

Bolton, M. D., Kolmer, J. A., & Garvin, D. F. (2008). Wheat leaf rust caused by Puccinia triticina. *Molecular Plant Pathology*, *9*(5), 563-575.

Bonman, J. M., Bockelman, H. E., Jin, Y., Hijmans, R. J., & Gironella, A. I. N. (2007). Geographic distribution of stem rust resistance in wheat landraces. *Crop Science*, *47*(5), 1955-1963.

Boyd, L. A., Ridout, C., O'Sullivan, D. M., Leach, J. E., & Leung, H. (2013). Plant–pathogen interactions: disease resistance in modern agriculture. *Trends in Genetics*, *29*(4), 233-240.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. John Wiley & Sons.

Carew, R., Meng, T., Florkowski, W. J., Smith, R., & Blair, D. (2017). Climate change impacts on hard red spring wheat yield and production risk: evidence from Manitoba, Canada. *Canadian Journal of Plant Science*, *98*(3), 782-795.

Chen, W., Wellings, C., Chen, X., Kang, Z., & Liu, T. (2014). Wheat stripe (yellow) rust caused by Puccinia striiformis f. sp. tritici. *Molecular Plant Pathology*, *15*(5), 433-446.

Chen, X. M., Jin, Y., & Singh, R. P. (2021). Recent advances in stem rust surveillance and control strategies in wheat. *Annual Review of Phytopathology*, 59, 201–219.

Chen, X., Wang, M., Wan, A., Bai, Q., Li, M., López, P. F., ... & Abdelrhim, A. S. (2021). Virulence characterization of Puccinia striiformis f. sp. tritici collections from six countries in 2013 to 2020. *Canadian Journal of Plant Pathology*, *43*(sup2), S308-S322.

Cummins, G. B. (1971). *The Rust Fungi of Cereals, Grasses and Bamboos.* Springer Science & Bussiness Media.

Cummins, G. B., & Hiratsuka, Y. (2003). *Illustrated genera of rust fungi* (No. Ed. 3). American Phytopathological Society (APS Press).

Duplessis, S., Cuomo, C. A., Lin, Y. C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., & Martin, F. (2011). Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proceedings of the National Academy of Sciences*, *108*(22), 9166-9171.

Duplessis, S., Joly, D. L., & Dodds, P. N. (2011). Rust effectors. *Effectors in Plant–Microbe Interactions*, 155-193.

Dyck, P. L. (1987). The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. *Genome*, *29*(3), 467-469.

Dyck, P. L., & Kerber, E. R. (1985). Resistance of the race-specific type. In *Diseases, distribution, epidemiology, and control* (pp. 469-500). Academic Press.

Einstein, J. P., Kumar, R., & Ndungu, M. (2022). Global trends in wheat production and food security implications. *International Journal of Agricultural Science*, 14(2), 115–123..

Ellis, J. G., Lagudah, E. S., Spielmeyer, W., & Dodds, P. N. (2014). The past, present and future of breeding rust resistant wheat. *Frontiers in Plant Science*, *5*, 641.

El-Sharkawy, H. H., Rashad, Y. M., & Ibrahim, S. A. (2018). Biocontrol of stem rust disease of wheat using arbuscular mycorrhizal fungi and Trichoderma spp. *Physiological and Molecular Plant Pathology*, *103*, 84-91.

Evers, J. B., Vos, J., Fournier, C., Andrieu, B., Chelle, M., & Struik, P. C. (2005). Towards a generic architectural model of tillering in Gramineae, as exemplified by spring wheat (Triticum aestivum). *New Phytologist*, *166*(3), 801-812.

Fahad, S., Hussain, S., Bano, A., Saud, S., Hassan, S., Shan, D., & Huang, J. (2015). Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Science and Pollution Research*, *22*(7), 4907-4921.

Fang, G., & Shen, K. (2018). Wheat straw pulping for paper and paperboard production. In *Global Wheat Production* (pp. 223-239). London, UK: IntechOpen.

FAO, F. (2018). Food and agriculture organization of the United Nations. *Rome, URL: http://faostat. fao. org*.

Faostat, F. A. O. (2019). Food and Agriculture Organization of the United Nations-Statistic Division https://www. fao. org/faost at/en/# data.

Fetch, T. G., Park, R. F., Pretorius, Z. A., & Depauw, R. M. (2021). Stem rust: its history in Kenya and research to combat a global wheat threat. *Canadian Journal of Plant Pathology*, *43*(sup2), S275-S297.

Fetch, T., Mitchell Fetch, J., Zegeye, T., & Xue, A. (2021). Races of Puccinia graminis on barley, oat, and wheat in Canada in 2013 and 2014. *Canadian Journal of Plant Pathology*, *43*(1), 101-107.

Fetch, T., Zegeye, T., Park, R. F., Hodson, D., & Wanyera, R. (2016). Detection of wheat stem rust races TTHSK and PTKTK in the Ug99 race group in Kenya in 2014. *Plant Disease*, *100*(7), 1495-1495.

Figueroa, M., Hammond-Kosack, K. E., & Solomon, P. S. (2018). A review of wheat diseases—a field perspective. *Molecular Plant Pathology*, *19*(6), 1523-1536.

Flint, M. L., & Dreistadt, S. H. (1998). *Natural enemies handbook: the illustrated guide to biological pest control* (Vol. 3386). Univ of California Press.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, *9*(1), 275-296.

Gallo, M. A., & Lawryk, N. J. (1991). Organic phosphorus pesticides. *Handbook of pesticide toxicology*, *2*, 917-1123.

García-Guzmán, G., & Dirzo, R. (2004). Incidence of leaf pathogens in the canopy of a Mexican tropical wet forest. *Plant Ecology*, *172*(1), 41-50.

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., & Citterio, S. (2018). Effect of soil pH on the growth, reproductive investment and pollen allergenicity of Ambrosia artemisiifolia L. *Frontiers in Plant Science*, *9*, 1335.

Gitachew, T., Tesfaye, K., & Fikre, A. (2019). *Wheat rusts in Ethiopia: Current status, challenges and future prospects*. Ethiopian Institute of Agricultural Research.

Gitonga, K. (2019) *Kenya Grain and Feed Annual: Kenya Imports of Corn, Wheat, and Rice Expected to Surge.* United States Department of Agriculture, Foreign Agricultural Service.

Goldhamer, D., Michailides, T., & Morgan, D. (2002). Buried drip irrigation reduces fungal disease in pistachio orchards. *California Agriculture*, *56*(4), 133-138.

Gordon, T., Jin, Y., Gale, S., Rouse, M., Stoxen, S., Wanyera, R., ... & Bonman, J. M. (2021). Identification of winter habit bread wheat landraces in the national small grains collection with resistance to emerging stem rust pathogen variants. *Plant Disease*, *105*(12), 3998-4005.

Grote, U., Fasse, A., Nguyen, T. T., & Erenstein, O. (2021). Food security and the dynamics of wheat and maize value Chains in Africa and Asia. *Frontiers in Sustainable Food Systems*, *4*, 317.

Gupta, A. K., Sauder, D. N., & Shear, N. H. (1994). Antifungal agents: an overview. Part I. *Journal of the American Academy of Dermatology*, *30*(5), 677-698.

Gutu, K., Tesfaye, T., Bacha, N., Negash, T., Kassa, D., Yirga, F., ... & Alemu, Z. (2022). Physiological Races of Puccinia graminis f. sp. tritici in Ethiopia in 2019/2020. *American Journal of Agriculture and Forestry*, *10*(2), 72-76.

Hare, R. A., & RA, M. (1979). Genetic and cytogenetic studies of durable adult-plant resistances in" Hope" and related cultivars to wheat rusts. *Euphytica*, *28*(3), 651-662.

Hei, N. B., Tsegaab, T., Getaneh, W., Girma, T., Obsa, C., Seyoum, A., ... & Yoseph, A. (2020). First report of Puccinia graminis f. sp. tritici race TTKTT in Ethiopia. *Plant Disease*, *104*(3), 982.

Hossain, A., da Silva, J. A. T., Lozovskaya, M. V., & Zvolinsky, V. P. (2012). The effect of high temperature stress on the phenology, growth and yield of five wheat (Triticum aestivum L.) varieties. *Asian and Australasian Journal of Plant Science and Biotechnology*, *6*(1), 14-23.

Hossain, M. A., Piyatida, P., da Silva, J. A. T., & Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany*, *2012*(1), 872875.

Hovmøller, M. S., Sørensen, C. K., Walter, S., & Justesen, A. F. (2011). Diversity of Puccinia striiformis on cereals and grasses. *Annual Review of Phytopathology*, *49*, 197-217.

Hovmøller, M. S., Yahyaoui, A. H., Milus, E. A., & Justesen, A. F. (2008). Rapid global spread of two aggressive strains of a wheat rust fungus. *Molecular Ecology*, *17*(17), 3818-3826.

Huerta-Espino, J., Singh, R., Crespo-Herrera, L. A., Villaseñor-Mir, H. E., Rodriguez-Garcia, M. F., Dreisigacker, S., ... & Lagudah, E. (2020). Adult plant slow rusting genes confer high levels of resistance to rusts in bread wheat cultivars from Mexico. *Frontiers in Plant Science*, *11*, 824.

ISRIC–World Soil Information. (2023). *Reference soil Kenya 36: Andosols* [Soil profile description]. ISRIC World Soil Museum.

Jennings, D. M., Ford-Lloyd, B. V., & Butler, G. M. (1989). An aniline blue squash technique for observation of urediniospore germ pores. *Mycological research*, *92*(2), 230-232.

Ji, Q., Xu., & Wang, K. (2013). Genetic transformation of major cereal crops. *The international journal of developmental biology*, 57(6-8), 495-508.

Jin, Y., Szabo, L. J., & Carson, M. (2010). Century-old mystery of Puccinia striiformis life history solved with the identification of Berberis as an alternate host. *Phytopathology*, *100*(5), 432-435.

Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R., & Fetch Jr, T. (2008). Detection of virulence to resistance gene Sr24 within race TTKS of Puccinia graminis f. sp. tritici. *Plant Disease*, *92*(6), 923-926..

Jin, Y., Szabo, L. J., Rouse, M. N., Fetch Jr, T., Pretorius, Z. A., Wanyera, R., & Njau, P. (2009). Detection of virulence to resistance gene Sr36 within the TTKS race lineage of Puccinia graminis f. sp. tritici. *Plant Disease*, *93*(4), 367-370.

Johnson, R., & Knott, D. R. (1992). Specificity in gene-for-gene interactions between plants and pathogens. *Plant pathology*, *41*(1), 1-4.

Johnson, R., & Taylor, A. J. (1972). Isolates of Puccinia striiformis collected in England from the wheat varieties Maris Beacon and Joss Cambier. *Nature*, *238*(5359), 105-106.

Kamwaga, J., Macharia, G., Boyd, L., Chiurugwi, T., Midgley, I., Canales, C., ... & Maina, I. (2016). *Kenya Wheat Production Handbook* (p. 78). Kenya Agricultural and Livestock Research Organization.

Kankwatsa, P., Singh, R. P., Hodson, D., Wanyera, R., & Njau, P. (2019). Status of wheat stem rust race Ug99 and TTKTT in eastern Africa and implications for resistance breeding. *Plant Disease*, 103(10), 2589–2597

Kenya National Bureau of Statistics. (2025). *Economic Survey 2025*. Government of Kenya. https://www.knbs.or.ke

Khan, M., Hussain, M., & Sajjid, M. (2006). A two environmental variable model to predict wheat leaf rust based on 10 years data. *Pakistan Journal of Phytopathology*, *8*, 114-116.

Kinyanjui, J. M., Mburu, D. M., & Kigomo, M. K. (2020). Trend and Variability in Interannual Air Temperature Over South West Mau Forest, 1985-2015. *Journal of Natural Sciences Research, 11(16),* 1-10

Kirby, E. J. M. (2002). Botany of the wheat plant. In B. C. Curtis (Ed), *Bread Wheat. Improvement and Production* (pp. 19-37.). Food and Agriculture Organization of the United Nation.

Klindworth, D. L., Rouse, M. N., Olivera, P. D., Jin, Y., Chu, C., Friesen, T. L., ... & Xu, S. S. (2025). Registration of four durum wheat lines carrying Sr13 alleles for resistance to stem rust. *Journal of Plant Registrations*, *19*(1), e20399.

Knott, D. R. (1982). Multigenic Inheritance of Stem Rust Resistance in Wheat 1. *Crop Science*, *22*(2), 393-399.

Knott, D. R. (2012). *The wheat rusts—breeding for resistance* (Vol. 12). Springer Science & Business Media.

Knott, D. R., & Padidam, M. (1988). Inheritance of resistance to stem rust in six wheat lines having adult plant resistance. *Genome*, *30*(3), 283-288.

Kumar, P., Yadava, R. K., Gollen, B., Kumar, S., Verma, R. K., & Yadav, S. (2011). Nutritional contents and medicinal properties of wheat: a review. *Life Sciences and Medicine Research*, *22*(1), 1-10.

Laido, G., Panio, G., Marone, D., Russo, M. A., Ficco, D. B., Giovanniello, V., ... & Mastrangelo, A. M. (2015). Identification of new resistance loci to African stem rust race TTKSK in tetraploid wheats based on linkage and genome-wide association mapping. *Frontiers in Plant Science*, *6*, 1033.

Langyintuo, A. S. (2020). *Wheat production in East Africa: Emerging threats and resilient farming strategies*. *African Journal of Agricultural and Resource Economics*, 15(2), 122–138.

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

Leonova, I. N., Skolotneva, E. S., Orlova, E. A., Orlovskaya, O. A., & Salina, E. A. (2020). Detection of genomic regions associated with resistance to stem rust in Russian spring wheat varieties and breeding germplasm. *International Journal of Molecular Sciences*, *21*(13), 4706.

Lewis, C. M., Persoons, A., Bebber, D. P., Kigathi, R. N., Maintz, J., Findlay, K., & Saunders, D. G. (2018). Potential for re-emergence of wheat stem rust in the United Kingdom. *Communications Biology*, *1*(1), 1-9.

Macharia, G., & Ngina, B. (2017). Wheat in Kenya: Past and twenty-first century breeding. *Wheat Improvement, Manaement and Utilizat*ion (pp. 3-5) Kenya Agricutural and Livestock Research Organization.

Mapuranga, J., Zhang, N., Zhang, L., Liu, W., Chang, J., & Yang, W. (2022). Harnessing genetic resistance to rusts in wheat and integrated rust management methods to develop more durable resistant cultivars. *Frontiers in Plant Science*, *13*, 951095.

McIntosh, R. A. (1988). The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. *Breeding strategies for resistance to the rusts of wheat.* (pp. 1-9) CIMMYT.

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C., Appels, R., & Xia, X. C. (2016). Laboratory Designators. *Annual Wheat Newsletter*, 102.

McIntosh, R. A., Wellings, C. R., & Park, R. F. (1995). *Wheat rusts: An atlas of resistance genes*. CSIRO publishing.

McVey, D. V., & Hamilton, K. (1985). Stem rust resistance gene from Triumph 64 identified in four other winter wheats. *Plant Disease*, *69*(3), 217-218.

Mehmood, S., Sajid, M., Zhao, J., Huang, L., & Kang, Z. (2020). Alternate Hosts of Puccinia striiformis f. sp. tritici and Their Role. *Pathogens*, *9*(6), 434.

Meyer, M., Burgin, L., Hort, M. C., Hodson, D. P., & Gilligan, C. A. (2017). Large-scale atmospheric dispersal simulations identify likely airborne incursion routes of wheat stem rust into Ethiopia. *Phytopathology*, *107*(10), 1175-1186.

Mojid, M. A., & Wyseure, G. (2011). Improving Water Regimes of Loamy Sand for Wheat Cultivation by Adding Silt Loam. *Environmental Control in Biology*, *50*(4), 347-362.

Moricca, S., & Ragazzi, A. (2008). Fungal endophytes in Mediterranean oak forests: a lesson from Discula quercina. *Phytopathology*, *98*(4), 380-386.

Mundt, C. C. (2018). Pyramiding for resistance durability: theory and practice. *Phytopathology*, *108*(7), 792-802.

Nagarajan, S., Kogel, K. H., & Zadoks, J. C. (2014). Epidemiological analysis of the damage potential of Pgt-Ug99 in Central East, North East Africa; Iran and Punjab (India). *Indian Phytopathology*, *67*(9), 26-32.

Nazari, K., Al-Maaroof, E. M., Kurtulus, E., Kavaz, H., Hodson, D., & Ozseven, I. (2021). First report of Ug99 race TTKTT of wheat stem rust (Puccinia graminis f. sp. tritici) in Iraq. *Plant Disease*, *105*(9), 2719.

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

Ngetich, K. F., Mucheru-Muna, M., Mugwe, J. N., Shisanya, C. A., Diels, J., & Mugendi, D. N. (2014). Length of growing season, rainfall temporal distribution, onset and cessation dates in the Kenyan highlands. *Agricultural and Forest Meteorology*, *188*, 24-32.

Noort, M. W. J., van der Kamp, J. W., & Luten, J. B. (2022). Wheat consumption and self-sufficiency in sub-Saharan Africa. *Food Security*, 14(3), 501–510.

Oerke, E. C. (2005). Crop losses to pests. *Journal of Agricultural Science*, 144(1), 31–43.

Oliveira, H. R., Hagenblad, J., Leino, M. W., Leigh, F. J., Lister, D. L., Penã-Chocarro, L., & Jones, M. K. (2014). Wheat in the Mediterranean revisited–tetraploid wheat landraces assessed with elite bread wheat Single Nucleotide Polymorphism markers. *BMC Genetics*, *15*(1), 1-13.

Olivera Firpo, P. D., Newcomb, M., Flath, K., Sommerfeldt-Impe, N., Szabo, L. J., Carter, M., ... & Jin, Y. (2017). Characterization of Puccinia graminis f. sp. tritici isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. *Plant Pathology*, *66*(8), 1258-1266.

Olivera, P., Newcomb, M., Szabo, L. J., Rouse, M., Johnson, J., Gale, S., ... & Jin, Y. (2015). Phenotypic and genotypic characterization of race TKTTF of Puccinia graminis f. sp. tritici that caused a wheat stem rust epidemic in southern Ethiopia in 2013–14. *Phytopathology*, *105*(7), 917-928.

Organisation météorologique mondiale. Working Group on Meteorological Factors Affecting the Epidemology of Wheat Rusts, & Hogg, W. H. (1969). *Meteorological factors affecting the epidemiology of wheat rusts*. Secretariat of the World Meteorological Organization.

Oyewole, C. (2016). *The wheat crop* (pp. 1-16). Technical report, Department of Crop production, Faculty of Agriculture, Kogi state University.

Park, R. F. (2015, January). Long term surveys of pathogen populations underpin sustained control of the rust diseases of wheat in Australia. In *Journal and Proceedings of the Royal Society of New South Wales* (Vol. 148, No. 455/456, pp. 15-27).

Park, R., Fetch, T., Hodson, D., Jin, Y., Nazari, K., Prashar, M., & Pretorius, Z. (2011). International surveillance of wheat rust pathogens: progress and challenges. *Euphytica*, *179*(1), 109-117.

Patpour, M., Hovmoller, M. S., Justesen, A. F., Newcomb, M., Olivera Firpo, P. D., Jin, Y., ... & Hodson, D. P. (2016). Emergence of virulence to SrTmp in the Ug99 race group of wheat stem rust, *Puccinia graminis* f. sp. *tritici*, in Africa. *Plant Disease*, *100*(3), 522

Paul, D., & Lade, H. (2014). Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. *Agronomy for Sustainable Development*, *34*(4), 737-752.

Peterson, R. F. (1965). Wheat growth and development under variable temperature conditions. *Canadian Journal of Plant Science*, 45(4), 271–277.

Peterson, R. F., Campbell, A. B., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian journal of research, 26*(5), 496-500.

Peturson, B. (1958). Wheat rust epidemics in western Canada in 1953, 1954 and 1955. *Canadian Journal of Plant Science*, *38*(1), 16-28.

Peturson, B., Forsyth, F. R., & Lyon, C. B. (1958). Chemical control of cereal rusts. 2. Control of leaf rust of wheat with experimental chemicals under field conditions. *Phytopathology*, *48*(12), 655-657.

Pinto, F. F., & Hurd, E. A. (1970). 70 years with wheat in Kenya. *East African Agricultural and Forestry Journal* (Special Issue).

Pranamika, S. and Saikia, M.K. (2013). Management of late blight of potato through chemicals.

IOSR Journal of Agricultural and Vetenary Science 2:23-36

Prank, M., Kenaley, S. C., Bergstrom, G. C., Acevedo, M., & Mahowald, N. M. (2019). Climate change impacts the spread potential of wheat stem rust, a significant crop disease. *Environmental Research Letters*, *14*(12), 124053.

Pretorius, Z. A., Bender, C. M., Visser, B., & Terefe, T. (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), 784-784.

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., & Payne, T. S. (2000). Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis. f. sp. tritici in Uganda. *Plant Disease*, *84*(2), 203-203.

Prévost, B. (1807). *Memoir on the Immediate Cause of Bunt Or Smut of Wheat: And of Several Other Diseases of Plants, and on Preventives of Bunt* (No. 6). American Phytopathological Society.

Rathmell, W. G., & Skidmore, A. M. (1982). Recent advances in the chemical control of cereal rust diseases. *Outlook on Agriculture*, *11*(1), 37-43.

Rodriguez-Algaba, J., Walter, S., Sørensen, C. K., Hovmøller, M. S., & Justesen, A. F. (2014). Sexual structures and recombination of the wheat rust fungus Puccinia striiformis on Berberis vulgaris. *Fungal Genetics and Biology*, *70*, 77-85.

Roelfs, A. P. (1982). Effects of Barberry eradication. *Plant Disease*, *66*(2), 177-187.

Roelfs, A. P. (1986). Development and impact of regional cereal rust epidemics. *Plant Disease Epidemiology*, *1*, 129-150.

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

Russell, P. E. (2005). A century of fungicide evolution. *The Journal of Agricultural Science, 143*(1), 11-25.

Saari, E. E., & Prescott, J. M. (1985). World distribution in relation to economic losses. In *Diseases, Distribution, Epidemiology, and Control* (pp. 259-298). Academic Press.

Sahri, A., Chentoufi, L., Arbaoui, M., Ardisson, M., Belqadi, L., Birouk, A., & Muller, M. H. (2014). Towards a comprehensive characterization of durum wheat landraces in Moroccan traditional agrosystems: analysing genetic diversity in the light of geography, farmers' taxonomy and tetraploid wheat domestication history. *BMC Evolutionary Biology*, *14*(1), 1-18.

Samborski, D. J. (1985). Wheat leaf rust. In *Diseases, Distribution, Epidemiology, and Control* (pp. 39-59). Academic Press.

Samborski, D. J., & Dyck, P. L. (1982). Enhancement of resistance to Puccinia recondita by interactions of resistance genes in wheat. *Canadian journal of Plant Pathology*, *4*(2), 152-156.

Savadi, S., Prasad, P., Kashyap, P. L., & Bhardwaj, S. C. (2018). Molecular breeding technologies and strategies for rust resistance in wheat (Triticum aestivum) for sustained food security. *Plant pathology*, *67*(4), 771-791.

Schumann, G. L., & Leonard, K. J. (2000). Stem rust of wheat (black rust). *The Plant Health Instructor*, *10*, 1094-2000.

Setter, T. L., & Carlton, B. M. (2000). *Leaf development and nutrient allocation in wheat*. *Crop Science*, 40(3), 765–771.

Shamanin, V. P., Pototskaya, I. V., Shepelev, S. S., Pozherukova, V. E., Salina, E. A., Skolotneva, E. S., ... & Morgounov, A. I. (2020). Stem rust in Western Siberia–race composition and effective resistance genes. *Vavilov Journal of Genetics and Breeding*, *24*(2), 131.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3/4), 591-611.

Shaw, D. E. (1970). Coffee eradication in a previously coffee rust infected area in Papua. *Papua and New Guinea Agricultural Journal*, *22*(1), 59-61.

Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. *Journal of experimental botany*, *53*(370), 947-958.

Shewry, P. R., D'Ovidio, R., Lafiandra, D., Jenkins, J. A., Mills, E. C., and Békés, F. (2009). Wheat grain proteins. *Wheat: Chemistry and Technology*, (Ed. 4), 223-298.

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*, *5*(3), 291-317.

Shrivastava, B., Jain, K. K., Kalra, A., & Kuhad, R. C. (2014). Bioprocessing of wheat straw into nutritionally rich and digested cattle feed. *Scientific Reports*, *4*(1), 1-9.

Si, B., Wang, H., Bai, J., Zhang, Y., & Cao, Y. (2022). Evaluating the Utility of Simplicillium lanosoniveum, a Hyperparasitic Fungus of Puccinia graminis f. sp. tritici, as a Biological Control Agent against Wheat Stem Rust. *Pathogens*, *12*(1), 22.

Simon, H., Ndegwa, M., & Musyoka, R. (2021). *Major constraints to wheat production in Kenya: A farmer's perspective*. *East African Agricultural and Forestry Journal*, 87(2), 145–154.

Simón, M. R., Börner, A., & Struik, P. C. (2021). Fungal wheat diseases: Etiology, breeding, and integrated management. *Frontiers in Plant Science*, *12*, 671060.

Singh, R. P., Herrera-Foessel, S., Huerta-Espino, J., Singh, S., Bhavani, S., Lan, C., & Basnet, B. R. (2014). Progress towards genetics and breeding for minor genes based resistance to Ug99 and other rusts in CIMMYT high-yielding spring wheat. *Journal of Integrative Agriculture*, *13*(2), 255-261.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., ... & Govindan, V. (2011). The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology*, *49*, 465-481.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., ... & Ward, R. W. (2008). Will stem rust destroy the world's wheat crop. *Advances in Agronomy*, *98*, 271-309.

Singh, R. P., Hodson, D. P., Jin, Y., Lagudah, E. S., Ayliffe, M. A., Bhavani, S., ... & Hovmøller, M. 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.

Singh, R. P., Huerta-Espino, J., Roelfs, A. P., & Curtis, B. C. (2002). The wheat rusts. *Growth*, *2*(25), 35.

Singh, R. P., William, H. M., Huerta-Espino, J., & Rosewarne, G. (2004, September). Wheat rust in Asia: meeting the challenges with old and new technologies. In *Proceedings of*

the 4th International Crop Science Congress (Vol. 26). Gosford, Australia: The Regional Institute Ltd.

Smith, K., Draper, M., Simmons, K., Bennett, R., Hebbar, P., Royer, M., & Murray, T. (2009). US preparations for potential introduction of Ug99 strains of wheat stem rust. *Outlooks on Pest Management*, *20*(4), 148-152.

Spanic, V., Rouse, M. N., Kolmer, J. A., & Anderson, J. A. (2015). Leaf and stem seedling rust resistance in wheat cultivars grown in Croatia. *Euphytica*, *203*(2), 437-448.

Stakman, E. C., Stewart, D. M., & Loegering, W. Q. (1962). *Identification of physiologic races of Puccinia graminis var. tritici*. Washington: USDA.

Tadesse, W., Bishaw, Z., & Assefa, S. (2019). Wheat production and breeding in sub-Saharan Africa: Challenges and opportunities in the face of climate change. *International Journal of Climate Change Strategies and Management*., 11(5), 695–715.

Terefe, T. G., Boshoff, W. H., Park, R. F., Pretorius, Z. A., & Visser, B. (2024). Wheat stem rust surveillance reveals two new races of Puccinia graminis f. sp. tritici in South Africa during 2016 to 2020. *Plant Disease*, *108*(1), 20-29.

Tyagi, M., Kayastha, A. M., & Sinha, B. (2000). The role of peroxidase and polyphenol oxidase isozymes in wheat resistance to Alternaria triticina. *Biologia Plantarum*, *43*(4), 559-562.

USDA reports (2019) Grain and Feed Annual, Kenya Imports of Corn, Wheat, and Rice Expected to Surge; https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Grain%20and%20Feed%20Annual_ Nairobi_Kenya_3-18-2019.pdf

Van der Plank, J. E. (1969). Pathogenic races, host resistance, and an analysis of pathogenicity. *Netherlands Journal of Plant Pathology*, *75*(1), 45-52.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Woo, S. L., Nigro, M., Marra, R., ... & Lorito, M. (2014). Trichoderma secondary metabolites active on plants and fungal pathogens. *The Open Mycology Journal*, *8*(1).

Wamalwa, M. N., Wanyera, R., Rodriguez-Algaba, J., Boyd, L. A., Owuoche, J., Ogendo, J., ... & Hovmøller, M. (2022). Distribution of Puccinia striiformis f. sp. tritici Races and Virulence in Wheat Growing Regions of Kenya from 1970 to 2014. *Plant Disease*, *106*(2), 701-710

Wamalwa, M. N., Wanyera, R., Rodriguez-Algaba, J., Boyd, L. A., Owuoche, J., Ogendo, J., ... & Hovmøller, M. (2022). Distribution of Puccinia striiformis f. sp. tritici Races and

Virulence in Wheat Growing Regions of Kenya from 1970 to 2014. *Plant Disease*, *106*(2), 701-710.

Wanyera, R., & Wamalwa, M. (2022). Past, Current and Future of Wheat Diseases in Kenya. In M. S. Khan (Ed), *Wheat-Recent Advances*. Intechopen

Wanyera, R., Kinyua, M. G., Jin, Y., & 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*(1), 113-113.

Wanyera, R., Wamalwa, M., Odemba, M., Wanga, H., Kinyanjui, P., Onyango, V., & Owuoche, J. (2016). Management of wheat rusts at different growth stages using Nativo 300 SC (trifloxystrobin 100g/L+ tebuconazole 200g/L) fungicide. *Australian Journal of Crop Science*, *10*(9), 1273-1280.

Wegulo, S. N., & Byamukama, E. (2012). *Rust diseases of wheat.* University of Nebraska Extension, Lincoln.

Wellman, F. L., & Echandi, E (1981). *Stakman-Craigie Symposium on Rust Diseases*. University of Minnesota Press.

Worku, D., Zerihun, T., Daniel, K., Habtemariam, Z., Dawit, A., & Wanyera, R. (2016). Development of wheat germplasm for stem rust resistance in eastern Africa. *African Crop Science Journal*, *24*(1), 25-33.

Yang, C., Hamel, C., Vujanovic, V., & Gan, Y. (2011). Fungicide: modes of action and possible impact on nontarget microorganisms. *International Scholarly Research Notices*, *2011*.

Yehuda, P. B., Eilam, T., Manisterski, J., Shimoni, A., & Anikster, Y. (2004). Leaf rust on Aegilops speltoides caused by a new forma specialis of Puccinia triticina. *Phytopathology*, *94*(1), 94-101.

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed research*, *14*(6), 415-421.

APPENDICES

Appendix A. Pedigree for 16th stem rust resistance screening nursery and checks

Entry	GID	PEDIGREE
6001	PBW343	NORD-DESPREZ/VG-1944//LALYANSORA//BLUEBIRD/3/YACO(SIB)/4/VI
6002	CACUKE	CANADIAN/CUNNINGHAM//KENNEDY
6003	ROBIN	BABAX/LR42//BABAX*2/3/TUKURU
6004	8780746	MUCUY*3//RL6077/AOC-YR
6005	8778945	COPIO/MUCUY
6006	8778988	SUP152/BAJ #1/5/PBW65/2*PASTOR/3/KIRITATI//PBW65/2*SERI.1B/4/DA
6007	8779055	MUCUY/3/SWSR22T.B./KACHU//2*KACHU
6008	8779079	KACHU//KIRITATI/2*TRCH/3/ABLEU
6009	8779084	KACHU/3/WHEAR//2*PRL/2*PASTOR/4/KACHU/KIRITATI
6010	8779296	BAJ #1*2/WHEAR*2/3/PRL/2*PASTOR*2//FH6-1-7
6011	8779366	KACHU/BECARD//WBLL1*2/BRAMBLING*2/3/KACHU/KIRITATI
6012	8779482	MUCUY*2/3/KACHU #1/KIRITATI//KACHU
6013	8779498	FRNCLN/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/5/2*SUP152
6014	8779700	KACHU/BECARD//WBLL1*2/BRAMBLING/3/MUNAL*2/WESTONIA/4/KA
6015	8779738	KACHU/DANPHE/3/2*KACHU//KIRITATI/2*TRCH
6016	8779815	MUTUS//ND643/2*WBLL1/3/2*KACHU//KIRITATI/2*TRCH
6017	8779838	SUP152/KENYA SUNBIRD/3/KACHU//KIRITATI/2*TRCH/4/SUP152/KENY
6018	8780240	MELON//FILIN/MILAN/3/FILIN/4/PRINIA/PASTOR//HUITES/3/MILAN/OTU
		LON//FILIN/MILAN/3/FILIN/6/2*ABLEU

Appendix A. Continued

Entry	GID	PEDIGREE
6021	8780524	CHYAK//ND643/2*WAXWING/3/ND643/2*WAXWING/4/2*ABLEU
6022	8780587	T 2003 (CRE7)//MUNAL*2/WESTONIA/3/NADI#2
6023	8776671	SUP152/BLOUK #1/3/SWSR22T.B./KACHU//2*KACHU
6024	8777193	ND643/2*WBLL1/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/5/K
		L/2*PASTOR
6030	8777482	ND643/2*WBLL1/4/CHIBIA//PRLII/CM65531/3/MISR
		2/5/BECARD/6/KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PA
		E1/3*CNO79//2*SERI
6031	8777487	PAURAQ/KENYA SUNBIRD//PAURAQUE #1/3/BORL14

6032	8777513	BAVIS/NAVJ07/3/FRANCOLIN #1*2//ND643/2*WBLL1
6033	8777527	KAKURU/KUTZ
6034	8777671	KACHU/3/WHEAR//2*PRL/2*PASTOR/4/KACHU/SAUAL
6035	8777672	KACHU/3/WHEAR//2*PRL/2*PASTOR/4/KACHU/SAUAL
6036	8777703	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/CIRO16/6/SWSR22T.B./ #1//WBLL1*2/KURUKU
6037	8777886	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/5/SERI.1B//KAUZ/HEVO/3/AMA U/KIRITATI
6038	8777906	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/5/SERI.1B//KAUZ/HEVO/3/AMA TENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65531/3/MISR 2

Appendix A. Continued

Entry	GID	PEDIGREE
6039	8777945	SOKOLL/WBLL1/6/OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.S
6040	8777950	TRCH/3/ROLF07/YANAC//TACUPETO F2001/BRAMBLING/4/PRL/2*PAST
6041	8777980	BECARD*2/5/BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/6/SW2148/2*ROLF NIA*2//SNLG
6042	8777993	KACHU/PAURAQ//PAURAQUE #1/3/BORL14
6043	8778010	SAUAL/YANAC//SAUAL/5/UP2338*2/SHAMA/3/MILAN/KAUZ//CHIL/CHU A/6/BORL14
6044	8778082	MARCHOUCH*4/SAADA/3/2*FRET2/KUKUNA//FRET2*2/4/CIRO16/5/BOR
6050	8780745	MUCUY*3//RL6077/AOC-YR
6051	8778320	QUAIU #2/BAVIS #1//BORL14
6052	8778326	BECARD #1/BAVIS/6/ROLF07/SAUAL*2/5/SERI.1B//KAUZ/HEVO/3/AMAD
6053	8778336	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1/4/SOKOL #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD #1//INQALAB 91*2/TU
6054	8778342	SOKOLL/92.001E7.32.5//SOKOLL/EXCALIBUR/3/KACHU/KIRITATI
6055	8778350	BAVIS #1*2/4/PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1/5/PRL/2*PAS
6056	8778400	FRET2/KUKUNA//FRET2/3/WHEAR/4/SAUAL/YANAC//SAUAL/5/SAUAL/ U*2/3/ND643//2*PRL/2*PASTOR
6057	8778420	KACHU/SAUAL*2/3/KINGBIRD #1//INQALAB 91*2/TUKURU/4/KASUKO
6058	8778453	BACOIS/3/2*PBW343*2/KUKUNA*2//FRTL/PIFED/4/KACHU/SAUAL*2//C

Appendix A. Continued

Entry	GID	PEDIGREE
6059	8778477	BECARD/AKURI*2/4/MUU#1//PBW343*2/KUKUNA/3/MUU/9/WBLL1/3/ST
		92/RAYON/5/TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON*2/8/TACU
		3//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PASTO
6060	8778492	BECARD/AKURI*2/4/MUU #1//PBW343*2/KUKUNA/3/MUU/5/KUTZ//KFA
6061	8778561	KASUKO/4/BECARD/AKURI*2/3/PBW343*2/KUKUNA*2//FRTL/PIFED
6062	8778594	ATTILA*2/PBW65//KACHU*2/3/FRANCOLIN #1//WBLL1*2/KIRITATI/4/K
6063	8778824	MUCUY*2/AMUR
6064	8781751	FRNCLN/3/ND643//2*PRL/2*PASTOR/4/FRANCOLIN #1*2/5/FRNCLN/NIIN
6065	8781927	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PASTOR/4/CHOIX
		*SERI*2/6/BORL14
6066	8781928	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PASTOR/4/CHOIX
		*SERI*2/6/BORL14
6067	8782069	MUTUS//ND643/2*WBLL1/3/BORL14/5/MUTUS/DANPHE#1/4/C80.1/3*BAT
		/3*QT4522//2*PASTOR
6068	8782113	KISKADEE#1/5/KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/6/WHEAR//2*PR
		DANPHE #1/4/C80.1/3*BATAVIA//2*WBLL1/3/C80.1/3*QT4522//2*PASTOR
6069	8782124	PRL/2*PASTOR//WAXWING*2/KRONSTADF2004/4/PBW343*2/KUKUNA/
		W343*2/KUKUNA/7/2*WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ
		2/KUKUNA*2//FRTL/PIFED

Appendix A. Continued

6070	8782130	ATTILA*2/PBW65/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*S
		14/8/MELON//FILIN/MILAN/3/FILIN/4/TRCH/SRTU//KACHU
6071	8782135	ATTILA*2/PBW65/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*S
		#1/7/BORL14/8/MELON//FILIN/MILAN/3/FILIN/4/TRCH/SRTU//KACHU
6072	8782141	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1/4/2*KACHU/5/2*SOKC
		2*BAU*2/4/PASTOR//MILAN/KAUZ/3/BAV92
6073	8782586	CROC_1/AE.SQUARROSA
		(205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/HUW234+LR34/PRINIA
		ROLF07/6/2*WBLL1*2/BRAMBLING//CHYAK
6074	8782588	CROC_1/AE.SQUARROSA

		(205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/HUW234+LR34/PRINIA
		ROLF07/6/2*WBLL1*2/BRAMBLING//CHYAK
6075	-	KFA/2*KACHU*2//QUELEA
6076	8782835	MUNAL #1/CIRO16*2//MISR 1
6077	8782880	SW2148/2*ROLF07/3/HUW234+LR34/PRINIA*2//SNLG/5/PASTOR//HXL757
		KOLL/3/PASTOR//HXL7573/2*BAU/6/WHEAR/SOKOLL/3/TRCH/SRTU//KA
6078	8782912	KACHU/SAUAL/3/TACUPETO
		F2001/BRAMBLING//KIRITATI*2/4/FRET2/TUKURU//FRET2/3/MUNAL #1
6079	8783201	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD
		91*2/TUKURU*2/5/NADI#2

Appendix A. Continued

6080	8783218	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD
		91*2/TUKURU*2/5/MUCUY
6081	8783222	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD
		91*2/TUKURU*2/5/MUCUY
6082	8783235	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD
		91*2/TUKURU*2/5/BECARD/CHYAK
6083	8783243	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD
		91*2/TUKURU*2/5/SUP152*2/TECUE #1
6084	8783489	FRET2/KUKUNA//FRET2/3/PARUS/4/FRET2*2/SHAMA*2/5/WBLL1/KUKU
		F2001/3/UP2338*2/VIVITSI/6/BAJ #1/SUP152/9/TC870344/GUI//TE
		87/AGR/3/2*WBLL1/8/BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6
		SQUARROSA (224)//OPATA/7/P
6085	8783632	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/SOKO
		/SOKOLL//SUNCO/2*PASTOR*2/8/CROSBILL
		#1/DANPHE/7/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS
		(TAUS)/4/WEAVER/5/2*KAUZ/6/PRL/2*PASTOR
6086	8783639	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/6/OASIS/5*BORL95/5/CNDO/R14
		Q/4/2*OCI*2/7/FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KI
		91*2/TUKURU
6087	8783677	BAVIS #1*2/4/PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1/5/2*NADI

Appendix A. Continued

Entry	GID	PEDIGREE
6088	8784017	SAUAL/YANAC//SAUAL/3/BECARD/QUAIU #1*2/4/PBW343*2/KUKUNA*2//FRTL/PIFED/3/KFA/2*KACHU
6089	8784092	KASUKO/5/FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KIN 91*2/TUKURU/6/KASUKO
6090	8784378	SPITFIRE/6/KS82W418/SPN/3/CHEN/AE.SQ//2*OPATA/4/FRET2/5/2*SOKO 2*BAU/7/MACE
6091	8785115	CASCBL/BAVIS//WA8189/9/TC870344/GUI//TEMPORALERA 87/AGR/3/2*WBLL1/8/BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6 SQUARROSA (224)//OPATA/7/PASTOR//MILAN/KAUZ/3/BAV92
6092	8785142	PRL/SARA//TSI/VEE#5/3/TILHI/4/ATTILA/2*PASTOR/6/YAR/AE.SQUARR (783)/4/GOV/AZ//MUS/3/SARA/5/MYNA/VUL//JUN/7/NELOKI/8/SWCL100 3/9/BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA/5/SAUAL/YANAC//SAU ASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KA
6093	8784809	FIDELIUS/6/ALTAR (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/MUTUS/7/BAJ #1/CIRO1
6094	8785878	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6095	8785438	SR47/5/3*SHORTENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65
6096	8785443	SR47/5/3*SHORTENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65
6097	8785496	W3763-SR35/3/3*SWSR22T.B./KACHU//2*KACHU
6098	8785948	W3763-SR35/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR

Appendix A. Continued

6099	8785514	W3763-SR35/4/3*KACHU/3/WHEAR//2*PRL/2*PASTOR
6100	-	W3763-SR35/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6101	8785707	SERI//T.DICOCCON PI94623/AE.SQUARROSA (1027)/3/NA 542/2*PASTOR/3/BACEU #1/5/NADI#2/7/NADI#2
6102	8785993	T.DICOCCON PI94623/AE.SQUARROSA (1027)//SERI/3/MUCUY/4/MUTUS
6103	8777506	TAM200/PASTOR//TOBA97/3/FRNCLN/4/WHEAR//2*PRL/2*PASTOR/5/BC
6104	8782419	CROC_1/AE.SQUARROSA(205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET HYAK/7/MOKUE #1
6105	8784397	WEDIN/5/TRCH/3/ROLF07/YANAC//TACUPETO F2001/BRAMBLING/4/PR
6106	8784401	WEDIN/5/TRCH/3/ROLF07/YANAC//TACUPETO F2001/BRAMBLING/4/PR

6107	8789894	MERCATO/VORB*2/4/SWSR22T.B.//TACUPETOF2001*2/BRAMBLING/3/2
		F2001*2/BRAMBLING
6108	8777479	ND643/2*WBLL1/4/CHIBIA//PRLII/CM65531/3/MISR 2/5/BECARD/6/PRL/2
6109	8789734	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/SAUAL/5/PBW343*2/KUKUNA//
		KUNA/6/KACHU/SAUAL/7/KACHU*2/3/ND643//2*PRL/2*PASTOR
6110	8789794	WBLL1*2/KUKUNA//KIRITATI/3/WBLL1*2/KUKUNA/4/KINGBIRD#1//IN
		WBLL1*2/BRAMBLING//KACHU/7/SHORTENEDSR26TRANSLOCATION/
		2/3/PASTOR/5/MUNAL/6/MUTUS*2/TECUE #1
6111	8790025	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/
		2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI*2/6/BORL14

Appendix A. Continued

6112	8790027	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PASTOR/4/CHOIX
		*SERI*2/6/BORL14
6113	8790046	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PASTOR/4/CHOIX
		*SERI*2/6/KINGBIRD #1//INQALAB 91*2/TUKURU
6114	8790053	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/SOKOLL/WBLL1/5/BORL14/6/KA
6115	8782393	CROC_1/AE.SQUARROSA
		(205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/CIRO16/6/BORL14/7/K
6116	8790089	PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1/4/HUW234+LR34/PRINIA//
		OLF07/5/WHEAR/SOKOLL/6/BORL14/7/KASUKO
6117	8790101	BAV92//IRENA/KAUZ/3/HUITES/4/PVN/5/CIRO16/6/MUCUY/7/MOKUE #1
6118	8790117	WBLL1/KUKUNA//TACUPETOF2001/3/BAJ#1*2/4/KINGBIRD#1/5/MELON
		/2*CIRO16/9/TACUPETOF2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS
		WEAVER/5/PASTOR/7/ROLF07*2/8/SUP152/MUU
6119	8790138	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS
		R/7/PBW343*2/KUKUNA*2//FRTL/PIFED/8/BORL14/9/KASUKO
6120	8790139	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS
		(TAUS)/4/WEAVER/5/2*PASTOR/7/PBW343*2/KUKUNA*2//FRTL/PIFED/8
6121	8790143	SAUAL*2/6/CND O/R143//ENTE/MEXI_2/3/AEGILOPS
		(TAUS)/4/WEAVER/5/2*PASTOR/7/PBW343*2/KUKUNA*2//FRTL/PIFED/8
6122	8790258	KENYA TAI*2/5/FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/K
		91*2/TUKURU

Appendix A. Continued

Entry	GID	PEDIGREE
6123	8790275	KENYA SUNBIRD/4/KACHU*2/3/ND643//2*PRL/2*PASTOR #1//WBLL1*2/KURUKU
6124	8790292	KASUKO*2/5/MUTUS/DANPHE #1/4/C80.1/3*BATAVIA//2*WBLL1/3/C80.
6125		KASUKO*2/5/MUTUS/DANPHE #1/4/C80.1/3*BATAVIA//2*WBLL1/3/C80.
6126	8790379	BAJ #1*2/PREMIO/4/BOKOTA*2/3/UP2338*2/KKTS*2//YANAC/9/KFA/2 F2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS (TAUS)/4/WEAVER/5/PASTOR/7/ROLF07
6127	8790382	BAJ #1*2/PREMIO/4/BOKOTA*2/3/UP2338*2/KKTS*2//YANAC/9/KFA/2 F2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS (TAUS)/4/WEAVER/5/PASTOR/7/ROLF07
6128	8790502	SPITFIRE/6/KS82W418/SPN/3/CHEN/AE.SQ//2*OPATA/4/FRET2/5/2*SOKO 2*BAU/7/MACE
6129	8790550	FD06150//KACHU/KIRITATI/3/KACHU/KIRITATI
6130	8790583	NSA04-6402/3/SWSR22T.B./2*BLOUK#1//WBLL1*2/KURUKU/4/SWSR22T. #1//WBLL1*2/KURUKU
6131	8790751	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6132	8790754	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6133	8790783	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6134	8790788	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6135	8790800	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR

Appendix A. Continued

6136	8790929	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6137	8790935	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6138	8790946	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6139	8790951	SR50/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6140	8790874	W3763-SR35/4/3*KACHU*2/3/ND643//2*PRL/2*PASTOR
6141	-	ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETOF2001*/BRAMBLING/5/P SAUAL
6142	-	ND643/2*WBLL1
6143	-	EMB16/CBRD//CBRD
6144		URES/JUN//KAUZ/3/Babax/4/Tilhi

GID-Genotype identification number (CYMMIT)

Appendix B. Infection types and co-efficient of infection for 16th SRRSN wheat lines evaluated against stem rust in the greenhouse against race TTKTT and field at KALRO Njoro

ENTRY	PBC	Disease reaction		Final disease severity				AUDPC		Coeffi
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6001	+	NG	0	70	S	40	MSS	735	309.17	70
6002	-	3+	NG	80	S	90	S	758.3333	764.17	80
6003	+	NG	3+	100	S	100	S	1003.333	1038.	90
6004	+	2+, 3	3	40	M	15	MS	320.8333	117.83	24
6005	-	3	3+	60	MSS	40	MSS	548.3333	303.33	54
6006	-	2	NG	30	M	10	MR	221.6667	75.83	18
6007	-	; 1	3	30	MS	20	M	234.5	134.17	24
6008	-	1	0 2	30	M	30	MSS	192.5	200.67	18
6009	-	3	3+/1	30	M	40	MSS	233.3333	374.5	18
6010	-	2-	2	20	M	15	M	116.6667	110.83	12
6011	-	2+	1	30	M	15	M	227.5	134.17	18
6012	-	2	2	40	M	40	MSS	332.5	292.83	24
6013	-	1	0 1	30	M	20	M	250.8333	164.5	18
6014	-	2	1+	30	M	40	MSS	268.3333	304.5	18
6015	-	;	0	10	MR	5	MR	64.16667	44.33	4
6016	-	; 1	0 1-	20	MS	15	M	134.1667	100.33	16
6017	+	1	0	15	MR	15	M	128.3333	101.5	6
6018	-	0, 1	3+	30	M	20	M	170.3333	141.166	18

Appendix B. Continued

ENTRY	PBC	Disease reaction		Final disease severity				AUDPC		Coefficie
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6019	-	2	2+	40	MS	40	MS	297.5	285.833	32
6020	-	3	3+	40	MS	20	MS	291.6667	210	32
6021	-	2	2+	40	MS	40	MSS	361.6667	285.833	32
6022	-	2, ;	0	15	RMR	5	RMR	75.83333	19.8333	4.5
6023	-	2+	0,1	40	MS	30	MS	320.8333	262.5	32
6024	-	2-	3,2	15	MR	15	M	105	101.5	6
6025	+	2-	0	30	MS	20	MS	205.3333	123.666	24
6026	-	1-	2	20	M	20	M	81.66667	100.333	12
6027	-	0,1	2	40	MS	40	MSS	420	332.5	32
6028	-	1	1	20	M	60	MSS	116.6667	333.6667	12
6029	-	0	NG	10	RMR	15	M	64.16667	77	3
6030	-	; 1	3	15	MR	15	M	140	116.6667	6
6031	-	0 ;	2	10	MR	15	M	52.5	67.66667	4
6032	-	;	0	30	M	15	M	180.8333	110.8333	18
6033	-	;	3	40	MSS	40	M	227.5	175	36
6034	-	3+	3-	30	MS	20	M	169.1667	170.3333	24
6035	+	2	0	30	M	40	M	204.1667	274.1667	18
6036	-	;	0 1-	20	M	15	M	87.5	75.83333	12
6037	+	NG	1	40	M	40	MSS	262.5	257.8333	24

Appendix B. Continued

ENTRY	PBC	Disease reaction		Final disease severity				AUDPC		Coefficie
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6038	-	0	0	20	M	15	MS	99.16667	64.16667	12
6039	+	3	3	20	M	50	MS	227.5	240.3333	12
6040	+	;	1-	20	MR	15	MR	151.6667	93.33333	8
6041	-	0 ;	0	15	MR	10	MR	81.66667	81.66667	6
6042	-	0, 1	2	30	M	50	MSS	186.6667	379.1667	18
6043	-	;	0	30	M	30	M	233.3333	151.6667	18
6044	+	1	2	30	M	40	MS	169.1667	217	18
6045	-	0	0	20	MS	20	M	99.16667	81.66667	16
6046	-	0	0	10	RMR	10	M	64.16667	56	3

6047	-	0 2	3+	50	MSS	50	MSS	420	408.3333	45
6048	-	3	3-	40	MS	50	MS	326.6667	381.5	32
6049	+	0	NG	30	M	40	MSS	210	187.8333	18
6050	+	NG	NG	20	MS	15	MS	192.5	129.5	16
6051	-	;	0	20	M	30	M	175	233.3333	12
6052	-	NG	0	40	MSS	30	MS	309.1667	245	36
6053	-	NG	2, 3+	50	MSS	50	MSS	466.6667	408.3333	45
6054	+	;	0 1	10	MR	15	M	52.5	87.5	4
6055	-	0, 1	0	15	M	15	M	75.83333	70	9
6056	-	0	3	10	MR	20	MS	70	116.6667	4

Appendix B. Continued

ENTRY	PBC	Disease reaction		Final disease severity				AUDPC		Coefficie
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6057	+	0	0	40	MS	40	MS	274.1667	246.1667	32
6058	+	0, 1	3+	40	MS	30	MS	285.8333	246.1667	32
6059	-	0	2/3+	20	M	30	M	180.8333	239.1667	12
6060	-	3	2+	30	M	20	MS	204.1667	182	18
6061	-	;	NG	60	MSS	40	MS	542.5	408.3333	54
6062	-	NG	0	30	M	40	MS	204.1667	217	18
6063	-	0	2,1	40	MS	30	MSS	215.8333	163.3333	32
6064	-	1	3	15	MR	20	M	81.66667	157.5	6
6065	-	3	0	15	MR	40	M	105	281.1667	6
6066	-	1-	0	10	MR	10	M	128.3333	75.83333	4
6067	-	1-	1-	30	MS	20	MS	210	165.6667	24
6068	-	1	NG	30	M	20	MS	140	154	18
6069	-	NG	0	50	MSS	60	MSS	344.1667	402.5	45
6070	-	0	2+	50	MSS	40	MSS	373.3333	315	45
6071	-	0,1	3+	20	MS	40	MSS	198.3333	205.3333	16
6072	-	0/1	1,2	20	MS	30	MS	87.5	152.8333	16
6073	-	0, ;	NG	30	M	15	MR	198.3333	123.6667	18
6074	-	0	;0	15	MR	15	MR	87.5	93.33333	6
6075		3	0	50	MSS	60	MS	425.8333	425.8333	45

Appendix B. Continued

ENTRY	PBC	Disease reaction		Final disease severity				AUDPC		Coefficien
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6076	-	2	3	40	MS	40	MSS	320.8333	287	32
6077	-	0	0 1	30	MS	10	MR	186.6667	71.16667	24
6078	-	0,1	0	40	MS	50	MS	373.3333	379.1667	32
6079	-	NG	2+	50	MSS	30	MS	420	199.5	45
6080	-	1	3	15	MR	10	M	52.5	58.33333	6
6081	-	1	1	10	RMR	5	M	70	29.16667	3
6082	-	0 1	2	30	MS	15	MS	151.6667	87.5	24
6083	-	0 1	3	50	MSS	40	M	408.3333	344.1667	45
6084	-	1+	0	5	RMR	15	M	29.16667	93.33333	1.5
6085	+	1	2	30	M	20	M	180.8333	14S0	18
6086	-	0	2	40	MSS	20	M	315	187.8333	36
6087	-	0	0	20	M	15	M	105	87.5	12
6088	+	3	2	30	MS	30	M	192.5	147	24
6089	-	1	0	60	MSS	40	MSS	595	367.5	54
6090	-	1+	3	30	MS	30	MS	163.3333	163.3333	24
6091	-	NG	0	50	MSS	50	MS	396.6667	332.5	45
6092	-	0	0	15	MR	15	M	75.83333	110.8333	6
6093	-	3	NG	40	MR	20	MS	280	140	16
6094	-	0	0	20	M	5	M	81.66667	52.5	12

Appendix B. Continued

ENTRY	PBC	Disease reaction		Final disease severity				AUDPC		Coefficien
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6095	+	0	0 1	15	MR	10	MR	70	75.83333	6
6096	+	0	0	15	MR	10	MR	75.83333	70	6
6097	-	0	0	30	M	10	MR	157.5	52.5	18
6098	-	0	0	15	M	5	M	81.66667	40.83333	9
6099	-	; 1	; 1	20	M	30	MS	157.5	140	12
6100	+	1	2	30	MS	40	MSS	239.1667	257.8333	24
6101	-	1	0	40	MS	30	MS	338.3333	170.3333	32
6102	-	2 1-	0	10	MR	20	M	70	116.6667	4
6103	+	0	0	50	MS	50	MS	332.5	269.5	40

6104	-	0	3 1	40	MSS	30	MSS	326.6667	186.6667	36
6105	-	1	3 1	60	MSS	60	MS	519.1667	379.1667	54
6106	+	1	0	40	MS	40	MSS	274.1667	246.1667	32
6107	+	;	0	15	RMR	5	RMR	87.5	31.5	4.5
6108	+	0	0 1	10	MR	10	MS	46.66667	58.33333	4
6109	-	NG	0	30	MS	40	MSS	215.8333	332.5	24
6110	-	1-	2	20	MS	30	MS	157.5	234.5	16
6111	-	1-	2	20	M	30	MS	87.5	145.8333	12
6112	-	0 1-	0	5	RMR	5	M	58.33333	40.83333	1.5
6113	-	1	0	10	R	5	M	46.66667	52.5	2

Appendix B. Continued

ENTRY		Disease reaction		Final disease severity				AUDPC		Coefficien
	PBC	Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6114	-	1-	0,1	50	MSS	40	MSS	361.6667	285.8333	45
6115	+	0	0	40	MS	60	MSS	402.5	501.6667	32
6116	-	1	3	40	MS	60	MSS	291.6667	414.1667	32
6117	-	1	3 2	20	M	30	M	134.1667	257.8333	12
6118	-	1	3	50	MSS	40	MS	367.5	256.6667	45
6119	+	3	1	40	M	30	MS	245	250.8333	24
6120	+	1	3	50	MSS	40	MSS	513.3333	350	45
6121	+	3	0	20	MS	40	MS	180.8333	309.1667	16
6122	-	1+	2	15	MS	20	MSS	70	93.33333	12
6123	+	1	2	10	RMR	20	M	70	99.16667	3
6124	-	3	3	40	MSS	50	MSS	280	344.1667	36
6125	-	2	3-	50	MSS	50	MSS	414.1667	332.5	45
6126	-	1	0	60	MSS	40	MSS	484.1667	285.8333	54
6127	-	3	0	60	MSS	60	MSS	606.6667	466.6667	54
6128	-	2	0	40	MSS	50	MSS	408.3333	420	36
6129	+	NG	0	40	MS	15	M	262.5	117.8333	32
6130	-	0	1-	30	M	15	MR	140	87.5	18

Appendix B. Continued

ENTRY	PBC	Disease reaction		Final disease severity				AUCPC		Coefficie
		Set 1	Set 2	2022 MS		2023 OS		2022 MS	2023 OS	2022 MS
6131	-	1	2	15	M	15	M	87.5	81.66667	9
6132	-	1+	2	15	M	15	M	93.33333	81.66667	9
6133	-	0 1+	0 1	10	RMR	20	M	58.33333	99.16667	3
6134	-	0 1+	0	5	MR	1	R	23.33333	3.5	2
6135	-	0 1+	0	10	RMR	10	M	52.5	58.33333	3
6136	-	0	0	15	MR	5	RMR	46.66667	17.5	6
6137	-	0	0 1-	10	MR	5	R	58.33333	12.83333	4
6138	-	0	0	10	MR	5	MR	70	12.83333	4
6139	-	0	0	10	RMR	5	R	64.16667	17.5	3
6140	-	0,1	2	15	MS	40	MR	75.83333	141.1667	12
6141	-	0	2	60	MSS	40	MR	548.3333	361.6667	54
6142	+	1	0,1	40	M	40	M	268.3333	246.1667	24
6143	-	3	1	30	MS	60	S	309.1667	466.6667	24
6144	-	2	3	40	MS	40	MSS	431.6667	361.6667	32

PBC=Pseudo black chaff, M=Moderately resistant, R=Resistant, M=Moderately resistant/Moderately susceptible reaction, MS=Moderately susceptible, MS=Moderately susceptible and S=Susceptible. '+' mean presence while '-' means absence of chlorosis. NG=No germination

Appendix C. Stem rust and yellow rust final disease severity and area under disease progress curve for 16th SRRSN lines evaluated at KALRO-Njoro

ENTRY	GID	FDS for Yellow rust				Mean AUDPC for Yr	FDS for Stem rust				Mean AUDPC for Sr
		2022 MS		2023 OS			2023 MS		2023 OS		
6001	PBW 343	30	MSS	10	MS	116.6667	70	S	40	MSS	522.0833

6002	Cacuke	40	MS	40	MS	254.9167	80	S	90	S	761.25
6003	Robin	80	S	60	MS	729.1667	100	S	100	S	1020.833
6004	8780746	40	MS	15	MS	259.5833	40	M	15	MS	219.3333
6005	8778945	10	M	5	RMR	42	60	MSS	40	MSS	425.8333
6006	8778988	10	M	5	RMR	46.66667	30	M	10	MR	148.75
6007	8779055	10	M	5	MR	21	30	MS	20	M	184.3333
6008	8779079	15	M	5	M	72.91667	30	M	30	MSS	196.5833
6009	8779084	15	M	10	M	70	30	M	40	MSS	303.9167
6010	8779296	15	M	10	M	102.0833	20	M	15	M	113.75
6011	8779366	30	M	15	MR	212.9167	30	M	15	M	180.8333
6012	8779482	40	MS	30	MS	312.0833	40	M	40	MSS	312.6667
6013	8779498	15	M	5	MS	52.5	30	M	20	M	207.6667
6014	8779700	40	MS	20	MS	285.8333	30	M	40	MSS	286.4167
6015	8779738	5	R	5	RMR	17.5	10	MR	5	MT	54.25
6016	8779815	50	MS	40	MS	344.1667	20	MS	15	M	117.25
6017	8779838	30	M	5	M	72.91667	15	MR	15	M	114.9167

Appendix C. Continued

ENTRY	GID	FDS for Yellow rust				Mean AUDPC for Yr	FDS for Stem rust		
		2022 MS		2023 OS			2022 MS		20
6018	8780240	15	M	15	MS	61.25	30	M	20
6019	8780339	30	M	20	M	172.0833	40	MS	40
6020	8780381	50	MS	30	MS	355.8333	40	MS	20
6021	8780524	20	M	10	M	129.5	40	MS	40
6022	8780587	10	M	0		26.25	15	RMR	5
6023	8776671	50	MS	20	MS	274.75	40	MS	30
6024	8777193	20	MS	5	RMR	116.6667	15	MR	15
6031	8777487	40	MS	30	MS	266	10	MR	15
6032	8777513	5	RMR	5	RMR	17.5	30	M	15
6033	8777527	15	M	1	MS	61.83333	40	MSS	40
6034	8777671	20	M	30	MS	201.25	30	MS	20
6035	8777672	10	M	1	M	32.66667	30	M	40
6036	8777703	10	M	5	RMR	55.41667	20	M	15
6037	8777886	15	M	10	M	81.66667	40	M	40
6038	8777906	5	M	5	M	8.75	20	M	15
6039	8777945	50	MS	15	M	280	20	M	50
6040	8777950	20	M	40	MS	236.25	20	MR	15

Appendix C. Continued

ENTRY	GID	FDS for Yellow rust				Mean AUDPC for Yr	FDS for Stem rust			
		2022 MS		2023 OS			2022 MS		2023 OS	
6041	8777980	20	M	20	MS	189.5833	15	MR	10	MR
6042	8777993	50	MS	40	MS	440.4167	30	M	50	MSS
6043	8778010	50	M	30	MS	317.9167	30	M	30	M
6044	8778082	30	M	5	M	110.8333	30	M	40	MS
6045	8778137	40	M	40	MS	285.8333	20	MS	20	M
6046	8778187	40	M	30	MS	215.8333	10	RMR	10	M
6047	8778236	10	MS	10	MS	32.08333	50	MSS	50	MSS

6048	8778241	5	M	15	MS	43.75	40	MS	50	MS
6049	8778268	0		0		0	30	M	40	MSS
6058	8778453	5	MR	5	M	35.58333	40	MS	30	MS
6059	8778477	60	MSS	10	M	293.4167	20	M	30	M
6060	8778492	10	M	10	M	68.83333	30	M	20	MS
6061	8778561	20	MS	5	M	109.0833	60	MSS	40	MS
6062	8778594	20	M	5	M	88.08333	30	M	40	MS
6063	8778824	15	M	5	R	43.75	40	MS	30	MSS
6064	8781751	5	MR	15	MS	32.08333	15	MR	20	M

Appendix C. Continued

ENTRY	GID	FDS for Yellow rust				Mean AUDPC for Yr	FDS for Stem rust			
		2022 MS		2023 OS			2022 MS		2023 OS	
6065	8781927	40	MS	20	M	210	15	MR	40	M
6066	8781928	20	M	10	M	96.25	10	MR	10	M
6067	8782069	15	M	0		40.83333	30	MS	20	MS
6068	8782113	10	M	0		14.58333	30	M	20	MS
6069	8782124	50	S	40	MS	350	50	MSS	60	MSS
6070	8782130	10	M	5	RMR	29.16667	50	MSS	40	MSS
6071	8782135	5	MR	5	RMR	26.25	20	MS	40	MSS
6072	8782141	15	M	5	M	47.25	20	MS	30	MS
6073	8782586	30	M	10	M	183.75	30	M	15	MR
6074	8782588	20	MS	15	M	142.9167	15	MR	15	MR
6084	8783489	15	M	5	M	77	5	RMR	15	M
6085	8783632	10	M	5	MR	49.58333	30	M	20	M
6086	8783639	0		5	MR	2.916667	40	MSS	20	M
6087	8783677	20	M	20	MS	129.5	20	M	15	M
6088	8784017	70	S	60	MS	627.0833	30	MS	30	M
6089	8784092	40	MS	40	MS	350	60	MSS	40	MSS
6090	8784378	40	MS	20	M	274.1667	30	MS	30	MS

Appendix C. Continued

ENTRY	GID	FDS for Yellow rust				Mean AUDPC_Yr	FDS for Stem rust				Mean AUDPC_Sr
		2022		2023	OS		2022		2023	OS	
		MS					MS				
6091	8785	2	M			72.91	5	M	5	M	364.5
	115	0	S	0		667	0	SS	0	S	833
6092	8785	4	M	3	M	224.5	1	M	1		93.33
	142	0	S	0	S	833	5	R	5	M	333
6093	8784	1		1			4	M	2	M	
	809	5	M	0	M	89.25	0	R	0	S	210
6094	8785	5	M		M	20.41	2				67.08
	878		S	5	S	667	0	M	5	M	333
6095	8785	0				2.916	1	M	1	M	72.91
	438			5	R	667	5	R	0	R	667
6096		0			R						
	8785				M	5.833	1	M	1	M	72.91
	443			5	R	333	5	R	0	R	667
6097		0			R						
	8785				M	5.833	3		1	M	
	496			5	R	333	0	M	0	R	105
6098	8785	3	M	2	M	239.1	1				
	948	0	S	0	S	667	5	M	5	M	61.25
6099	8785	4	M	4	M		2		3	M	148.7
	514	0	S	0	S	350	0	M	0	S	5
6100	-	2	M	5	R	74.66	3	M	4	M	248.5
		0	S		M	667	0	S	0	SS	
					R						
6101	8785	5	M	0		14.58	4	M	3	M	254.3
	707		R			333	0	S	0	S	333
6102	8785	1	M	1	M	42	1	M	2	M	93.33
	993	0		0	S		0	R	0		333
6103	8777	5	M	3	M	288.7	5	M	5	M	301

	506	0	S	0	S	5	0	S	0	S	
6104	8782	5	R	5	M	11.66	4	M	3	M	256.6
	419		M		R	667	0	SS	0	SS	667
			R								
6105	8784	1	M	5	M	78.75	6	M	6	M	449.1
	397	5					0	SS	0	S	667
6106	8784	1	M	5	M	74.08	4	M	4	M	260.1
	401	0			S	333	0	S	0	SS	667
6107	8789	5	R	0		8.75	1	R	5	R	59.5
	894						5	M		M	
								R		R	
6108	8777	5	M	1	M	3.5	1	M	1	M	52.5
	479		R		R		0	R	0	S	

Appendix C. Continued

ENTRY	GID	FDS for Yellow rust				Mean AUDPC_Yr	FDS for Stem rust				M A
		2022 MS		2023 OS			2022 MS		2023 OS		
6109	8789734	40	MS	20	MS	239.1667	30	MS	40	MSS	
6110	8789794	30	M	15	MR	177.9167	20	MS	30	MS	
6111	8790025	20	M	15	M	99.16667	20	M	30	MS	
6112	8790027	40	M	15	MS	198.3333	5	RMR	5	M	
6113	8790046	40	M	60	MS	332.5	10	R	5	M	
6114	8790053	30	MS	10	M	177.9167	50	MSS	40	MSS	
6115	8782393	50	MS	30	MS	326.6667	40	MS	60	MSS	
6116	8790089	50	MS	30	MS	352.9167	40	MS	60	MSS	
6117	8790101	40	M	15	M	190.75	20	M	30	M	
6118	8790117	10	M	5	M	49.58333	50	MSS	40	MS	
6119	8790138	50	MS	20	M	282.9167	40	M	30	MS	
6120	8790139	15	M	5	MR	70	50	MSS	40	MSS	
6121	8790143	50	MS	30	MS	323.75	20	MS	40	MS	
6122	8790258	5	R	5	RMR	8.75	15	MS	20	MSS	
6123	8790275	20	MS	10	MS	113.75	10	RMR	20	M	
6124	8790292	50	M	20	M	247.9167	40	MSS	50	MSS	

6125	-	50	MSS	20	MS	306.25	50	MSS	50	MSS
6126	8790379	0		5	M	2.916667	60	MSS	40	MSS
6127	8790382	10	M	0		26.25	60	MSS	60	MSS

Appendix C. Continued

ENTRY	GID	FDS for Yellow rust				Mean AUDPC_Yr	FDS for Stem rust			
		2022 MS		2023 OS			2022 MS		2023 OS	
6128	8790502	40	MS	40	MS	288.75	40	MSS	50	MSS
6129	8790550	15	M	5	MR	58.33333	40	MS	15	M
6130	8790583	15	M	5	MS	79.33333	30	M	15	MR
6131	8790751	30	MS	20	MS	180.8333	15	M	15	M
6132	8790754	30	M	30	MS	204.1667	15	M	15	M
6133	8790783	50	MS	40	MS	315	10	RMR	20	M
6134	8790788	10	M	5	R	20.41667	5	MR	1	R
6135	8790800	30	MS	30	MS	176.75	10	RMR	10	M
6136	8790929	5	M	5	R	12.25	15	MR	5	RMR
6137	8790935	10	M	0		17.5	10	MR	5	R
6138	8790946	5	MS	0		2.916667	10	MR	5	MR
6139	8790951	5	MS	5	R	14.58333	10	RMR	5	R
6140	8790874	40	MS	30	MS	312.0833	15	MS	40	MR
6141	-	5	MR	5	M	26.25	60	MSS	40	MR
6142	-	30	M	5	RMR	125.4167	40	M	40	M
6143	-	70	S	60	MS	600.8333	30	MS	60	S
6144	-	70	S	60	MS	618.3333	40	MS	40	MSS

2022 MS – 2022 Main season from July – October, 2023 OS – 2023 Off-season from January - May

Appendix D. Means for yield and yield related traits for 16th SRRSN wheat lines along with checks, PBW 343, K. Robin and Cacuke evaluated at KALRO, Njoro over two seasons main season (July – October, 2022) and off season (January – May, 2023)

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6001	77.00	89.27	83.13	8.47	8.73	8.60	120.00	124.00	122.00
6002	79.80	91.93	85.87	9.53	9.93	9.73	124.33	119.67	122.00

6003	82.30	83.97	83.13	10.80	9.60	10.20	128.00	123.33	125.67
6004	74.53	99.13	86.83	9.33	9.13	9.23	129.00	133.67	131.33
6005	76.33	92.13	84.23	9.47	9.00	9.23	124.67	123.33	124.00
6006	79.87	93.00	86.43	9.00	8.07	8.53	121.67	120.33	121.00
6007	80.20	90.53	85.37	9.40	9.53	9.47	122.00	122.67	122.33
6008	79.47	81.13	80.30	7.80	7.00	7.40	119.00	120.33	119.67
6009	82.00	88.87	85.43	8.80	8.00	8.40	122.00	122.67	122.33
6010	77.33	89.73	83.53	9.40	9.13	9.27	120.00	121.00	120.50
6011	73.07	94.60	83.83	9.47	9.80	9.63	125.67	121.00	123.33
6012	71.87	89.20	80.53	8.53	9.00	8.77	120.67	121.00	120.83
6013	82.40	89.53	85.97	9.60	9.47	9.53	123.33	121.00	122.17
6014	79.73	92.07	85.90	8.47	9.00	8.73	120.00	119.67	119.83
6015	78.13	83.60	80.87	9.47	10.47	9.97	123.67	125.67	124.67
6016	70.53	91.13	80.83	8.73	8.53	8.63	124.00	121.00	122.50
6017	76.67	90.87	83.77	9.40	10.33	9.87	124.00	123.33	123.67
6018	86.13	95.33	90.73	9.67	9.60	9.63	123.33	121.67	122.50

Appendix D. Continued

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6019	82.33	93.67	88.00	8.93	13.87	11.40	124.67	122.67	123.67
6020	87.60	90.47	89.03	9.33	8.53	8.93	123.33	121.00	122.17
6021	88.20	97.40	92.80	9.13	9.20	9.17	124.00	123.67	123.83
6022	84.47	93.07	88.77	8.80	9.00	8.90	123.33	124.33	123.83
6023	78.60	88.27	83.43	8.60	8.27	8.43	122.67	122.00	122.33
6024	83.67	96.00	89.83	9.27	8.60	8.93	120.67	121.67	121.17
6030	82.07	94.80	88.43	9.40	9.33	9.37	124.33	113.67	119.00
6031	80.07	95.00	87.53	9.07	10.07	9.57	124.00	122.00	123.00
6032	83.20	96.73	89.97	10.87	9.53	10.20	123.33	126.33	124.83
6033	88.80	100.73	94.77	9.80	9.40	9.60	123.33	127.00	125.17
6034	84.93	95.53	90.23	9.07	9.07	9.07	124.00	122.67	123.33
6035	88.27	93.60	90.93	9.73	9.73	9.73	123.33	122.67	123.00
6036	87.00	93.20	90.10	9.80	9.40	9.60	123.33	122.00	122.67
6037	81.73	91.27	86.50	9.67	8.80	9.23	122.67	123.33	123.00

6038	85.87	90.47	88.17	10.33	9.53	9.93	124.00	123.33	123.67
6039	86.73	93.53	90.13	9.47	9.60	9.53	120.33	123.33	121.83
6040	86.07	91.47	88.77	10.60	10.00	10.30	245.67	121.00	183.33
6041	89.07	91.93	90.50	10.13	9.93	10.03	123.33	123.33	123.33
6042	84.80	84.87	84.83	9.40	8.93	9.17	119.33	119.67	119.50

Appendix D. Continued

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6043	84.13	85.80	84.97	9.33	8.40	8.87	123.33	122.00	122.67
6044	86.27	95.07	90.67	8.20	8.60	8.40	124.00	122.00	123.00
6045	80.20	90.20	85.20	8.87	9.00	8.93	124.00	121.00	122.50
6046	81.60	94.73	88.17	9.47	9.47	9.47	124.00	125.00	124.50
6047	88.00	92.80	90.40	10.13	9.67	9.90	123.33	122.67	123.00
6048	84.73	93.47	89.10	10.27	9.67	9.97	124.00	116.67	120.33
6049	90.07	95.33	92.70	9.93	8.60	9.27	124.00	126.33	125.17
6050	77.73	101.60	89.67	9.00	9.60	9.30	129.67	132.67	131.17
6051	83.13	87.47	85.30	9.67	9.27	9.47	120.00	120.33	120.17
6052	88.13	95.20	91.67	10.27	9.93	10.10	121.67	123.33	122.50
6053	90.87	94.73	92.80	9.13	8.80	8.97	123.67	121.00	122.33
6054	83.67	87.53	85.60	10.20	8.87	9.53	124.00	123.33	123.67
6055	81.67	86.33	84.00	8.87	8.27	8.57	122.67	127.33	125.00
6056	83.87	90.73	87.30	9.40	9.13	9.27	124.67	122.33	123.50
6057	79.33	85.87	82.60	9.40	9.47	9.43	123.33	120.33	121.83
6058	126.73	89.60	108.17	9.53	8.93	9.23	129.00	126.33	127.67
6059	81.07	89.73	85.40	8.53	8.80	8.67	121.67	121.00	121.33
6060	79.00	92.13	85.57	8.47	8.40	8.43	126.33	130.33	128.33
6061	80.60	89.87	85.23	9.73	9.33	9.53	124.00	122.67	123.33

Appendix D. Continued

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6062	76.93	90.80	83.87	8.93	8.53	8.73	127.33	127.67	127.50
6063	81.47	101.93	91.70	9.40	9.47	9.43	128.67	129.67	129.17
6064	82.07	96.13	89.10	9.20	9.40	9.30	129.00	126.33	127.67

6065	80.27	94.07	87.17	9.00	9.47	9.23	125.67	125.00	125.33
6066	78.47	86.40	82.43	9.13	9.53	9.33	124.67	126.67	125.67
6067	87.93	99.47	93.70	10.27	10.27	10.27	123.33	125.33	124.33
6068	82.40	99.60	91.00	10.60	14.53	12.57	125.00	127.33	126.17
6069	86.53	94.53	90.53	8.80	8.73	8.77	127.33	125.00	126.17
6070	85.00	92.53	88.77	8.60	8.47	8.53	125.67	125.67	125.67
6071	83.93	90.87	87.40	8.67	8.40	8.53	124.00	125.67	124.83
6072	85.40	94.33	89.87	8.87	9.00	8.93	127.33	124.33	125.83
6073	79.13	89.60	84.37	8.67	8.60	8.63	122.67	119.67	121.17
6074	79.47	90.20	84.83	8.87	8.93	8.90	119.33	120.33	119.83
6075	75.73	90.53	83.13	9.87	9.13	9.50	119.33	123.67	121.50
6076	80.07	92.20	86.13	7.67	8.27	7.97	122.67	121.00	121.83
6077	87.20	104.20	95.70	10.60	11.07	10.83	123.33	125.00	124.17
6078	81.73	92.47	87.10	8.60	8.73	8.67	123.33	120.33	121.83
6079	86.73	93.00	89.87	9.47	9.07	9.27	123.33	122.67	123.00
6080	81.40	90.87	86.13	10.07	9.27	9.67	124.00	124.67	124.33

Appendix D. Continued

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6081	81.07	87.40	84.23	9.80	9.20	9.50	123.33	125.00	124.17
6082	81.00	87.53	84.27	8.87	8.67	8.77	123.33	124.67	124.00
6083	87.40	91.27	89.33	9.60	8.20	8.90	122.67	122.67	122.67
6084	84.27	93.80	89.03	9.33	9.27	9.30	127.33	130.33	128.83
6085	80.00	91.20	85.60	9.00	8.67	8.83	126.33	125.67	126.00
6086	83.60	86.67	85.13	8.47	8.00	8.23	121.67	123.33	122.50
6087	82.53	149.20	115.87	9.20	9.40	9.30	122.33	125.00	123.67
6088	76.80	94.53	85.67	8.20	9.07	8.63	126.33	122.33	124.33
6089	85.07	93.87	89.47	8.93	8.87	8.90	126.00	121.00	123.50
6090	82.07	98.27	90.17	9.73	9.60	9.67	124.00	124.00	124.00
6091	87.33	91.40	89.37	14.60	8.93	11.77	123.33	120.33	121.83
6092	84.40	95.47	89.93	14.27	8.33	11.30	125.00	122.67	123.83
6093	83.27	92.27	87.77	10.27	9.33	9.80	120.67	123.33	122.00
6094	87.27	95.20	91.23	10.20	9.27	9.73	121.67	124.00	122.83

6095	92.47	96.67	94.57	11.27	9.27	10.27	128.00	127.33	127.67
6096	96.60	97.87	97.23	10.73	9.80	10.27	130.33	127.33	128.83
6097	94.80	99.07	96.93	10.93	10.40	10.67	128.00	129.67	128.83
6098	77.33	88.53	82.93	8.67	9.13	8.90	123.33	125.33	124.33
6099	82.93	96.73	89.83	8.60	9.13	8.87	124.00	124.00	124.00

Appendix D. Continued

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6100	86.33	92.80	89.57	9.80	8.80	9.30	123.33	118.33	120.83
6101	86.20	93.60	89.90	9.27	8.87	9.07	123.33	122.67	123.00
6102	80.40	93.73	87.07	8.87	9.13	9.00	123.33	125.67	124.50
6103	84.60	91.40	88.00	9.33	9.00	9.17	114.00	119.67	116.83
6104	82.87	85.60	84.23	9.87	8.80	9.33	127.33	128.67	128.00
6105	83.73	92.53	88.13	8.67	8.67	8.67	124.33	123.33	123.83
6106	87.40	96.07	91.73	9.60	9.80	9.70	126.33	125.00	125.67
6107	83.67	97.40	90.53	9.47	9.53	9.50	128.00	130.67	129.33
6108	84.00	91.93	87.97	9.07	8.80	8.93	126.33	132.67	129.50
6109	78.87	90.40	84.63	8.93	9.13	9.03	116.00	121.67	118.83
6110	82.27	90.27	86.27	10.00	9.93	9.97	123.00	122.00	122.50
6111	82.67	98.07	90.37	9.00	10.07	9.53	122.33	122.33	122.33
6112	78.53	96.73	87.63	8.93	9.07	9.00	125.00	121.67	123.33
6113	77.53	97.53	87.53	9.13	9.60	9.37	126.33	125.33	125.83
6114	81.80	87.13	84.47	9.67	9.27	9.47	125.33	119.67	122.50
6115	79.80	88.53	84.17	9.80	10.60	10.20	124.00	119.67	121.83
6116	82.20	104.73	93.47	9.60	10.13	9.87	126.33	122.33	124.33
6117	81.27	90.07	85.67	9.00	8.73	8.87	121.00	123.67	122.33
6118	83.00	87.53	85.27	9.40	9.07	9.23	122.67	125.67	124.17

Appendix D. Continued

ENTRY	plant height (cm)			Spike length (cm)			Maturity (Days)		
	MS	OS	Mean	MS	OS	Mean	MS	OS	Mean
6119	83.47	93.53	88.50	8.20	8.60	8.40	127.33	124.00	125.67
6120	86.73	93.47	90.10	9.53	9.47	9.50	123.33	127.33	125.33
6121	79.53	91.00	85.27	7.93	8.27	8.10	124.67	125.00	124.83

6122	91.27	101.73	96.50	10.53	10.00	10.27	123.33	118.00	120.67
6123	85.67	99.60	92.63	9.67	9.27	9.47	124.00	127.33	125.67
6124	83.87	92.80	88.33	10.20	10.07	10.13	125.00	123.33	124.17
6129	87.20	96.73	91.97	10.33	9.67	10.00	124.67	129.67	127.17
6130	87.27	94.07	90.67	9.20	8.87	9.03	124.00	127.00	125.50
6131	88.53	94.00	91.27	8.80	9.40	9.10	124.67	124.00	124.33
6132	87.87	96.60	92.23	9.67	9.73	9.70	124.67	124.00	124.33
6133	82.40	95.07	88.73	9.00	9.00	9.00	121.00	121.00	121.00
6134	88.40	94.80	91.60	10.13	9.47	9.80	124.67	124.00	124.33
6135	84.40	98.27	91.33	9.53	9.33	9.43	124.67	121.00	122.83
6136	85.47	103.27	94.37	10.13	10.13	10.13	125.67	124.00	124.83
6137	85.07	87.87	86.47	10.07	9.27	9.67	124.00	129.00	126.50
6138	80.67	92.53	86.60	10.00	9.67	9.83	124.00	129.67	126.83
6139	84.33	93.53	88.93	9.80	9.93	9.87	124.00	128.00	126.00
6140	80.40	91.47	85.93	9.33	9.13	9.23	124.67	125.00	124.83
6141	84.53	95.20	89.87	10.13	10.20	10.17	124.00	125.67	124.83
6142	88.80	98.93	93.87	10.13	10.07	10.10	123.33	123.33	123.33
6143	76.67	92.07	84.37	8.13	9.20	8.67	119.33	123.67	121.50
6144	91.33	100.93	96.13	9.00	9.53	9.27	122.33	124.33	123.33

Appendix D. Continued

	Biomass t ha^{-1}			Yield t ha^{-1}			1000-Kernel weight			Seeds spike^{-1}		
ENTRY	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	MS	OS	Mean
6001	37.86	65.48	51.67	2.04	3.02	2.53	22.14	23.76	22.95	34.67	46.00	40.33
6002	31.43	53.57	42.50	0.75	0.85	0.80	20.68	17.90	19.29	25.67	44.27	34.97
6003	40.67	20.33	30.50	2.86	4.05	3.45	20.03	25.24	22.64	52.00	36.33	44.17
6004	30.24	143.57	86.90	2.26	4.66	3.44	27.84	26.18	27.01	39.33	42.60	40.97
6005	60.71	96.07	78.39	4.18	4.88	4.53	33.95	31.73	32.84	33.00	45.60	39.30
6006	52.14	91.79	71.96	5.26	8.06	5.67	36.45	33.34	34.89	43.00	43.53	43.27
6007	60.00	84.82	72.41	5.53	4.74	7.14	36.28	36.49	36.39	53.33	53.33	53.33
6008	40.00	68.10	54.05	5.38	5.28	5.33	39.48	35.05	37.27	32.00	37.60	34.80
6009	70.48	66.25	68.36	5.22	4.22	4.72	36.67	30.11	33.39	47.67	42.47	45.07
6010	63.33	93.10	78.21	5.80	7.38	6.59	37.35	36.88	37.12	49.67	42.07	45.87
6011	29.05	91.67	60.36	2.12	4.97	3.55	29.69	30.67	30.18	41.67	52.13	46.90
6012	37.14	80.24	58.69	2.98	4.34	3.66	27.59	28.77	28.18	49.67	52.03	50.85

	81.6		75.5	5.7	6.3		33.3	34.2	33.8	34.3	50.0	42.1
6013	7	69.46	7	0	9	6.05	6	4	0	3	0	7
	34.7		49.4	3.6	4.5		35.1	33.5	34.3	44.3	31.4	37.8
6014	6	64.05	0	9	8	4.13	2	0	1	3	0	7

Appendix D. Continued

							1000-Kernel			Seeds spike^{-1}		
	Biomass t ha^{-1}			Yield t ha^{-1}			weight					
ENTRY	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	MS	OS	Mean
	78.3		75.7	4.6	7.2		35.1	35.1	35.1	55.3	46.9	51.1
6015	3	73.21	7	7	4	5.95	6	1	3	3	3	3
	38.1		58.8	3.1	4.7		37.8	28.2	33.0	43.3	48.6	45.9
6016	0	79.52	1	2	7	3.94	2	4	3	3	0	7
	56.1		65.6	4.8	6.4		35.3	34.5	34.9	61.0	53.0	57.0
6017	9	75.00	0	2	0	5.61	7	1	4	0	0	0
	73.3		77.5	6.8	5.6		42.2	35.0	38.6	49.0	51.9	50.4
6018	3	81.67	0	1	2	6.22	3	7	5	0	3	7
	58.1		69.5	4.9	4.1		39.5	28.1	33.8	50.3	40.3	45.3
6019	0	80.95	2	6	4	4.55	0	3	1	3	3	3
	75.4		86.7	6.4	6.9		33.6	32.9	33.3	53.6	47.0	50.3
6024	8	97.98	3	8	5	6.71	4	8	1	7	0	3
	50.0		62.0	5.7	4.9		31.3	30.5	30.9	49.6	38.1	43.9
6025	0	74.17	8	5	5	5.35	7	7	7	7	3	0
	65.4		64.8	5.6	6.7		46.4	41.6	44.0	26.6	44.2	35.4
6026	8	64.29	8	6	6	6.21	2	5	4	7	7	7
	54.2		60.7	7.1	3.4		31.7	29.1	30.4	43.0	38.1	40.5
6027	9	67.14	1	4	6	5.30	0	9	5	0	3	7
6028	23.2	83.81	53.5	3.1	4.9	4.04	29.6	32.2	30.9	40.3	47.7	44.0

	9		5	8	1		4	4	4	3	3	3
	93.1		92.3	6.4	6.3		36.9	40.3	38.6	53.0	44.4	48.7
6029	0	91.61	5	5	4	6.39	3	3	3	0	0	0
	32.3		42.9	4.6	4.6		40.8	34.5	37.6	44.0	42.5	43.2
6030	8	53.57	8	8	7	4.67	2	2	7	0	3	7
	50.7		73.8	5.0	5.1		41.0	32.7	36.8	36.3	50.0	43.1
6031	1	97.02	7	6	9	5.12	6	1	8	3	0	7
	98.3		98.1	7.7	7.3		45.1	38.0	41.5	29.3	50.7	40.0
6032	3	98.04	8	5	2	7.54	0	4	7	3	3	3
	77.3		76.9	5.4	6.1		39.1	35.4	37.2	49.6	39.8	44.7
6033	8	76.61	9	1	8	5.80	1	6	8	7	0	3
	62.1	108.5	85.3	5.6	6.1		37.8	33.9	35.8	39.0	56.5	47.7
6034	4	7	6	6	1	5.88	0	1	5	0	3	7
	76.1		73.1	5.9	7.6		41.7	41.3	41.5	60.3	49.5	54.9
6035	9	70.00	0	7	8	6.82	9	2	5	3	3	3
	89.5		91.9	5.5	7.1		38.1	32.5	35.3	46.6	53.0	49.8
6036	2	94.46	9	6	1	6.33	6	0	3	7	0	3
	60.9		73.6	6.2	6.0		38.6	35.7	37.2	38.6	51.0	44.8
6037	5	86.43	9	7	6	6.16	5	5	0	7	7	7

Appendix D. Continued

	Biomass t ha^{-1}			Yield t ha^{-1}			1000-Kernel weight			Seeds spike^{-1}		
ENTRY	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	MS	OS	Mean
	91.9		89.0	7.1	7.2		41.4	43.2	42.3	31.0	43.1	37.0
6038	0	86.25	8	3	9	7.21	3	1	2	0	3	7
	51.6		63.1	4.2	7.2		36.2	33.6	34.9	40.3	43.6	42.0
6039	7	74.52	0	6	3	5.75	7	5	6	3	7	0
	78.3		68.6	5.4	4.6		37.0	32.4	34.7	49.6	53.0	51.3
6040	3	58.93	3	1	8	5.05	9	9	9	7	7	7
	74.7		75.6	5.5	4.1		35.0	32.2	33.6	41.0	44.4	42.7
6043	6	76.43	0	8	1	4.85	8	0	4	0	7	3
6044	64.5	84.17	74.3	6.4	5.1	5.77	36.7	31.6	34.2	41.3	43.1	42.2

	2		5	1	3		6	4	0	3	3	3
	44.7	106.0	75.4	7.1	5.9		35.6	30.1	32.9	43.3	44.6	44.0
6045	6	7	2	2	1	6.51	6	7	1	3	7	0
	54.2		68.3	5.3	6.4		36.3	33.3	34.8	48.6	52.4	50.5
6046	9	82.50	9	2	9	5.90	3	8	6	7	0	3
	83.2		86.2	5.9	5.3		34.4	27.7	31.1	56.3	51.8	54.0
6047	1	89.29	5	0	1	5.61	5	8	2	3	0	7
	51.4		72.0	5.0	5.0		30.0	27.8	28.9	51.3	47.0	49.1
6048	3	92.74	8	0	3	5.02	8	0	4	3	0	7
	79.7	107.8	93.8	8.1	6.1		38.1	31.3	34.7	50.3	52.0	51.2
6049	6	6	1	8	1	7.14	7	2	4	3	7	0
	49.5	119.8	84.6	3.7	3.8		31.5	28.2	29.8	42.6	44.2	43.4
6050	2	2	7	2	4	3.78	7	1	9	7	0	3
	65.0		64.1	5.6	4.4		42.9	33.8	38.4	53.3	42.4	47.8
6051	0	63.21	1	5	8	5.06	6	6	1	3	0	7
	72.8		97.2	6.2	5.9		44.8	38.5	41.6	32.6	51.2	41.9
6052	6	58.39	0	9	2	6.11	4	4	9	7	7	7
	71.9	122.5	97.2	6.0	4.8		39.2	32.7	35.9	47.0	53.2	50.1
6053	0	0	0	6	3	5.44	4	0	7	0	7	3
	50.4		62.3	6.5	4.5		43.0	41.1	42.1	44.0	55.7	49.8
6054	8	74.29	8	7	2	5.55	8	5	1	0	0	5
	67.8		83.5	6.6	4.8		45.4	41.3	43.3	41.6	46.1	43.9
6055	6	99.17	1	3	4	5.74	2	1	6	7	3	0
	58.5	100.7	79.6	5.4	6.0		35.6	27.0	31.3	45.6	50.8	48.2
6056	7	1	4	2	7	5.75	0	9	5	7	0	3
	63.1		78.1	7.9	5.1		39.7	30.1	34.9	49.0	50.8	49.9
6057	0	93.21	5	3	8	6.56	8	4	6	0	7	3
	41.1		60.7	4.2	6.0		39.9	32.4	36.2	49.6	47.0	48.3
6058	9	80.36	7	5	8	5.16	9	5	2	7	7	7

Appendix D. Continued

ENTRY	Biomass t ha^{-1}			Yield t ha^{-1}			1000-Kernel weight			Seeds spike^{-1}		
	S1	S2	Mea	S1	S2	Mea	S1	S2	Mea	MS	OS	Mea

			n			n			n			n
6059	53.33	63.10	58.21	4.15	5.76	4.95	32.90	37.46	35.18	42.00	39.70	40.85
6060	52.14	101.07	76.61	4.76	5.25	5.01	36.98	29.86	33.42	40.33	48.13	44.23
6061	64.76	92.14	78.45	4.84	4.31	4.57	31.34	25.57	28.45	34.67	44.80	39.73
6062	45.00	93.10	69.05	5.43	3.53	4.48	34.30	29.69	31.99	48.67	46.47	47.57
6063	55.24	94.40	74.82	6.00	4.19	5.09	37.00	27.26	32.13	37.33	42.47	39.90
6064	50.48	128.69	89.58	5.59	7.08	6.33	42.92	39.93	41.42	46.67	49.73	48.20
6065	39.76	75.12	57.44	3.96	6.30	5.13	34.11	25.31	29.71	39.00	49.67	44.33
6066	45.24	112.98	79.11	4.44	5.98	5.21	35.51	31.89	33.70	55.33	54.13	54.73
6067	82.62	120.24	101.43	6.29	4.94	5.62	36.75	37.01	36.88	55.33	55.70	55.52
6068	41.14	100.00	70.57	5.29	8.43	6.86	39.46	36.66	38.01	53.67	47.27	50.47
6077	53.10	74.82	63.96	6.39	10.28	8.34	41.42	38.14	39.78	49.67	48.60	49.13
6078	40.71	60.12	50.42	5.53	5.30	5.41	36.47	34.66	35.57	38.33	47.33	42.83
6079	68.10	78.93	73.51	6.61	4.96	5.78	42.63	35.19	38.91	47.00	42.73	44.87
6080	77.62	85.36	81.49	6.83	8.05	7.44	44.05	41.27	42.66	44.00	46.07	45.03
6081	77.14	90.12	83.63	7.18	10.48	8.83	42.73	44.14	43.43	53.00	42.80	47.90
6082	58.57	73.93	66.25	6.27	8.55	7.41	36.91	36.81	36.86	36.67	47.07	41.87

6083	52.86	80.60	66.73	5.99	7.84	6.92	38.13	33.28	35.70	45.67	47.67	46.67
6084	51.43	101.31	76.37	6.29	6.19	6.24	37.51	38.87	38.19	44.00	43.00	43.50
6085	51.19	108.33	79.76	6.27	6.02	6.15	39.27	34.00	36.63	45.00	0.00	22.50

Appendix D. Continued

ENTRY	Biomass t ha^{-1}			Yield t ha^{-1}			1000-Kernel weight			Seeds spike^{-1}		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	MS	OS	Mean
6086	78.81	102.62	90.71	6.96	6.41	6.69	39.37	36.96	38.16	43.33	41.00	42.17
6087	58.81	84.29	71.55	6.12	6.31	6.21	38.42	34.46	36.44	48.00	46.07	47.03
6088	26.79	69.46	48.13	1.61	2.26	1.93	21.18	17.72	19.45	34.67	46.53	40.60
6089	45.00	65.48	55.24	3.02	2.04	2.53	24.52	17.40	20.96	31.33	44.27	37.80
6090	36.67	86.79	61.73	3.69	2.97	3.33	33.60	22.81	28.20	45.33	52.07	48.70
6091	81.19	76.19	78.69	7.21	5.06	6.14	35.29	34.50	34.89	50.00	41.67	45.83
6092	36.67	91.90	64.29	4.04	4.72	4.38	33.97	33.61	33.79	38.67	48.13	43.40
6093	49.05	90.36	69.70	5.46	4.57	5.02	36.51	32.72	34.62	48.33	51.13	49.73
6094	64.52	111.79	88.15	5.95	8.40	7.17	36.64	37.93	37.28	52.33	46.53	49.43
6095	88.81	85.00	86.90	8.61	7.17	7.89	40.22	39.14	39.68	59.33	46.27	52.80
6105	60.71	93.81	77.26	5.21	4.40	4.80	33.37	30.73	32.05	47.67	49.40	48.53

6106	96.43	105.36	100.89	6.69	4.44	5.56	39.62	31.40	35.51	45.67	47.27	46.47
6107	76.43	161.43	118.93	7.42	7.73	7.58	35.75	35.14	35.44	49.67	46.40	48.03
6108	72.14	108.33	90.24	6.70	4.97	5.83	41.78	40.34	41.06	45.67	49.50	47.58
6109	59.52	87.62	73.57	5.54	3.14	4.34	28.49	21.73	25.11	41.67	61.10	51.38
6110	60.00	69.52	64.76	5.90	4.07	4.99	40.82	28.61	34.71	40.67	52.27	46.47
6111	66.43	69.29	67.86	7.87	6.26	7.06	36.55	31.33	33.94	34.00	53.13	43.57
6112	77.86	105.83	91.85	6.62	6.74	6.68	32.55	31.49	32.02	47.33	46.60	46.97
6113	44.52	82.26	63.39	5.47	5.09	5.28	32.89	29.95	31.42	45.00	52.67	48.83

Appendix D. Continued

	Biomass t ha^{-1}			Yield t ha^{-1}			1000-Kernel weight			Seeds spike^{-1}		
ENTRY	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	MS	OS	Mean
6114	56.67	86.07	71.37	4.63	3.98	4.30	32.02	30.30	31.16	45.33	43.87	44.60
6115	50.00	71.55	60.77	3.82	2.24	3.03	27.69	22.37	25.03	50.00	66.00	58.00
6116	48.21	81.31	64.76	3.44	2.41	2.93	29.00	25.19	27.09	49.67	48.40	49.03
6117	52.38	70.71	61.55	5.55	5.77	5.66	36.08	34.09	35.08	37.33	41.20	39.27
6118	70.71	74.40	72.56	7.00	3.79	5.39	33.66	27.47	30.56	43.00	41.87	42.43
6119	40.71	69.40	55.06	3.95	3.25	3.60	29.15	26.77	27.96	36.00	45.87	40.93

	67.1			6.3			39.2	35.2	37.2	46.0	43.7	44.8
6120	4	81.19	74.17	2	4.92	5.62	6	9	7	0	3	7
	44.0			4.3			28.6	22.8	25.7	39.3	50.3	44.8
6121	5	71.43	57.74	2	2.46	3.39	6	8	7	3	3	3
	78.1	113.9		7.5			44.0	38.5	41.3	52.3	48.8	50.5
6122	0	3	96.01	5	7.05	7.30	1	9	0	3	0	7
	61.1	113.8		6.5			37.5	37.7	37.6	48.0	47.3	47.6
6123	9	1	87.50	0	6.33	6.41	0	1	0	0	0	5
	55.9			5.5			38.3	32.0	35.1	46.3	42.5	44.4
6132	5	85.36	70.65	8	6.79	6.19	1	1	6	3	3	3
	55.7			4.6			33.8	28.5	31.2	41.6	42.1	41.9
6133	1	80.60	68.15	9	6.29	5.49	9	9	4	7	3	0
	75.0	157.5	116.2	7.5			38.7	37.7	38.2	53.3	50.1	51.7
6134	0	0	5	0	7.07	7.29	1	5	3	3	3	3
	47.8			5.5			38.6	29.4	34.0	47.6	42.5	45.1
6135	6	96.43	72.14	0	5.12	5.31	2	8	5	7	3	0
	84.0	170.3	127.2	8.0			40.5	39.8	40.2	51.6	44.6	48.1
6136	5	6	0	5	7.13	7.59	7	5	1	7	0	3
	92.6			8.1			36.5	35.5	36.0	45.0	47.0	46.0
6137	2	97.14	94.88	6	7.05	7.61	2	5	3	0	7	3
	66.4			5.8			36.8	40.2	38.5	49.6	43.5	46.6
6138	3	80.89	73.66	2	8.64	7.23	7	2	4	7	3	0
	68.8	109.4		6.8	10.0		37.6	38.6	38.1	49.0	55.6	52.3
6139	1	6	89.14	8	5	8.46	6	1	3	0	7	3
	36.9	124.6		4.7			32.7	28.4	30.5	36.6	41.8	39.2
6140	0	4	80.77	1	4.48	4.59	5	2	8	7	0	3
	40.3			4.8			31.9	28.8	30.3	51.6	54.8	53.2
6141	6	93.57	66.96	2	3.61	4.21	5	4	9	7	0	3
	70.2			5.7			30.5	27.9	29.2	49.3	46.4	47.8
6142	4	96.43	83.33	4	3.84	4.79	9	7	8	3	0	7
	46.4			1.9			24.2	21.3	22.8	31.6	44.3	38.0
6143	3	69.29	57.86	6	2.75	2.35	8	8	3	7	3	0
6144	35.2	25.89	30.57	2.6	3.34	2.99	28.2	26.2	27.2	39.6	50.6	45.1

4	4	4	0	2	7	0	3

MS = Main season, July – October, 2022, OS = off season, January – May, 2023

Appendix E. Temperature and rainfall variations during wheat growing seasons at KALRO Njoro

Season	Month	Temperature °C		Mean	Rainfall (mm)
		Mean maximum temperature	Mean minimum temperature		
2022 Main season	July	21.96774	9.129032	15.54839	21
	August	21.09677	9.709677	15.40323	165.5
	September	22.13333	8.833333	15.48333	231.2
	October	23.33333	8.6	15.96667	78.1
	November	22.03333	9.83333	15.93333	123.6
	Mean	22.1129	9.221075		total=629.4

2023 Off-season	January	25.35	7.419	16.3845	0.7
	February	27.57	7.554	17.562	0
	March	27	9.967	18.4835	59.3
	April	23.83	10.07	16.95	142.7
	May	23.9	9.871	16.885	113.2
	Mean	25.53	8.9762		total=315.9

Source: KALRO, Njoro meteorological station, (2022, 2023)

Appendix F. Research article

Vol. 21(5), pp. 378-407, May, 2025
DOI: 10.5897/AJAR2024.16771
Article Number: D583A0773221
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Agricultural Research

Full Length Research Paper

Screening for stem rust (*Puccinia graminis* f.sp. *tritici*) resistance in selected wheat (*Triticum aestivum* L.) lines

Millicent Achieng[1], Alex Machio Kange[2] and Miriam Karwitha Charimbu[1]*

[1]Department of Crops, Horticulture and Soils, Egerton University, P. O. Box 536-20115, Njoro, Kenya. 2Department of [2]Agriculture and Natural Science, Bomet University College, P. O. Box 701 – 202040 Bomet, Bomet, Kenya

Received 26 August, 2024; Accepted 21 October, 2024.

Stem rust, caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*), is a major threat to wheat (*Triticum aestivum* L.) production due to increased susceptibility. This study aimed to identify wheat lines in the 16th Stem Rust Resistance Screening Nursery (SRRSN) with seedling and adult plant resistance (APR) to wheat stem rust. A two-season field experiment was conducted at (KALRO) Njoro using a 12×12 partially balanced lattice design under stem rust pressure. The results showed significant ($p \leq 0.001$) effects of season, genotype, and their interaction on plant height, 1000 kernel weight (TKW), biomass, and Area Under Disease Progress Curve (AUDPC). 54.86% of the wheat genotypes had an AUDPC of ≤200, 52.78% had a CI of ≤20, and 60.42% had FDS of ≤30 indicating resistant. Grain yield ranged from 0.8 to 9.05 t ha^{-1} and TKW from 20.03 to 46.42 g. Genotypes 6022, 6095, 6096, 6107, 6134, 6136, 6137, 6138, and 6139 showed APR and high yield potential. At seedling stage, about 72.7% of the lines were resistant to stem rust race TTKTT while 27.3% of the lines were susceptible. These high-yielding and APR lines can be valuable in breeding programs for stem rust resistance.

Key words: Genotypes, screening, stem rust, resistance, virulence.

Appendix G. Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **796929**

Date of Issue: **28/May/2024**

RESEARCH LICENSE

This is to Certify that Ms.. Millicent Achieng of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: RACE IDENTIFICATION AND SCREENING OF SELECTED WHEAT (Triticum aestivum L.) LINES FOR RESISTANCE TO STEM RUST (Puccinia graminis f.sp. tritici) for the period ending : 28/May/2025.

License No: **NACOSTI/P/24/35469**

796929

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY &