

**DETECTION OF PATHOLOGICAL ISOLATES OF STEM RUST FROM
SELECTED WHEAT FIELDS AND VARIETIES' REACTIONS IN
TANZANIA**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN CROP SCIENCE OF SOKOINE UNIVERSITY OF
AGRICULTURE. MOROGORO, TANZANIA.**

2013

ABSTRACT

This study was conducted to determine status of wheat stem rust disease in Tanzania and how the available wheat varieties react to the prevailing isolates of this disease. The study aimed at identifying isolates groups and resistant varieties, and draw implications for farmers, research and extension. Wheat stem rust disease survey was conducted in twenty fields from Manyara, Mbeya, Iringa Kilimanjaro and Arusha regions. Of all the fields surveyed 60% were infected by stem rust disease with severity between 26 – 50%. Fifteen percent of fields were affected at 11 – 25% disease severity and 10% had 51 – 75%. The lowest disease severity of 5% was scored in Iringa region whilst in northern highlands none of the field was infected at a severity level of less than 15%. Seventeen isolates of *Puccinia graminis* f. sp. *tritici* collected from the selected regions were all virulent to Viri wheat variety. Isolates from Manyara and Arusha were more virulent followed by those from Kilimanjaro, Mbeya and Iringa. The most destructive isolate encountered in different regions caused various severity levels on Viri wheat variety with average severity of 37.5%. Most varieties (Mamba, Mbayuwayu, Selian, Juhudi, Azimio 87, Chiriku, and Riziki) under production were susceptible to wheat stem rust disease. Sifa variety response to wheat stem rust isolates both in the field and under controlled environment indicated an outstanding resistant to the most aggressive isolates and is thus recommended for disease management. Technology transfer is significantly important for farmers to get rid of using susceptible varieties e.g. Viri. Plant protection strategies such as crop rotation and weeding are not implemented accordingly in some areas; such cases need to be addressed as well to improve wheat production in Tanzania.

DECLARATION

I, **Agatha Aloyce**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

.....

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.....

Date

The above declaration is confirmed by

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Date

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ACKNOWLEDGEMENTS

First and foremost I am thankful to God for giving me the inspiration to move on amidst hardships. To you O God, I ascribe all the greatness and glory.

I am thankful to the Tanzania Commission for Science and Technology (COSTECH) for financial support. Special appreciation is extended to my employer for granting me the study leave.

I am greatly indebted to Dr. D. P. Mamiro for her vital supervision, guidance, support, generosity, precious inspiration and encouragement during my study which was a tool for timely completion. I am sincerely grateful for her special interest in my work and for her profound scientific advice. Her constructive critique, comments, stimulating discussions, intellectual advice, critical remarks in reviewing dissertation have been of great value to me. It was an enjoyable working experience with her. She educated me on the importance of collecting as much data as possible in order to have a better explanation of the results. She was always ready when I needed her help. In the finishing stages, her weekly, daily and sometimes hourly attentions were crucial and immeasurable. Few times in life it is possible to find people with such good combination of professional capacity and human qualities such as D. P. Mamiro.

The Department of Crop Science and Production staff, Sokoine University of Agriculture (SUA), led by Prof. C. L. Rweyemamu, all classmates and interested personnel such as W. Adeniji is acknowledged for their enormous support. I extend

my thanks to the postgraduate staff, SUA for providing tremendous guidance. Instructions given during dissertation writing, editing and manuscript organization were immense and indispensable.

My profound gratitude is to all wheat stakeholders in different regions for their input and support given during field survey. Farmers in Manyara, Arusha, Kilimanjaro, Mbeya and Iringa regions were always willing to help, communicate and give suggestions. I am obliged to Mr. Mamuya and Dr. C. Kuwite of Agricultural Research Institute (ARI) Selian, and Mr. Nyagasi and A. Elanga of ARI Uyole and Mr. Alphonse Mnyaw of Hydom Lutheran Hospital Farm (HLHF), for generously sharing all their expertise, experience and wheat seed provision.

I am very proud of my father Aloyce Gabriel and my mother Emeliana Salu to see this dissertation completed. Without my parents, my brothers Thobias, Gabriel, Augustino, Mathias and Emanuel and sister Valeria I would neither end up being a pathologist nor a researcher. To all, very warm and sincere thanks for everything you gave me on my way. This appreciation is extended to my brother and sister in law Paskali and Paskalina who stayed with my family. The love they always showed to me was a source of encouragement.

Many people have helped me at different stages of this work but it is impossible to adequately acknowledge everyone who contributed to this work. Should I miss your name please do not consider it as an act of ingratitude. I consider this achievement is part of your assistance and I am grateful to everyone whose contribution has enabled this work to be concluded fruitfully in time.

Lastly, is a note of special thanks to my beloved family for the perseverance they went through during my absence from home. The good understanding and precious help from my lovely husband J. Francis, who was patient with me, will be remembered. I was often absent for weeks away in the field and worse when I had to leave you with our children Francis and Emiliana. Francis and Emiliana do not take it as a deliberate action of leaving you but rather building up your future.

DEDICATION

This work is dedicated to my father Aloyce Gabriel, my mother Emeliana Salu, my husband Joseph Francis and my children Francis and Emiliana.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
ARI	Agricultural Research Institute
ASPS	Agricultural Sector Programme Support
CABI	Commonwealth Agricultural Bureaux (CAB) International
CIMMYT	International Maize and Wheat Improvement Center
COSTECH	Tanzania Commission for Science and Technology
CRBD	Complete randomized block design
CV	Coefficient of variation
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO statistical database
Fig.	Figure
f. sp.	<i>Forma specialis</i> i.e. special form
GDP	Gross domestic profit
GIS	Geographic information system
GPS	Global positioning system
HLHF	Hydom Lutheran Hospital Farm
ICRISAT	International Crops Research Institute for Semi - Arid Tropics
µm	micrometre
MN	Minnesota
MOAC	Ministry of Agriculture and Cooperatives
NRAES	Natural Resource, Agriculture and Engineering Service
p	level of statistical significance
RCBD	Randomized complete block design

SARI	Selian Agricultural Research Institute
s.e	standard error
St.	Saint
SUA	Sokoine University of Agriculture
Ug99	Uganda 1999 (Wheat stem rust isolate identified and named in Uganda in 1999)
UK	United Kingdom
URT	United Republic of Tanzania
USA	United States of America
USDA	United States Department of Agriculture

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.), the most prominent crop species is the first cereal crop to be cultivated and domesticated in the world. Wheat is highly nutritious, such that besides being rich in carbohydrates, it has protein content that exceeds all other cereal crops (Singh *et al.*, 2008). The annual production of wheat globally exceeds 600 million tones; nearly half of which is from developing countries (FAO, 2002). Wheat is grown on about 225 million hectares worldwide from the equator to latitudes of 60° N and 44° S and at altitudes 3000 metre above sea level (m.a.s.l) (Aquino *et al.*, 2002).

In Tanzania, wheat is mainly produced in Manyara, Arusha, Kilimanjaro Mbeya and Iringa regions (Mgonja, 1990). The wheat industry in Tanzania is growing tremendously, most millers are increasing their capacity and new mills are being commissioned. The rapid growth of wheat industry is mainly due to rapid urbanization associated with increased consumption of wheat products in the urban areas of developing countries (Pingali, 1999; Singh *et al.*, 2008). In Tanzania wheat is consumed as whole grains, bread, pancakes, biscuits and macaroni.

The global wheat production increased from 2.26 t ha⁻¹ in 1980 to 2.57 t ha⁻¹ in 1994 (Pingali, 1999). In contrast, Tanzania wheat grain yield decreased from 2 t ha⁻¹ in 1980 (Nyaki, 1993) to less than 1.3 t ha⁻¹ in 1998 (FAO, 2002). In the southern highlands of Tanzania for example, the genetic yield potential of the currently improved wheat varieties is 4 t ha⁻¹ while farmers' yields are only 0.4 t ha⁻¹ (Mussei

et al., 2001). Wheat production in Tanzania is below the projected demand and production has been either decreasing or stagnated during 1980 to 2000 (Table 1.1) while consumption was increasing from 6.7 to 10.9 kg person⁻¹ year⁻¹ (FAO, 2002). According to URT (2009) in 2008/09 cropping season, the estimated demand for wheat was 204 156 tones whilst production was only 97 901 tones (Table 1.2), resulting into increased imports (Kilima, 2006).

The causes of low wheat yields are complex and include losses due to disease, weed and insect pests (Loewen – Rudgers, 1993). Most fungal pathogens reported on wheat globally occur in Tanzania, and usually infect susceptible varieties concomitantly. Rusts are among the fungal diseases that are feared by wheat farmers in Tanzania. Wheat rusts can cause losses ranging from slight to complete destruction of a wheat crop depending on the disease severity and variety grown (Singh *et al.*, 2008). Rusts also lower crop's forage value, predispose plants to other diseases and weaken stems so plants lodge; causing mechanical harvesting impossible (Agrios, 2004).

Wheat rust pathogens are important because of their wide distribution, capacity to form new isolates that can attack previously resistant varieties, ability to move long distances and potential to develop rapidly under optimal growth conditions that result in serious yield losses (CIMMYT, 2005). Wheat stem rust fungus (*Puccinia graminis* f. sp. *tritici*) has been continuously producing new isolates that can attack previously resistant varieties (Sing *et al.*, 2008).

Scientific information on wheat stem rust disease distribution, incidence and severity in the wheat growing areas of Tanzania is lacking. There is no study that has investigated the relationship between disease severity and the agro – ecological location of the fields. Such knowledge gaps have hindered efforts to increase wheat production in Tanzania. Additionally, most models relating yield to disease incorporate information on disease incidence and severity (Braun *et al.*, 2010). Such information can be obtained through disease surveys (Hodson *et al.*, 2005). Surveys can also reveal the effectiveness and desirability of existing or recommended disease management practices such as improved varieties (Mussei *et al.*, 2001).

Scientific information on the prevailing isolates of stem rust and reaction of varieties against these isolates which is a devise for plant protection and breeding strategies are also lacking in Tanzania. The isolates are detected when previously resistant variety becomes infected and they differ in their pathogenicity to their host (Agrios, 2004; Singh *et al.*, 2008). Stem rust pustules on a susceptible variety are quite large and lesions often elongate, tearing the cuticle and epidermis as they erupt (Agrios, 2004; Peterson *et al.*, 1948). Depending on the resistance of each variety relative to the virulence of the specific isolate of the pathogen, lesions vary considerably in size and number (Stakman *et al.*, 1962; Brown and Hormoller, 2002). The generation time (period from inoculation to sporulation) can be isolate specific and thus isolates can be grouped as slow, normal or fast (Stakman *et al.*, 1962; Jin *et al.*, 2007b).

1.1 Objectives

1.1.1 Overall objective

The overall objective of this study was to ascertain the current status of wheat stem rust disease and identify varieties that are resistant to isolates found in Tanzania.

1.1.2 Specific objectives

- i. To determine wheat stem rust disease distribution, incidence and severity in selected wheat fields in Tanzania
- ii. To identify possible differential varieties and categorize isolates on their virulence
- iii. To evaluate varietal reactions against the most virulent wheat stem rust isolates

Table 1.1: Wheat production, area, yield, imports and consumption in Tanzania 1980 - 2000

Year	1980	1985	1990	1995	2000
Production (t)	90 000	72 000	83 700	84 000	90 000
Harvested area (ha)	64 000	42 450	50 300	54 500	60 000
Yield (kg ha ⁻¹)	1 406	1 696	1 664	1 541	1 500
Import (t)	23 496	46 000	50 000	47 000	317 676
Consumption (kg yr ⁻¹)	6.7	5.9	5.0	4.9	10.9

Source: FAO (2002)

Table 1.2: Regional wheat production in Tanzania for 2008/09 season

Region	Area (ha)	Yield (t ha ⁻¹)	Production (t)
Arusha	249 497	0.8	25 211
Iringa	401 095	0.9	8 068
Kilimanjaro	271 322	0.5	4 903
Manyara	380 989	0.6	58 563
Mbeya	320 251	1.0	752
Morogoro	335 434	0.8	33
Rukwa	280 757	1.3	278
Tanga	25 026	0.3	92

Source: URT (2009)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wheat Production in Tanzania: Potentials and Challenges

About 280 000 ha in Tanzania are suitable for wheat production but only 71 000 ha (25%) is being cultivated every year (FAO, 2002) and production falls short of requirements (Kilima, 2008). Tanzania relies heavily on imports of wheat and wheat products to meet the demand, in 2001 for instance, Tanzania produced 90 000 t and imported 60 000 t of wheat to satisfy its demand. Present potential requirements for wheat production such as suitable lands and climate should be utilized to meet the country demand and produce surplus for export. A neighbour country, Kenya has been producing surplus for export since 1968 having more than 160 000 ha of wheat under production each year (FAO, 2002).

Different countries have adopted green revolution to increase crop production including wheat. In Mexico for example, green revolution increased wheat production between 1950 and 1980 (Pingali, 1999). Adoption of green revolution in India enabled India to increase its production from 11 million tonnes in 1967 to more than 68 million tonnes of wheat in 2001 (FAO, 2002). Although Tanzania has potentials which can be utilized to increase wheat production, such increase has not been realized. Success of green revolution depended upon free international movement of wheat germplasm and production technologies.

2.2 Wheat Diseases

Globally important fungal diseases of wheat, caused by biotrophs, include rusts, powdery mildew, bunts and smuts; whereas, those caused by hemibiotrophs include

Septoria tritici leaf blotch, *Septoria nodorum* blotch, spot blotch, tan spot, and Fusarium head blight. The biotrophs are highly specialized and significant variation exists in the pathogen population for virulence to a particular variety.

2.3 Types of Wheat Rust Diseases

Wheat rusts which comprise of stem rust (*Puccinia graminis* f.sp. *tritici*), leaf rust (*Puccinia recondite*) and stripe rust (*Puccinia striiformis*) are known to be the most important diseases of wheat worldwide (Keiper *et al.*, 2006). The morphology and optimal growth conditions differ between the *Puccinia* spp. (Wiese, 1987). They are obligate pathogens of living tissue and thus require a host as a “green bridge” in order to survive until the next growing season (Staples, 2003). The rusts may also survive as dormant mycelia on self-sown wheat during the offseason. When sporulation occurs, the epidermis of the plant bursts open to release the spores. The damage to the epidermis reduces the ability of the plant to photosynthesise and increases the rate of transpiration and respiration. This reduces the yield from the plant. The degree to which any plant becomes infected is dependent on a variety of factors including cultivar, pathotype and chemical control methods (Wiese, 1987).

The *Puccinia* spp. has lifecycle that consists of both sexual and asexual reproduction (Fig. 2.1). Asexual reproduction occurs primarily on wheat, while sexual reproduction takes place on an alternative host. For stem rust this alternative host is *Barberis vulgaris* and for leaf rust it is species of the genus *Thalictrum*, and the alternative host for yellow rust has yet to be identified. None of these alternative hosts occur in Tanzania, and the wheat rusts have not been noted on indigenous species.

Urediniospores, formed by the uredinium on infected plants, are tiny single celled, dikaryotic spores that are released in million. They are dispersed by wind and water and can spread the infection over vast distances (Keiper *et al.*, 2006). Germination of the urediniospore on a susceptible host plant requires water, and so is usually started by rain or heavy dew. Germ-tubes grow from the infection site until they reach the stomata, where an apressorium is formed. The formation of a structure bordering the membrane inside the stomata is indicative of a successful infection. This structure then forms haustorium mother cells as well as mycelia. The speed at which the infection proceeds is dependent on the rust pathotype, cultivar as well as the environmental conditions such as temperature and can vary between five and eight days. The urediniospores can re-infect the wheat plant and asexual reproduction can continue indefinitely (Wiese, 1987; Knott, 1989).

Sexual reproduction, which allows the pathogen to survive periods of environmental stress and introduces variation, can occur after urediniospore formation is completed. Dikaryotic teliospores form. The teliospores germinate, fusing the dikaryon into a single nucleus which then undergoes meiosis producing a pro-mycelium that consists of four haploid basidiospores. These basidiospores can only infect the alternative host and not wheat. Once the host has been infected by the basidiospores, positive or negative pycnia are formed. Positive pycnia fuse with negative pycnia to form a dikaryon again. The dikaryon develops to form an aecium which produces aeciospores that can infect wheat (Wiese, 1987; Knott, 1989). Within each special form (f. sp.) of rust there are many pathogenic isolates. These can attack only certain species within the genus and can be detected and identified by the set of susceptible varieties they infect (Agrios, 2004).

2.3.1 Wheat leaf rust disease

Wheat leaf rust disease flourishes in regions where wheat becomes ripe late in the season (Wiese, 1987). One of the typical signs of leaf rust infection is the formation of orange uredia on the dorsal surface of the leaf. The uredia are up to 1.5 mm in diameter and produce vast numbers of spherical spores of around 20 µm diameter. Telia develop as the growing season ends and produce black teliospores (Wiese, 1987; Knott, 1989). Leaf rust disease occurs on wheat and triticale. Infection sites are on leaf surfaces and sheaths. Infection at temperatures between 16 - 27°C causes significant yield losses, by reducing kernels number, quality, and weight (Agrios, 2004).

2.3.2 Wheat stripe rust disease

Wheat stripe rust disease is distinguished by the presence of light yellow, straight-sided pustules that occur in stripes on leaves and heads. Pustules arrangement into stripes is an important distinguishing characteristic of this disease. As the pustules mature, yellow-orange spores are produced. As the disease progresses, tissues around the pustules turn brown and dry resulting in a scorched appearance. Leaves chlorosis can be quite evident and fields with severe symptoms may be easily detectable from a distance. While leaf- and stem rust are able to tolerate a wide temperature range, yellow rust cannot. Its optimum temperature for infection is between 9°C and 11°C and optimum development occurs at temperatures below 23°C (Wiese, 1987; Knott, 1989).

2.3.3 Wheat stem rust disease

2.3.3.1 Characteristics of wheat stem rust disease

Wheat stem rust disease mostly occurs on both stem and the leaves of wheat plants while leaf and stripe rust occur on the leaves. Urediniospores are dark reddish brown and may occur on both sides of leaves, on the stem and spikes. With light infection the urediniospores are usually separated and scattered, but with heavy infection they may coalesce (Agrios, 2004). Stem rust pustules on leaves develop mostly on the lower side, but may penetrate and produce limited sporulation on the upper side. On the leaf sheath and glumes, pustules rupture the epidermis and give a ragged appearance. The uredia are quite large, about 3 mm by 10 mm in size; the urediniospores are oval and 15 - 20 µm by 40 - 60 µm in size (Wiese, 1987; Knott, 1989). Stem rust disease is not restricted to wheat, it also occurs on rye and triticale (Pretorius *et al.*, 2000). As infected plants mature, uredinia convert into telia; changing colour from red into dark brown to black, thus the disease is also called black rust disease (Roelfs and Groth, 1988).

2.3.3.2 Epidemiology of wheat stem rust disease

Puccinia graminis f. sp. *tritici* survives the same environmental conditions that wheat crop survives. Cool nights followed by warm days (19 - 30°C) and long wet duration of canopy are ideal conditions for wheat stem rust disease. New pustules are produced every 7 - 10 days during the growing season and severe rusting occurs 14 - 17 days after the initial infection (Agrios, 2004). The minimum, optimum and maximum temperatures for spore germination are 2°, 15° to 24°, and 30°C, respectively and for sporulation are 5°, 30° and 40°C, respectively (Roelfs *et al.*,

1992), thus providing a wide range of favourable environmental conditions. Maximum infection is obtained within 8 to 12 hours of dew at 18°C followed by light provision while the dew slowly dries and the temperature rises to 30°C (Luig, 1985). Stem rust pathogen grows between 15°C and 40°C with an optimal growth occurring at 26°C (Pretorius *et al.*, 2000). Wheat stem rust disease is more important late in the growing season, on late-sown and late maturing wheat cultivars (Sing *et al.*, 2008). In the greenhouse, reduced light is often the reason for reduced infection.

2.3.3.3 Historical significance of wheat stem rust disease

Wheat stem rust disease is historically known to cause severe devastation and is the most feared disease in various countries in all continents where wheat is grown (Saari and Prescott, 1985). The major wheat stem rust disease epidemics occurred in Ethiopia during 1993 and 1994 when a popular wheat variety “Enkoy” suffered major losses (Shank, 1994). In 1998, severe wheat stem rust disease infections were observed on wheat in Uganda, and a race, designated as Ug99 with virulence on over 90% of wheat varieties in the world was recorded (Pretorius *et al.*, 2000). Race Ug99 was subsequently detected in Kenya and Ethiopia in 2005 and in Sudan and Yemen in 2006 (Wanyera *et al.*, 2006). A new variant of this race was detected in Kenya in 2006 (Jin *et al.*, 2007b), causing major losses in 2007 wheat cropping season.

In Tanzania, the team of Tanzanian and international scientists has confirmed three variants in the Ug99 lineage in wheat stem rust samples collected during August, 2009 (Ravi *et al.*, 2011). Races confirmed by the USDA Cereal Disease Laboratory,

St. Paul, MN, USA were; TTKSK, TTKST and TTTSK. Races TTKSK and TTTSK were present in samples from the Hanang, and Ngorongoro areas, whilst race TTKST was present in the Karatu and Monduli areas.

2.3.3.4 Pathology and dissemination modes of wheat stem rust isolates

Wheat stem rust fungus (*Puccinia graminis* f.sp. *tritici*) is heteroecious, alternating between a telial host in Poaceae and an aecial host in Berberidaceae, and macrocyclic, with five spore states that are distinct in morphology and function. Wheat stem rusts produce large numbers of urediniospores during the crop season and wind dispersion transmit these urediniospores onto the same or new host plants in the vicinity or distantly. Urediniospores disseminate to newly emerged tissues of the same plant or adjacent plants to cause new infections, or can be transported through wind in long distances (Luig, 1985).

In the case of long-distance dispersal, spore depositions on crops in a new area are often associated with rain showers. Brown and Hovmoller (2002) reported that wheat stem rust is one pathogenic group with reasonably strong evidence for unassisted, long-distance dispersal under natural airborne conditions. The introduction of a wheat stem rust into Australia from southern Africa in 1969 provides strong evidence for being unassisted natural long distance wind-borne dispersals (Steele *et al.*, 2001). The enabling factor in this mode of dispersal for rusts is the robust nature of spores ensuring protection against environmental damage. Deposition in new areas is primarily through rain-scrubbing of airborne spores onto susceptible hosts. Assisted long-distance dispersal, typically on travellers clothing or

infected plant material, is another increasingly important element in the colonization of new areas by pathogens. Steele *et al.* (2001) insisted that despite strict phytosanitary regulations, increasing globalization and air travel both increase the risk of pathogen spread. More recently, concerns over none accidental release of plant pathogens as a form of “agricultural bio-terrorism” have arisen, with wheat stem rust considered one pathogen of concern primarily due to its known ability to cause devastating production losses to a major food staple (Leonard, 2001).

The second major mode of dispersal for pathogens like rusts is stepwise range expansion. This typically occurs over shorter distances, within a country or a region, and has a much higher probability than the first described dispersal mode. This probably represents the most common mode of dispersal for rust pathogens (Singh *et al.*, 2008).

The third mode of dispersal, extinction, and re-colonization, could be a sub-mechanism of stepwise range expansion which occurs in areas that have unsuitable conditions for year-round survival. Typically these are temperate areas where hosts are absent during winter or summer (Stakman, 1962; Brown and Hovmoller, 2002). Urediniospores from these areas are then blown to wheat fields in other areas and initiate disease.

2.3.3.5 Causes of variation in wheat stem rust isolates

Wheat stem rust fungi are constantly changing with time and from one area to another. Evolution of new virulence through migration, mutation, recombination of

existing virulence genes and their selection has been more frequent in rust fungi (Singh *et al.*, 2008). Mutation towards virulence in existing populations followed by selection on susceptible hosts is at present considered to be the most important evolution mechanism for wheat stem rust pathogen to acquire new virulence to overcome varietal resistant. Where an alternate host is present, it is possible to have new combinations of virulence through sexual recombination; however, it is limited at present to few areas of the world. Rare asexual recombination is also known to occur through exchange of nuclei between conjugating hypha of two isolates that have by chance infected same tissues.

2.3.3.6 Detection efforts to mitigate wheat stem rust isolates

Various efforts have been in place in different countries to control wheat stem rust disease. Research findings from Peterson *et al.* (1948) and Roelfs *et al.* (1992) reported that wheat stem rust disease response has two components (i) severity based on modified Cobb's scale in which infection on plants are rated on by using a given scale and (ii) host reaction whereby plants are assessed for their response to disease after inoculation i.e. resistant/susceptible.

Efforts to find a solution to wheat stem rust disease also initiated global collaboration among wheat scientists who grow and evaluate wheat germplasm for resistance to this disease. At present wheat scientists use wheat varieties to determine avirulence and or virulence characteristics of an isolate. Isolates vary in their ability to infect different wheat varieties. Detection and spread in East Africa of Ug99, is of high significance as most wheat varieties currently grown are highly susceptible to this race and the environment is conducive to disease epidemics.

Identifying resistant varieties in a relatively short time and replacing the susceptible varieties is the strategy to mitigate potential losses.

2.4 Wheat Varieties Improvement Against Wheat Stem Rust Disease

Strong emphases to identify resistance to wheat stem rust disease and to breed resistant wheat varieties were initially given in the USA, Canada, Australia, and Europe. Efforts to find a solution to the wheat stem rust disease problems facilitated global collaboration amongst wheat scientists who shared, grew, and evaluated wheat germplasm in the quest of finding different sources of resistance to this disease. Resistant wheat materials developed at Njoro, Kenya through the support from Canadian scientists in 1960s and 1970s contributed substantially to international breeding efforts. Resistance from Hope and Chris formed the foundation of the high-yielding, semi dwarf wheat varieties that led to “Green Revolution” (CIMMYT, 2005).

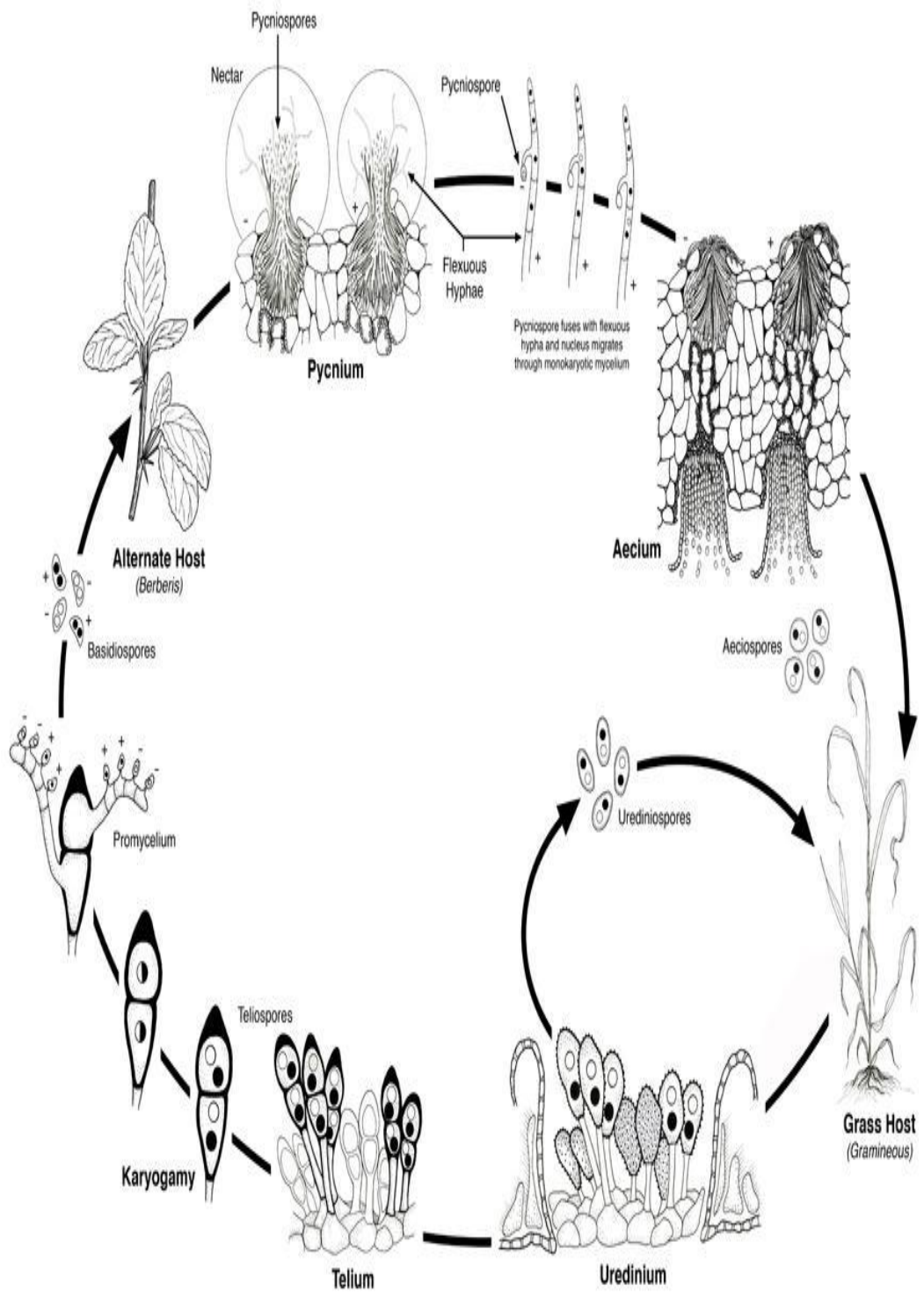


Figure 2.1: Wheat rusts life cycle
 Source: Agrios (2004)

CHAPTER THREE

3.0 OCCURRENCE, INCIDENCE AND SEVERITY OF WHEAT STEM RUST DISEASE IN TANZANIA

3.1 Abstract

A wheat stem rust disease survey was conducted in five farmers' fields of Manyara, Mbeya and Iringa and three and two fields in Kilimanjaro and Arusha regions respectively. Through discussion with farmers and researcher important information on variety cultivated, field size under wheat production, and plant protection strategies versus wheat stem rust disease were captured. Averages of 10 plants were assessed along a diagonal within a 1 m x 1 m quadrant for disease incidence whilst disease severity was assessed on a 0 to 9 scale. Wheat stem rust disease survey revealed various levels of disease infection in all fields surveyed. The allotment of mean disease severity in all the fields surveyed was significantly different at 5% probability level ranging from 23 - 44 %. The most infected fields were from Arusha and Manyara regions with mean severity of 48% and 44% respectively followed by Kilimanjaro (41.67%), Mbeya (25%) and Iringa (23%) regions. Fields in the northern highlands had consistently suffered with maximum wheat stem rust disease as compared to the fields in southern highlands. Disease management practices such as the use of fungicides, improved varieties and crop rotation were some of the common methods used to manage disease. Wheat stem rust disease is widespread with more even distribution pattern in the northern than in the southern highlands and the disease is of an economic importance in key wheat producing regions of Tanzania.

3.2 Introduction

Wheat is considered the fifth most important cereal crop in Tanzania after maize (*Zea mays*), sorghum (*Sorghum vulgare*), pearl millet (*Pennisetum glaucum*) and rice (*Oryza sativa*) (FAO, 1996; MOAC, 2000; FAO, 2010). Famous regions for wheat production in Tanzania are Manyara, Arusha and Kilimanjaro in the northern and Mbeya and Iringa in southern highlands. According to Kuwite *et al.* (2010) wheat grain yield in average is 1.6 t ha⁻¹ compared to the world production that reached an average of 2.7 t ha⁻¹ for the same period. Among other pests, wheat production in Tanzania is constrained largely by wheat stem rust disease (*Puccinia graminis* f. sp. *tritici*), which is prevalent in most wheat growing regions (Ndondi *et al.*, 2001; Kuwite, 2008).

Wheat stem rust fungus infects stem, leaves, sheaths, glumes, and awns during booting stage to 3 weeks before harvesting. Stem rust disease of wheat apart from lowering crop's forage value and predisposing plants to other diseases also interfere the physiological processes of plant by weakening stems so plants lodge; causing mechanical harvesting impossible and consequently reduced or no yield is obtained in endemic situations (Agrios, 2004). Rust pustules associated with this disease are dark reddish brown and may occur on both sides of leaves, on the stem, leaves and spikes (Roelfs *et al.*, 1992). With light infection the urediniospores are usually separated and scattered, but with heavy infection they may coalesce. The disease is more important late in the growing season, on late-sown and maturing wheat cultivars as previously reported by Sing *et al.* (2008).

Disease severity increases in the presence of long period of favourable conditions (moist and average temperatures of 20°C) and when a local inoculum source is available, causing complete yield losses (Sing *et al.*, 2008). With the temperatures of between 19 - 22 °C complete yield losses occur (Sing *et al.*, 2008). In Tanzania, during 1997/98 cropping season, significant yield losses due to wheat stem rust disease was reported by farmers. This was associated with the “el – nino rains”. In addition, under high infection yield losses of between 30 to 40 percent was reported for most fields in Tanzania (Loewen – Rudger, 1993). Also yield losses of 70% were estimated in Hanang wheat complex in 2000 to 2006 cropping seasons for the susceptible cultivar Mbayuwayu (Farm report, 2006 unpublished report). However, when susceptible varieties are cultivated yield losses of 100% were common on farmers fields (Ndoni *et al.*, 2001).

Farmers’ concern for wheat stem rust disease is probably due to the fact that a healthy crop 3 weeks before harvest could be reduced to a black tangle of broken stems and shrivelled grains by harvest if infected by un-managed disease. In addition, crop management practices are not effective in reducing the scourge of this disease due to the limitation associated with such control measures. Wheat stem rust disease has been problematic since when wheat production begun in Tanzania (Kuwite, 2008). Since then in the absence of resistant varieties, wheat production has continuously been threatened by wheat stem rust disease until present. The disease has a wide distribution and occurs in almost all wheat- growing areas in Tanzania.

Quantitative information on wheat stem rust disease distribution, incidence and severity in the wheat growing areas of Tanzania is scanty. There is inadequate information on the incidence and severity of wheat stem rust disease, and association between wheat rust disease severity in farmers' fields and the agro – ecological location of the fields. Such knowledge gaps have hindered efforts to assess the economic importance of disease and development of control mechanism (Braun *et al.*, 2010; FAO, 2010). Such information can be obtained through disease surveys (Hodson *et al.*, 2005). This survey was undertaken to evaluate the distribution, incidence and severity of wheat stem rust disease on farmers' fields in major wheat growing regions of Tanzania.

3.3 Materials and Methods

3.3.1 Location and description of the experimental sites

In 2012 wheat growing season, a wheat stem rust disease survey was carried out in fields in Mbeya, Iringa, Arusha, Kilimanjaro and Manyara regions. Mbeya and Iringa regions are found in the southern highlands while Kilimanjaro, Arusha and Manyara and located in the northern zone of Tanzania. Figure 3.1 shows surveyed regions of Mbeya (latitude 8° 54' 0" S longitude 33° 27' 0" E), Iringa (latitude 9° 22' 19" S longitude 34° 29' 44" E), Arusha (latitude 3° 0' S longitude 36° 0' E), Manyara (latitude 4° 45' longitude S 36° 0' 40" E) and Kilimanjaro (latitude 3° 20' 05.58" S longitude 37° 20' 25.37" E) regions.

Often, mean rainfall in northern Tanzania is characterized by bi-modal pattern whereby long rains begin from March and ends in May, while short rains are expected from October to December. The single rainfall pattern is experienced in

southern highlands where it starts from November and ends in May. The wheat production seasons relatively differ from one region to another as it is determined by the onset of rainfall and it influences disease occurrence and development. During 2012 wheat growing season when this study for example was conducted, Manyara wheat production season started in mid-January to July while for Kilimanjaro and Arusha regions wheat was grown between mid March to September. In Mbeya and Iringa regions, wheat production season began in April to October for 2011/12 season.

3.3.2 Field survey

Wheat stem rust disease survey was conducted in wheat fields of Mbeya, Iringa, Kilimanjaro, Arusha and Manyara regions between April and June, 2012. In Manyara region, fields were surveyed in mid April while in Arusha and Kilimanjaro regions this activity was conducted in mid May, 2012. Mbeya and Iringa regions were surveyed in early June, 2012. The survey was timed to coincide with booting stage and ripening stage (just 3 weeks before harvest) when most wheat fields were infected by the wheat stem rust disease. Five fields were selected from Manyara, Mbeya and Iringa regions where as three fields were selected from Kilimanjaro and two from Arusha. The selection of number of fields was based on wheat production history and to ensure the representation of different ecological backgrounds (Table 3.1). Fields were sampled purposively to get sufficient stem rust disease inocula between booting and ripening stages.

3.3.3 Data collection

Discussion was conducted with farmers and researcher to capture information on variety cultivated, field size under wheat production, and plant protection strategies versus wheat stem rust disease. In each field the incidence and severity of wheat stem rust disease were assessed in five 1 m x 1 m quadrants, one at each corner and at the centre of the field. An average of 10 plants was assessed along a diagonal within a quadrant. The number of plants within each quadrant showing symptoms of wheat stem rust disease was scored and recorded. Disease severity was assessed on a 0 to 9 scale (McNeal *et al.*, 1971), where 0 = healthy stems, 1 = 1 to 10% stem area with rust pustules, 3 = 11 to 25%, 5 = 26 to 50%, 7 = 51 to 75%, and 9 = > 75% of stem area affected (almost lodging to lodging).

Plant stems bearing wheat stem rust pustules were collected from each field as source of inocula for further research work (Plate 3.1A and 3.1B). To be location specific for the collected samples, global positioning system (GPS) was used (Plate 3.1C). Within the surveyed fields of wheat, other frequently occurring pests were recorded.

3.3.4 Data analysis

Wheat stem rust disease severity and incidence were assessed from each field. The disease incidence was determined by taking the number of infected plants in each quadrant as a percentage of the total number of plants sampled in that quadrant as follows:-

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants in a quadrant}}{\text{Total number of plants sampled in a quadrant}} \times 100$$

The mean rating for disease incidence, severity and variety reaction of 50 plants (ten from each quadrant) was calculated from each field as shown below:-

$$\text{Mean rating of disease incidence per field} = \frac{\text{Total disease incidence scored}}{50 \text{ plants}}$$

$$\text{Mean rating of disease severity per field} = \frac{\text{Total disease severity scored}}{50 \text{ plants}}$$

$$\text{Mean rating of variety reaction per field} = \frac{\text{Total variety reaction scored}}{50 \text{ plants}}$$

The number of fields within each severity category was expressed as a percentage of the total number of fields surveyed to obtain severity frequency distribution by using the following formula:-

$$\% \text{ of number of fields in a category} = \frac{\text{Number of field in severity category}}{\text{Total number of fields surveyed}} \times 100$$

Before analysis, data on disease severity was transformed to normalize and make variance relatively independent of the mean. Analysis of variance was used to determine whether the proportion disease severity differed between regions in the distribution of disease severity levels. Means were separated using the Bonferroni's test ($p \leq 0.05$). Gen Stat software V.13 statistical package (VSN International, UK) was used to facilitate the analysis with the factorial type of statistical model:-

$$X_{ijk} = \mu + R_i + F_j + (RF)_{ij} + e_{ijk}$$

Where: X = general mean common to all observations,
 R_i = effect of i th level of factor R (Region),
 F_j = effect of j th level of factor F (field),
 $(RR/FF)_{ij}$ = Interaction effect of a given disease severity score from one region/field with another,
 E_{ijk} = random error.

3.4 Results

3.4.1 Distribution of Wheat Stem Rust Disease

Wheat stem rust disease survey undertaken in the key wheat growing regions in Tanzania revealed disease infection in all fields of the northern regions and in some of fields from southern regions. Neither of the fields surveyed was wheat stem rust disease – free nor lodging in Manyara, Arusha and Kilimanjaro regions (Table 3.2). One field in Mbeya and two in Iringa regions were not infected by wheat stem rust disease at all.

Large number of plants killed by wheat stem rust disease was scored in the fields where Mamba, Mbayuwayu, Juhudi or local varieties were grown in all regions as shown by disease incidence and severity (Table 3.2). Disease incidence and severity was less frequent in fields of Riziki, Karioga, and Njombe 7 and rarely observed in the field of Sifa (Table 3.2).

3.4.2 Extent of variation in disease severity between the regions, agro – ecological zones and national wise

There was marked difference in the level of disease infection in the fields of different regions (Fig. 3.2). While some fields in Iringa and Mbeya were not infected

by wheat stem rust disease all fields surveyed in Manyara, Arusha and Kilimanjaro had infection at different severity levels as shown in Fig. 3.3. Generally many fields in all regions were affected moderately (score 5 on 0 – 9 scale) at 26 – 50% disease severity. Large number (35%) of fields affected were those of Manyara, followed by fields of Mbeya (25%), Kilimanjaro and Iringa (15%) and Arusha (10%). National wise disease severity was higher (26 – 50%) in 60 % of fields, moderate (11 – 25%) in 15% and highest (51 – 75%) in 10% of fields (Fig. 3.3).

The allotment of mean disease severity in all fields surveyed were significantly ($p \leq 0.05$) different ranging from 23 - 44 % (Table 3.3). The most infected fields were from Arusha and Manyara with mean severity of 48% and 44% respectively followed by Kilimanjaro (41.67%), Mbeya (25%) and Iringa (23%). It was thus evident that disease severity varied with the agro-ecological location of the fields. Fields in the northern highlands had consistently suffered with maximum wheat stem rust disease as compared to the fields in southern highlands (Table 3.3). Disease severity was insignificantly different ($p \leq 0.05$) from one region to another within similar agro-ecological region except for the fields in Kilimanjaro region which deviated from those of Manyara and Arusha as well as from Mbeya and Iringa regions (Table 3.3).

3.5 Other Pests of Wheat Observed During the Field Survey

During the field survey there were other wheat pests observed in different fields. The commonly occurring pests were such as weeds: black jack (*Bidens pilosa*), couch grass (*Elymus repens*), nutsedges (*Cyperus* spp.), Guasa (*Galinsoga*

parviflora), dayflowers (*Commelina* spp.) and *Oxalis* spp. Others were birds such as yellow weaver (*Parapercis* spp.) and red-billed quelea quelea (*Quelea* spp.); insects were aphids and caterpillars of various insects. Other pests included rodents, diseases like wheat stripe rust disease, wheat leaf rust disease, wheat smut disease, wheat take all disease, wheat crown root rot disease, wheat streak mosaic disease and wheat bacterial leaf blight disease.

3.6 Discussion

One of the reasons for the more widespread of wheat stem rust disease in the northern zone than in the southern zone could be the contribution of the varieties grown. Wheat stem rust disease appeared to be more severe in all the varieties prevalent in northern highlands. Prevalent varieties in northern highlands were Mamba, Mbayuwayu, Chiriku and Riziki while Juhudi and Sifa were popular in the southern highlands. Ngamia and Kariega varieties were imported from Kenya and one of the reasons for their preference was resistance to wheat stem rust disease.

Reasons for the deviations in disease severity observed in Kilimanjaro fields to those of Manyara and Arusha regions could be inoculum load, favourable environmental and climatic conditions for infection and disease development, variety grown and plant protection measures (Sing *et al.*, 2008). The differences in disease severity within similar agro-ecological region when a similar variety is grown are indication of pathogen variability (Agrios, 2004; Jain *et al.*, 2009).

In large scale fields such as the Hanang wheat complex area of northern zone, crop rotation to minimize inocula build up is not practiced. There is thus more chance of

disease occurrence in such areas than is crop rotation was done. On the other hand, farmers in the southern highlands elaborated that crop rotation is regularly practiced since after wheat harvest they normally grow potato before next wheat season. This practice probably contributed to the low wheat stem rust disease incidence and severity. According to Joseph *et al.* (2007) crop rotation also improves management of plant diseases through manipulation of host factors such as crop and cultivar selection; interruption of disease cycles through crop rotation, fungicide application, and removal of weeds and volunteer crop plants.

The field survey findings showed that most of the fields which were severely affected by wheat stem rust disease in the southern highlands were those which crop rotation is not practised. Lack of crop rotation can lead to serious problems for example, the build up of disease of a critical crop, or imbalances in soil nutrients (Charles *et al.*, 2009). Such problems can result in an inability to meet the demands or in additional labour and expense.

Field sizes in the surveyed regions varied from less than a hectare to more than 500 ha. Smallholder farmers owned less field sizes while fields of up to 539 ha were under commercial wheat production like those owned by the HLHF. Field sizes influenced disease control strategies, for instance, large fields that were committed in annual wheat production disease control measures such as crop rotation were uncommon.

Discussion with farmers during the survey revealed that disease management practices such as the use of fungicides, improved varieties which are early maturing and resistant to wheat stem rust disease and crop rotation were some of the common methods used to control wheat stem rust disease. The use of fungicides which was the popular strategies in large scale wheat production was effective but its efficacy may be limited by both the economic and pest resistant factors (Boshoff *et al.*, 2003; FAOSTAT, 2009). Crop rotation which was commonly practiced by small scale farmers was challenged by land scarcity issues whilst use of improved wheat varieties which is a better option than other methods is limited by the availability of such varieties as reported by Ndoni (2003) and Ravi *et al.* (2006). This study found that some farmers are using varieties (Ngamia and Karioga) from Kenya for the reason that they are resistant to stem rust as compared to those used in Tanzania.

Wheat stripe rust disease and oxalis weeds were especially pronounced and economically important in Mbeya and yellow weavers in fields of Hanang wheat complex, the rest of the pests were equally distributed in many wheat fields surveyed. Birds were regularly present around the Hanang wheat complex due to the fact that the fields were surrounded by many forest trees in which birds usually dwell. The concern about wheat stripe rust disease being common in Mbeya can be due to susceptibility of varieties and favorable environmental conditions for disease development (Table 3.1). Cooler areas of temperature range of 9°C and 11°C such as Mbeya region are suitable for wheat stripe rust disease development (Agrios, 2004). The case of oxalis weed species was uniquely important because there was a concern from farmers as to whether rusts attacking their wheat were associated with

those on weeds. Previous knowledge on host specificity at this point may or may not be valid due to the fact that isolates may change and pathogens may co-exist.

3.7 Conclusions

It is evident from this study that wheat stem rust disease is widespread in most fields in Tanzania. Disease distribution and severity differed with the agro – ecological location of fields of which distribution was more frequent and even in the northern highlands than in southern highlands. Disease severity was also comparably higher in most fields of northern than those in the southern regions of Tanzania.

The study has quantified information on wheat stem rust disease distribution, incidence and severity in the wheat growing areas of Tanzania. The information gained has potentially indicated that wheat stem rust disease is of economic importance in the key wheat producing regions of Tanzania and requires management.

Field survey revealed that the use of fungicides, resistant varieties and crop rotation are effective and desirable as disease management strategies for wheat stem rust disease. However, the above measures were not properly implemented because of limitations such as land scarcity and unavailability of resistant varieties and unaffordable costs for fungicides.

Effective use of each strategy thus require adequate understanding of the production system before making recommendation of the given control measures.

Table 3.1: A summary description of the climatic conditions taken during wheat stem rust disease survey in Tanzania, 2012

Region	General description	Relative humidity (%)	Altitude (m)	Mean Temperature (°C)	Mean annual rainfall (mm)
Arusha	Warm-humid	> 70	1350–1520	19.3 – 20.4	1052
Kilimanjaro	Warm-humid	60 – 75	1340 -1480	19.8 – 21.5	927
Manyara	Warm-humid	50 – 65	1380 – 1785	20.3 – 22.4	983
Iringa	Cold-humid	>75	1580 – 2945	11 – 12	661
Mbeya	Cold-humid	>75	1230 – 1800	9 – 11	969

Table 3.2: Status of wheat stem rust disease in the selected wheat fields in Tanzania

Region	District	Variety	Location		Disease incidence (%)	Disease severity (%) ¹	
Manyara	Hanang	Kariega	4°28'33''S	35°00'14''E	1773.94m.a.s.l	20ab	20ab
	Hanang	Mamba	4°26'32''S	35°12'25''E	1776.07m.a.s.l	60ef	60ef
	Hanang	Ngamia	4°37'15''S	35°12'25''E	1764.79m.a.s.l	10a	15a
	Babati	Mbayuwayu	4°23'24''S	35°36'21''E	1398.06m.a.s.l	50de	45d
	Katesh	Local	4°53'58''S	35°28'33''E	1781.62m.a.s.l	30bc	50de
Arusha	Karatu	Mbayuwayu	3°21'37''S	35°33'12''E	1534.17m.a.s.l	30bc	40cd
	Monduli	Selian	3°32'42''S	36°10'56''E	1369.98m.a.s.l	50de	60ef
Kilimanjaro	Hai	Mbayuwayu	3°55'09''S	37°43'32''E	1343.59m.a.s.l	25b	30bc
	Hai	Selian	3°24'11''S	37°19'36''E	1482.61m.a.s.l	40cd	35c
	Hai	Riziki	3°06'18''S	37°51'31''E	1398.27m.a.s.l	20ab	15a
Mbeya	Mbeya rural	Juhudi	5°54'29''S	36°22'07''E	1756.79m.a.s.l	90f	75f
	Mbeya urban	Juhudi	5°56'46''S	36°53'51''E	1784.67m.a.s.l	60ef	50de
	Mbeya urban	Sifa	5°14'27''S	36°12'53''E	1801.43m.a.s.l	10a	30bc
	Mbeya urban	Sifa	8°01'03''S	34°53'47''E	1234.51m.a.s.l	10a	5a
Iringa	Njombe	Juhudi	9°33'47''S	34°47'31''E	1581.54m.a.s.l	10a	25b
	Njombe	Njombe 7	9°59'58''S	34°12'08''E	1965.32m.a.s.l	30bc	15a
	Makete	Local	9°19'10''S	34°43'10''E	2943.33m.a.s.l	40cd	25b
s.e					2.34	0.99	
CV (%)					16.30	27.52	

¹Average levels of disease severity under a 0 – 9 scale (McNeal *et al.*, 1971), where 0 = healthy stems, 1 = 1 to 10% stem area with rust pustules, 3 = 11 to 25%, 5 = 26 to 50%, 7 = 51 to 75%, and 9 = > 75% of stem area affected (almost lodging to lodging)

Table 3.3: Differences in mean disease severity of wheat stem rust between selected regions and agro – ecological zones of Tanzania

Agro – ecological location	Region	Mean disease severity (%)
Northern highlands	Manyara	44.00c
	Arusha	48.00c
	Kilimanjaro	41.67b
Southern highlands	Mbeya	25.00a
	Iringa	23.00a
	s.e	3.43
	CV (%)	29.80

Means in the same column followed by the same letter do not differ significantly according to Bonferroni test ($p \leq 0.05$).

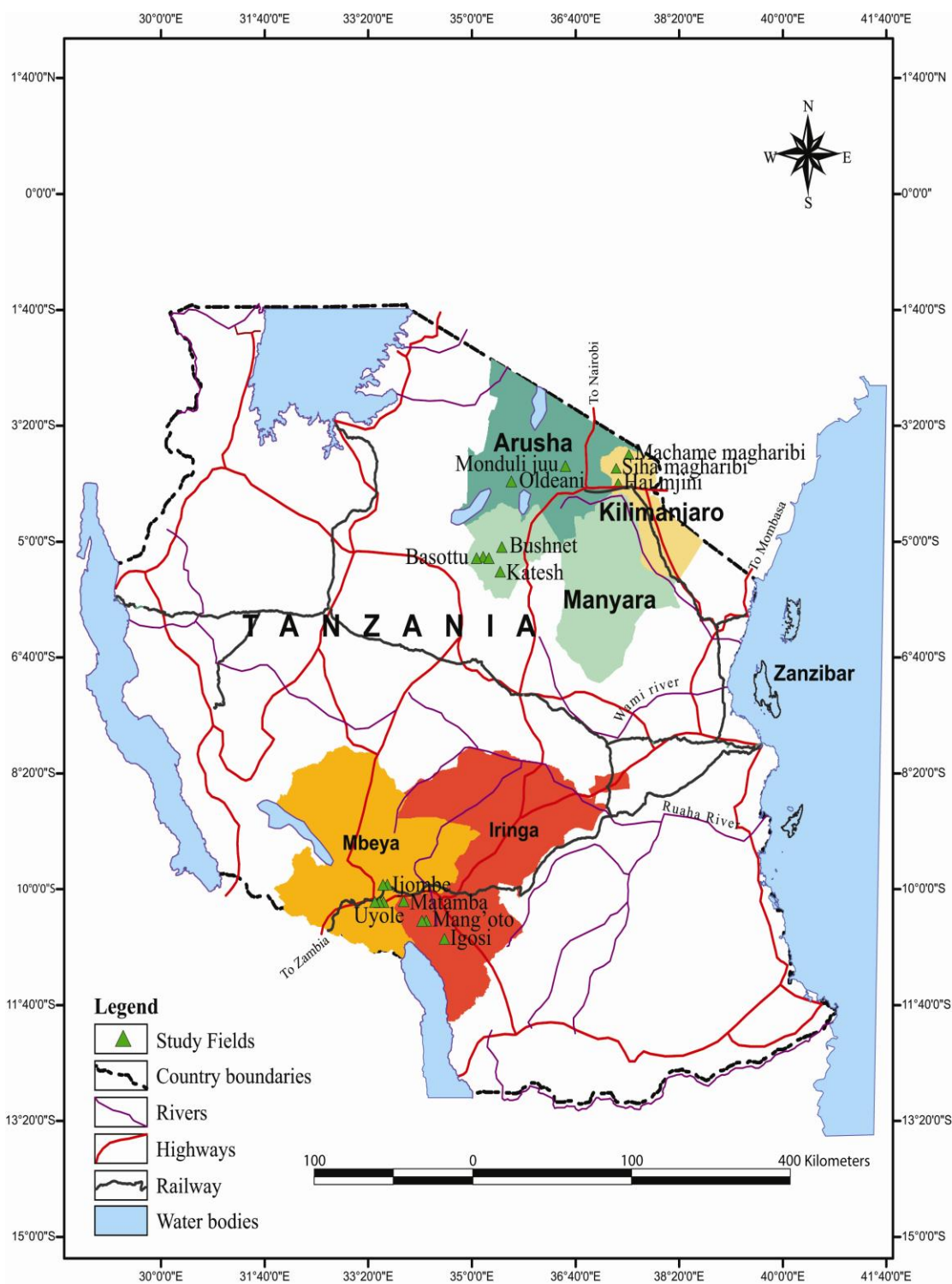


Figure 3.1: Map of Tanzania showing surveyed regions and locations for the collected isolates of wheat stem rust disease

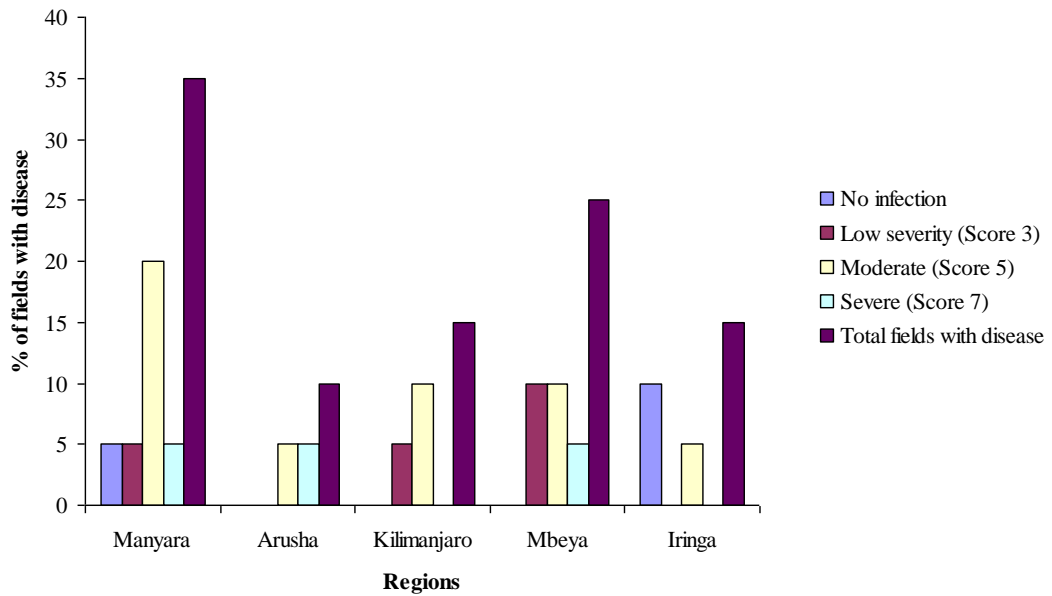


Figure 3.2: Fields with no infection, low, moderate and severe levels of wheat stem rust disease and total fields with disease in each of the surveyed regions

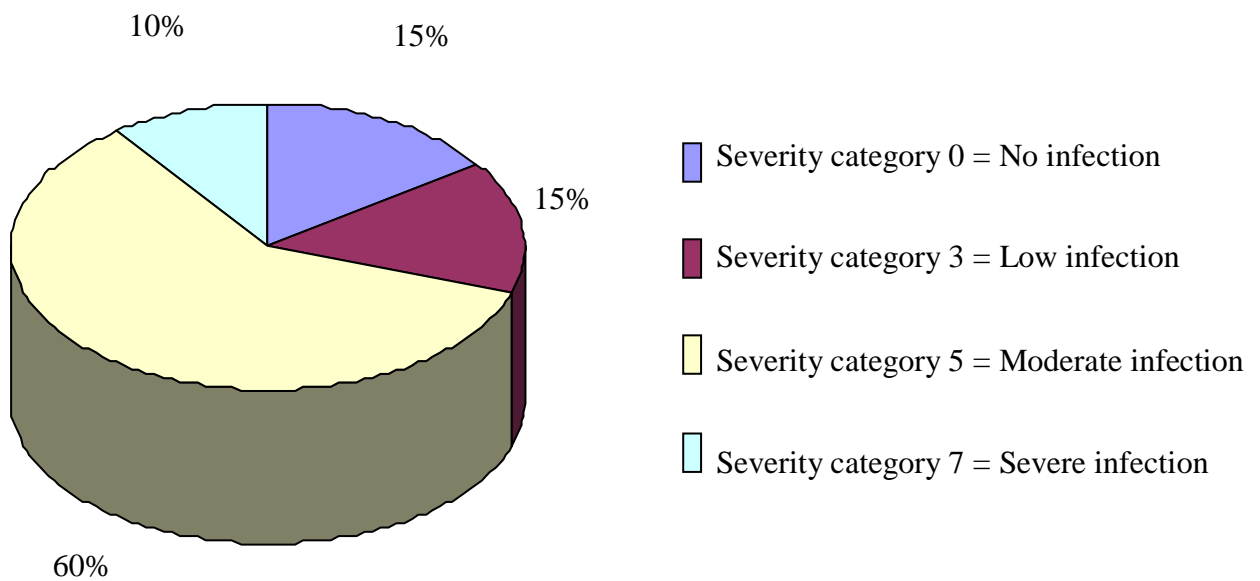


Figure 3.3: Proportion of fields infected by wheat stem rust disease at different severity categories in Tanzania

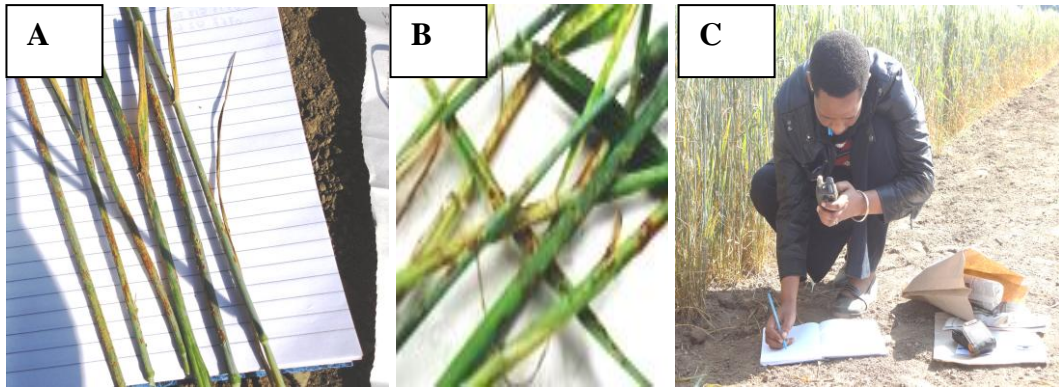


Plate 3.1: Inocula collected from southern (A) and northern (B) highlands and geo-referencing of isolates (C)

CHAPTER FOUR

4.0 DETECTION OF DIFFERENTIAL VARIETIES AND PATHOLOGICAL ISOLATES OF WHEAT STEM RUST DISEASE IN TANZANIA

4.1 Abstract

Screen house experiment was laid out with 18 wheat varieties to determine differential varieties and pathological groups of wheat stem rust disease in Tanzania. 10 seedlings per variety were raised 8 days prior inoculation with 10 inocula from most infected fields of Manyara, Mbeya, Iringa, Kilimanjaro and Arusha. 18 wheat varieties were evaluated for their response to wheat stem rust disease and infection type recorded. Infection type showed that there is variability on the way varieties responded to the isolates. No spores were observed on resistant variety while spore number and size on moderately resistant group were fewer and smaller than those on susceptible varieties. Varieties were thus grouped based on their infection types. Viri was identified as the most susceptible variety. In pathogenicity experiment, 10 seedlings of Viri were inoculated when 8 days old. Generation time was recorded from 7 - 17 days after inoculation, infection types were scored on a 0 – 4 scale and severity was rated on a 0 – 9 scale. Generation time significantly differed with isolates, fast (sporulated 7 – 10 days after inoculation) and normal (11 – 14 days) isolates were encountered in all regions whilst slow (15 – 17 days) were present in samples of Mbeya. Based on infection type, isolates were classified as those causing susceptible and moderately resistant infection types. Isolates were classified based on generation time, infection type and disease severity of which most aggressive isolates from each region was identified. Presence of fast sporulating isolates in all

the regions could be one of the root causes of maximum disease severity in the field. Variations in virulence attributes of *Puccinia graminis* f. sp. *tritici* on the same variety confirmed presence of different isolates in Tanzania.

4.2 Introduction

Existing varieties are threatened by the establishment of new isolates of pathogens such as *Puccinia graminis* f. sp. *tritici* (Ravi *et al.*, 2011) The presence of a new isolate explains why resistant varieties became susceptible after a while; why variety resistance change from time to time and why a variety that was resistant in one geographic area became susceptible in another (Agrios, 2004). In 1999, for example, a new, more virulent strain of wheat stem rust pathogen, known as Ug99, was isolated in Eastern Africa, Yemen and Iran (Singh *et al.*, 2008). If a new isolate arises in an area, given time it could spread throughout the epidemiologic (Chakraborty *et al.*, 2006) region or beyond with huge crop losses.

The isolates are detected when previously resistant variety becomes infected and they differ in their pathogenicity to their host (Agrios, 2004; Singh *et al.*, 2008). Wheat stem rust pustules on a susceptible wheat variety are quite large and lesions often elongate, tearing the cuticle and epidermis as they erupt (Agrios, 2004). Depending on the resistance of each variety relative to the virulence of the specific isolate of the pathogen, lesions vary considerably in size and colour (Brown and Hovmoller, 2002). The generation time can be isolate specific and thus isolates can be grouped as slow, normal or fast of which a reduction in the length of the sporulating period is a virulence factor (Agrios, 2004).

Certain varieties have in several crops known to be used as differentials for a particular disease. For wheat despite of the fact that there are many varieties available in the germplasm no attempt has been in place 'till when this study was conducted' as wheat stem rust disease is concerned in Tanzania. Fortunately, currently there are some differentials from CIMMYT are being evaluated by National Research Program under Selian Agriculture Research Institute (SARI) in Monduli district, Arusha Region. Differential varieties which segregate isolates reaction are crucial for isolates characterization and screening experiments. There is paucity of scientific information on the differential wheat varieties and virulence levels of prevailing isolates of wheat stem rust disease in Tanzania. This is imperative for plant protection and development of resistant varieties. This study was carried out to investigate the likely differential wheat varieties and pathotypic groups of wheat stem rust isolates in Tanzania.

4.3 Materials and Methods

4.3.1 Location and description of the experimental site

Screen house experiments were conducted at Sokoine University of Agriculture (SUA) at the Department of Crop Science and Production (6° 45" S, 37° 40" E, 525 m.a.s.l). The experiments were carried out during the period between April and June, 2012.

4.3.2 Collection of isolates

Wheat stems bearing wheat stem rust teliospores were collected from wheat fields of Arusha, Manyara, Kilimanjaro, Mbeya and Iringa region during stem rust disease survey. Infected stems were collected from five quadrants of 1 m x 1 m, one at each

corner and at the centre of the field. Infected stem were then rapped in a paper and taken to the screen house. In the screen house rust spore were each dissolved in sterile distilled water and inoculated on susceptible wheat variety (Viri) for multiplication and storage purposes.

4.3.3 Experiment to establish possible differential varieties

Screen house experiment was laid out in a split plot design with three replications using 18 wheat varieties obtained from Selian Agricultural Research Institute, Department of Wheat Research and Hydom Lutheran Hospital Farm (HLHF) (Table 4.1). Additional informational on the important attributes of these varieties during their release are also provided in Table 4.2. Ten seedlings of each variety were raised 8 days prior inoculation in plastic pots of 1 litre (L) size filled with 1 kg of pasteurized loam soil. Watering of seedlings was done after every other day to ensure adequate moisture and relative humidity by pouring water on the floor and walls during the afternoon when it was dry and hot.

Eight day old seedlings were inoculated with ten different types of inocula collected from the most infected fields from Manyara, Mbeya, Iringa, Kilimanjaro and Arusha regions as shown in Table 4.3. Choice of number of inocula included in the study depended on the number of fields infected in the region where they were collected from. Spore suspension was prepared by using sterile distilled water and by using hand sprayer eight days old seedlings were inoculated (Plate 4.1A – B). Plants were incubated for 24 h in a polythene chamber then removed and placed on screen house bench for disease development (Plate 4.1C - D).

4.3.4 Experiment to establish categories of isolates

A pathogenicity test experiment was laid out in a screen house in a randomized complete block design (RCBD) with three replicates. Ten seedlings of the most susceptible wheat variety, Viri, were inoculated using seventeen inocula (Table 4.4) of stem rust disease from each field when they were 8 days old. The seedlings were incubated by covering by using polyethylene sheet for 24 h under screen house conditions.

4.4 Data Collection

4.4.1 Possible differential varieties

There were 18 wheat varieties of which each variety was evaluated for its response to wheat stem rust disease 14 - 17 days after inoculation. Spore number on leaves and size were assessed and infection type recorded (Plate 4.1E). Scoring was on leaves because plants were at seedlings stage (Jin *et al.*, 2007b). Infection type was recorded on a 0 to 4 scale as described by Stakman *et al.* (1962), whereby varieties that expressed 0 and 1 types of infection were considered resistant, 2 infection type were taken as moderately resistant while those scored 3 and 4 infection types were taken as susceptible.

4.4.2 Categorization of isolates

The generation time was observed and recorded (Jin, 2007b) whereby sporulation duration of 7 – 10 , 11 – 14 and 15 – 17 days after inoculation were designated to fast, normal and slow growing isolate groups respectively. Infection types (0 and 1 = less aggressive, 2 = moderately aggressive and 3 and 4 = aggressive) were

characterized as described by Stakman *et al.*, (1962). Averages of 10 seedlings were assessed for disease incidence and severity. The number of plants within each pot showing symptoms of wheat stem rust disease was recorded. The disease incidence was determined by taking the number of infected plants in each pot as a percentage of the total number of plants sampled in that pot. Average disease incidence was then calculated for isolates from each field in a region. Disease severity was rated using the Cobb scale on a 0 – 9 scale (McNeal *et al.*, 1971), where 0 = healthy seedling, 1 = 1 to 10% seedling area with rust pustules, 3 = 11 to 25%, 5 = 26 to 50%, 7 = 51 to 75%, and 9 = > 75% of seedling area affected.

4.5 Data Analysis

4.5.1 Possible differential varieties

Data of infection types scored for different varieties were summarized by calculating the average infection type caused by isolates from each region on respective variety. Based on the means of infection type the most susceptible variety was identified as that variety with the highest infection type score on average.

4.5.2 Categorization of isolates

Before analysis data on disease severity were transformed to normalize and make variance relatively independent of the mean. Results of disease severity and infection type were subjected to analysis of variance (ANOVA) of complete randomized block design (CRBD) model and means were separated using the Bonferroni's test ($p \leq 0.05$). Gen Stat software V.13 statistical package (VSN International, UK) was used to facilitate the analysis with the following statistical CRBD models:-

$$Y_{ij} = \mu + T_i + B_j + e_{ij};$$

Where: μ = overall experimental mean,

T_i = effect of the respective isolate amongst the isolates

B_j = effect due to blocks

e_{ij} = random error effect.

Isolates were therefore, classified based on the generation time, infection type and disease severity of which the most aggressive isolates from each region were identified based on the means of these virulence traits.

4.6 Results

4.6.1 Possible differential varieties to wheat stem rust disease in Tanzania

Wheat varieties evaluated segregated in their response to isolates as indicated by infection types. The average infection type varied among the varieties within each region, and among the regions. The average infection type was low (1.37) and high (3.03) over regions (Table 4.7). Distinctively with isolate from Mbeya, variety Kweche recorded the lowest infection type compared to other varieties. Mamba and Viri varieties recorded the highest infection type following infection with isolates from Manyara and Mbeya (Table 4.5). Additionally, no spores were observed on Sifa, which showed a consistently low infection rate across isolate from the regions. Spore number and size on moderately resistant group were comparatively fewer and smaller than those displayed by susceptible group (Plate 4.2).

4.6.2 Pathological isolates of wheat stem rust disease

Results of the pathogenicity test for 17 wheat stem rust isolates on Viri wheat variety indicated a mean generation time of approximately 7 days, infection type of 2.18 and disease severity of 20.88% (Table 4.5). The implication is that isolates were in average fast sporulating; causing aggressive infection type that affected about 27.06% of seedlings at 11 – 25% disease severity. Out of five seedlings evaluated in every pot at least 2 were infected by wheat stem rust isolates in which maximum disease incidence was 60%.

Disease severity significantly varied at $p \leq 0.05$ with isolates in the range of 10 to 37.50% (Table 4.6). Isolates collected from fields number 2 and 7 infected Viri severely (37.50%) while less severity of 10.00% was caused by isolate collected from field number 18 (Table 4.6). The rest of the isolates infected Viri variety in the following order; isolates from fields number 4 and 9 (27.50%), 6 (25.00%), 2, 3, 9 and 12 (22.50%), 11, 13 and 14 (15.00), 5, 10, 16 and 19 (12.50%).

Mean separation indicated that generation time between isolates significantly differed for approximately 8 to 17 days (Table 4.6). Two isolates from Manyara and Arusha were the fastest in sporulation while the slowest isolates that sporulated about 17 days after inoculation was collected from Iringa region. The means of infection type were statistically insignificant at $p \leq 0.05$ among isolates (Table 4.6). However, based on the scale of infection type described by Agrios (2004) and Stakman *et al.* (1962), isolates collected from field number 1, 2, 3, 6, 7, 11, 12 and 19 caused less aggressive (0 and 1 scoring) type of infection whereas isolates from

field number 4, 5, 8, 9, 10, 13, 14, 16 and 18 caused moderately aggressive (scored 2) infection type (Table 4.6). Isolates were then named based on names of districts where they were collected (Table 4.6).

Based on generation time, infection type and disease severity the most virulent isolates from each region were identified as those resourced from field numbered 1, 7, 8, 12, and 19 i.e. Hanang 1, Monduli 1, Hai 1, Mbeya urban 1, and Makete 1 respectively. Wheat varieties evaluated segregated in their response to isolates as indicated by infection types. The average infection type varied among the varieties within each region, and among the regions. The average infection type was low (1.37) and high (3.03) over regions (Table 4.7).

There was a wide range of differences in isolates generation time, infection type and disease severity when wheat variety Viri was uniformly inoculated and then observed for the virulence 14 – 17 days later. Most isolates required 7 – 10 days to infect Viri; another group of isolates were those with normal generation time of 11 – 14 days while the last class represented isolates which sporulated after 15 – 17 days after inoculation (Jin *et al.*, 2007b). Fast and normal isolates were encountered in all the surveyed regions at various numbers whilst slow group of isolates were present from isolates samples collected from Mbeya region (Fig. 4.1). Figure 4.2 is a frequency distribution for above described isolate groups. Frequency plot of mean generation time was highly skewed to the left indicating the prevalence of fast isolate group as compared to the rest (Fig. 4.2).

Another grouping criterion for isolates depended on type of infection caused by respective isolate on Viri. The number in each group of isolates in different regions varied as shown in Fig. 4.3. All isolates from Arusha caused aggressive infection type and none of the isolates was unaggressive to Viri wheat variety (Fig. 4.3). At this point two major groups of wheat stem rust isolates were identified as those causing moderately aggressive and aggressive infection types (Fig. 4.4).

Final approach attempted in isolates categorization was that which considered number and area of seedling infected with rust pustules. Most seedlings were infected by isolates pustules at severity range of 11 - 25% with exceptional of isolates collected from Iringa which caused less disease severity (1 – 10%) as presented in Fig. 4.5. None of the isolates left Viri seedlings uninfected (0% score) or caused disease severity greater than 51%. Isolates categorization according to disease severity resulted into three isolates groups from different regions (Fig. 4.6).

4.7 Discussion

The average infection type among the varieties over isolates from five regions varied. There were varieties which exhibited resistant, moderately resistant and susceptible type of infection. Varieties with low (score 0 and 1) infection rate were regarded as resistant (R) while those with high (3 and 4) infection rate were regarded as susceptible (S). This implied differential response among varieties and could imply the possibility of exploiting the resistant genotype as donor parent for the purpose of introgression of the resistant gene into commercial or widely accepted varieties (Abede *et al.*, 2012). According to the infection type described by Agrios

(2004) and grading scale of Stakman *et al.* (1962), varieties can thus be grouped based on their infection types in which the first group can be resistant type of infection, group 2 moderately resistant and group 3 susceptible infection types (Table 4.7). Some of these varieties could possibly serve as differentials for wheat stem rust disease (Belayneh *et al.*, 2009) though; more work is needed to confirm such preliminary findings. At this point farmers' argument that wheat varieties, Ngamia and Karioga sourced from Kenya were resistant to wheat stem rust disease was not valid as both varieties showed symptoms and were scored with infection type of 1.67 – 2.43 among varieties. Additionally, the variety Viri which was highly infected was identified as the most susceptible variety to be used during pathogenicity experiment. Viri variety was resistant to none of the isolates and thus not recommended for cultivation in any of the regions.

According to the infection type range Sifa variety was grouped as resistant as it recorded low infection type range. This category was followed by 10 genotypes: Azimio 87, Juhudi, Rv 593, Kweche, Riziki-C2, Riziki-C1, Kware, Mbayuwayu, Karioga and Ngamia which recorded infection type between 1.67 and 2.43, thus classified as moderately resistant. At the extreme of this category were 7 varieties: Lumbesa, Njombe 7, Chiriku, Viri, Tausi, Joli and Mamba, which recorded infection types ranging between 2.53 and 3.03, similar observations were reported in Ethiopia by Beteselassie *et al.* (2007). There were comparatively less disease severity scores in screen house experiments than in the fields. Similar findings were reported by Luig (1985) in which it was highlighted that reduced light to be reason for poor infection rates in the screen house.

Isolates virulence also varied with geographical locations of wheat field in which most isolates obtained from northern (Arusha, Manyara and Kilimanjaro) Tanzania took few days to sporulate when compared to those isolates obtained from southern part of the country (Mbeya and Iringa). It was not surprising to recognize that most isolates sporulated in shorter period of time, showed aggressive type of infection (Belayneh and Emebet, 2005) and resulted into maximum disease severity. During the field survey farmers from the northern highlands for example, highlighted that nowadays wheat stem rust disease spread faster than ever before. Farmers even reported that the situation became worse since after the rains popularly known as El – nino in 1997/98 in which stem rust disease became economically important in Hanang wheat complex for example (Joseph M. personal communication, 2012).

Climatic factors can have an important impact on disease risk (Coakley, 2004). Climate influences all stages of host and pathogen life cycles as well as development of disease. Disease severity over a period can fluctuate according to climatic variation (Braun *et al.*, 2010). The most likely impacts would be shift in the geographical distribution of the host and pathogen, change in the physiology of host-pathogen interactions and change in crop losses. With climate change therefore, some plant pathogens become more aggressive while other new invasive are been reported. Usha and Parimal (2008) reported that a rise in temperature above 20°C can inactivate temperature sensitive resistance to stem rust in oat cultivars.

Conversation with farmers is in consistence with the trend of result obtained for generation time, infection type and disease severity among isolates received from

different fields in Manyara, Arusha, Kilimanjaro, Mbeya and Iringa regions. Presence of fast sporulating isolates in all regions surveyed could be one of the root causes of maximum disease severity in the fields. In this study, these isolates are categorized as most virulent. Significant differences recorded for infection type and disease severity caused by different isolates on similar variety were evidences that prevailing isolates of wheat stem rust in Tanzania are different as reported by Jin *et al.* (2007b) and Agrios (2004).

4.8 Conclusions and Recommendations

Variations in virulence attributes of *Puccinia graminis* f. sp. *tritici* on the same variety confirmed the presence of different isolates in Tanzania. Common isolates were those which are fast sporulating (7 – 10 days) followed by normal (11 – 14 days) and few slow (15 – 17 days) spore forming isolates. Isolates from the northern zone were relatively more aggressive than those from southern highlands and this can be the reason why most fields in the northern were infected as compared to those of southern highlands. The following remarks can be made out of this study:-

- i. Appearance of new pathogenic isolates of *Puccinia graminis* f. sp. *tritici* might be due to the effect of changes in climatic factors.
- ii. It is also suspected that some of the isolates could be the variants of Ug99.
- iii. Race analysis study using molecular techniques is vital to figure out the differences and similarities detected within the isolate groups.

The study has also contributed to the knowledge of differential wheat varieties by identifying some wheat varieties that could be potentially donor parents or serve as differentials for wheat stem rust disease and therefore:-

- i. These varieties can potentially add to those imported through CIMMYT.
- ii. In-depth study is recommended to build on these findings especially gene characterization for the detected differential varieties.

Table 4.1: Wheat varieties, year of release and seed source used in this study

S/n	Variety	Year released	Seed source	Wheat stem rust disease quality at the time of release
1	Mamba	1973	ARI Selian	Resistant to stripe rust
2	Kweche	1975	ARI Selian	Susceptible to stripe rust
3	Joli	1977	ARI Selian	Moderately resistant to stripe rust
4	Viri	1983	ARI Selian	Moderately susceptible to stem rust
5	Mbayuwayu	1987	ARI Selian	Moderately resistant to stem rust
6	Azimio 87	1987	ARI Selian	Resistant to stem rust
7	Tausi	1987	ARI Selian	Susceptible to stem rust
8	Juhudi	1987	ARI Selian	Resistant to stem leaf rust
9	Njombe 7	1987	ARI Selian	Resistant to all rusts
10	Kware	1989	ARI Selian	Moderate resistant to stem rust
11	Chiriku	2002	ARI Selian	Slow rusting
12	Sifa	2004	ARI Selian	Moderate resistant stem rust
13	Riziki-C2	2006	ARI Selian	Moderate resistant to stem rust
14	Riziki-C1	2006	ARI Selian	Moderate resistant to stem rust
15	Lumbesa	2006	ARI Selian	Moderate resistant to stem rust
16	Rv 593	---	ARI Selian	No known
17	Kariega ¹	-	Farmers	The study found farmers using them after importing from Kenya for the reason that are resistant to stem rust disease.
18	Ngamia	-	Farmers	

Source: Tanzania national variety list (2008)

--- A breeding line

- Varieties not released in Tanzania

Table 4.2: Inocula used for the identification of possible differential varieties

Inocula location	Field identity	District	Region	Disease severity (%)
4°26'32''S 35°12'25''E	1776.07m.a.s.l	2 Hanang	Manyara	60ef
4°23'24''S 35°36'21''E	1398.06m.a.s.l	4 Babati	Manyara	45d
4°53'58''S 35°28'33''E	1781.62m.a.s.l	5 Katesh	Manyara	50de
3°32'42''S 36°10'56''E	1369.98m.a.s.l	7 Monduli	Arusha	60ef
3°55'09''S 37°43'32''E	1343.59m.a.s.l	8 Hai	Kilimanjaro	30bc
3°24'11''S 37°19'36''E	1482.61m.a.s.l	9 Hai	Kilimanjaro	35c
5°54'29''S 36°22'07''E	1756.79m.a.s.l	11 Mbeya urban	Mbeya	75f
5°56'46''S 36°53'51''E	1784.67m.a.s.l	12 Mbeya urban	Mbeya	50de
9°33'47''S 34°47'31''E	1581.54 m.a.s.l	16 Njombe	Iringa	15a
9°19'10''S 34°43'10''E	2943.33m.a.s.l	19 Makete	Iringa	25b

Table 4.3: Inocula used for the identification of the most virulent isolates

Inocula location			Region	District	Disease severity (%)
4°28'33''S	35°00'14''E	1773.94m.a.s.l	Manyara	Hanang	20ab
4°26'32''S	35°12'25''E	1776.07m.a.s.l		Hanang	60ef
4°37'15''S	35°12'25''E	1764.79m.a.s.l		Hanang	15a
4°23'24''S	35°36'21''E	1398.06m.a.s.l	Arusha	Babati	45d
4°53'58''S	35°28'33''E	1781.62m.a.s.l		Katesh	50de
3°21'37''S	35°33'12''E	1534.17m.a.s.l	Kilimanjaro	Karatu	40cd
3°32'42''S	36°10'56''E	1369.98m.a.s.l		Monduli	60ef
3°55'09''S	37°43'32''E	1343.59m.a.s.l		Hai	30bc
3°24'11''S	37°19'36''E	1482.61m.a.s.l	Mbeya	Hai	35c
3°06'18''S	37°51'31''E	1398.27m.a.s.l		Hai	15a
5°54'29''S	36°22'07''E	1756.79m.a.s.l		Mbeya rural	75f
5°56'46''S	36°53'51''E	1784.67m.a.s.l	Iringa	Mbeya urban	50de
5°14'27''S	36°12'53''E	1801.43m.a.s.l		Mbeya urban	30bc
8°01'03''S	34°53'47''E	1234.51m.a.s.l		Mbeya urban	5a
9°33'47''S	34°47'31''E	1581.54m.a.s.l	Makete	Njombe	25b
9°59'58''S	34°12'08''E	1965.32m.a.s.l		Njombe	15a
9°19'10''S	34°43'10''E	2943.33m.a.s.l		Makete	25a
s.e					0.99
CV (%)					27.52

Table 4.4: The mean reaction to infection type expressed by different varieties after inoculation with inocula from different regions

Varieties	Average infection type grading / region					Mean ¹	Reaction to infection
	Manyara	Arusha	Kilimanjaro	Mbeya	Iringa		
Mamba	3.67	3	2.5	3.33	2.5	3.00c	S
Kweche	2.33	3	1.5	0	1.5	1.67ab	MR
Joli	2.33	3	2.5	2.33	2.5	2.53c	S
Viri	3.67	3	2.5	3	3	3.03c	S
Mbayuwayu	2	2	1.5	2.67	2	2.03ab	MR
Azimio 87	3	2	2.5	2.33	2	2.37ab	MR
Juhudi	2.33	3	2.5	2.33	2	2.43ab	MR
Njombe 7	3	2	2	3	3.5	2.70c	S
Kware	3	2	2.5	2	2.5	2.40ab	MR
Chiriku	3	3	2.5	3.67	2.5	2.93c	S
Sifa	2	1	2	1.33	0.5	1.37a	R
Riziki C1	3	2	1.5	2.67	2	2.23ab	MR
Riziki C2	3	2	2	3	2	2.40ab	MR
Tausi	3	2	2.5	3.67	3	2.83c	S
RV 593	2	2	1.5	3.33	2	2.17ab	MR
Lumbesa	3	3	2	3.67	2.5	2.83c	S
Kariega	2.33	2	2	2.67	2.5	2.30ab	MR
Ngamia	2	2	2	3.33	2.5	2.37ab	MR
s.e						0.86	
CV (%)						21.53	

¹Reaction to infection type in average grading on a 0 – 4 scale (Stakman *et al.*, 1962) in which 0 and 1 = Resistant (R) types of reaction to infection, 2 = moderately resistant (MR) and 3 and 4 = susceptible (S) reaction to infection types.

Table 4.5: Virulence of wheat stem rust isolates from the various fields of the major wheat growing regions in Tanzania

Regions	Average scores for virulence traits of isolates per field																			
	Generation time (days) ¹					Infection type ²					Disease incidence ³ (%)					Disease severity ⁴ (%)				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Manyara	8a	7a	9a	8a	14b	3a	4a	2b	3a	2b	30bc	60ef	20ab	40cd	20ab	35c	45d	5a	30bc	15a
Arusha	10a	7a	-	-	-	3a	3a	-	-	-	20ab	50de	-	-	-	25b	40cd	-	-	-
Kilimanjaro	8a	8a	13b	-	-	3a	3a	2b	-	-	30bc	40cd	30bc	-	-	25b	20ab	5a	-	-
Mbeya	10a	9a	13b	16c	-	3a	3a	2b	2b	-	10a	60ef	30bc	-	-	15a	40cd	20ab	10a	-
Iringa	11b	-	9a	8a	-	2b	-	2b	3a	-	10a	-	10a	-	-	5a	-	5a	15a	-
Average					7.39						2.18				27.06					20.88
s.e					19.32						0.75				0.09					15.39
CV (%)					10.97						22.30				26.54					17.88

¹Isolates groups based on generation time 7 – 10 days of sporulation after inoculation = fast, 11 – 14 = normal and 15 – 17 = slow

²Isolate categories according to infection type 0 – 1 = less aggressive; 2 = moderately aggressive; 3 – 4 = aggressive

³Disease incidence taken as percentage of infected seedlings out of five seedlings sampled per pot.

⁴Disease severity on a 0 – 9 scale where 0 = healthy seedling, 1 = 1 to 10% seedling area with rust pustules, 3 = 11 to 25%, 5 = 26 to 50%, 7 = 51 to 75%, and 9 = > 75% of seedling area affected.

Table 4.6: Mean separation for the virulence attributes of wheat stem rust isolates from selected wheat fields in Tanzania

Field ¹	Suggested isolate names ²	Generation time (days) ³	Infection type ³	Disease severity (%) ²
1	Hanang 1	9.00abc	2.50a	22.50abcd
2	Hanang 2	8.50ab	3.50a	37.50d
3	Hanang 3	9.50abcd	2.50a	22.50abcd
4	Babati 1	7.50a	2.00a	27.50cd
5	Katesh 1	13.50abcde	2.00a	12.50abc
6	Karatu 1	12.50abcde	2.50a	25.00bcd
7	Monduli 1	7.50a	2.50a	37.50d
8	Hai 1	9.50abcd	1.50a	22.50abcd
9	Hai 2	9.00abc	1.50a	27.50cd
10	Hai 3	15.00de	1.50a	12.50abc
11	Mbeya rural 1	9.50abcd	2.50a	17.50abc
12	Mbeya urban 1	9.50abcd	3.50a	22.50abcd
13	Mbeya urban 2	12.00abcde	1.50a	17.50abc
14	Mbeya urban 3	14.50cde	2.00a	15.00abc
16	Njombe 1	16.50e	2.00a	12.50abc
18	Njombe 2	8.00ab	2.00a	10.00abc
19	Makete 1	9.00abc	2.50a	12.50ac
	s.e	3.84	1.93	2.66
	CV (%)	11.70	27.80	23.20

¹Field numbers 1 – 5, 6 -7, 8 – 10, 11 – 14 and 16, 18 and 19 were located in Manyara, Arusha, Kilimanjaro, Mbeya and Iringa regions respectively. Farm number 15 of Mbeya and 17 and 20 of Iringa regions were free from wheat stem rust disease, hence no isolates.

²Isolates named based on districts' names where they were collected

³Means in the same column and row followed by the same letter (s) do not differ significantly according to Bonferroni test ($p \leq 0.05$).

Table 4.7: Varietal grouping according to the infection types exhibited

Group	Variety	Infection type range
1	Sifa	0 – 1.37a
2	Azimio 87, Juhudi, Rv 593, Kweche, Riziki-C2, Riziki-C1, Kware, Mbayuwayu, Kariaga, and Ngamia	1.67 – 2.43ab
3	Lumbesa, Njombe 7, Chiriku, Viri, Tausi, Joli, and Mamba	2.53 – 3.03c
	s.e	0.19
	CV (%)	24.66

Infection types on a 0 – 4 scale in which 0 and 1 = less aggressive; 2 = moderately aggressive and 3 and 4 = aggressive

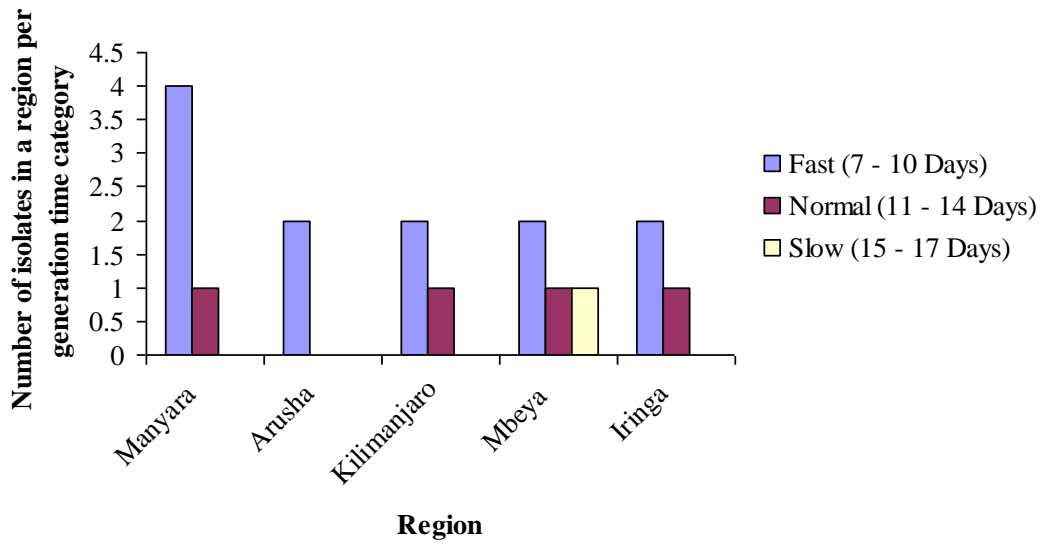


Figure 4.1: Groups of wheat stem rust isolates in Tanzania based on generation time

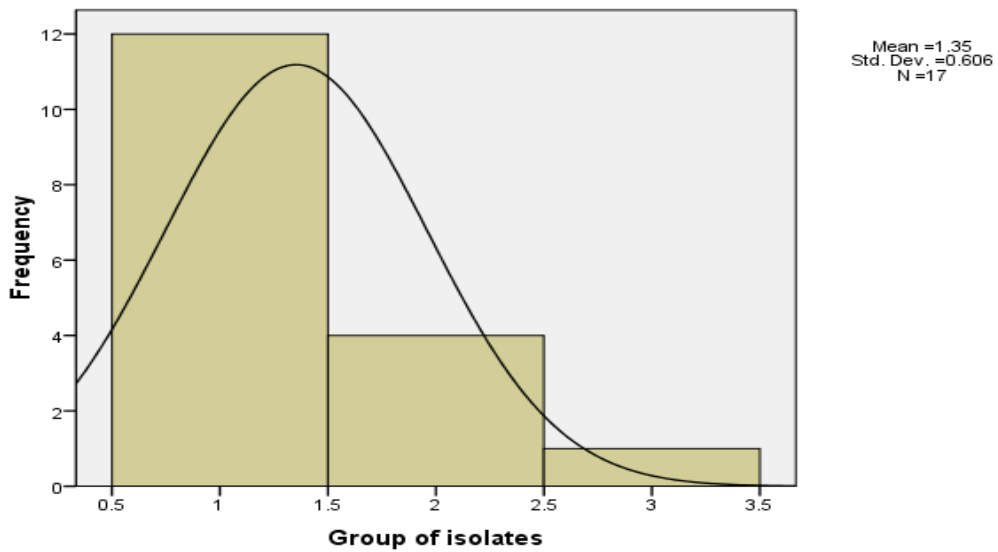


Figure 4.2: Groups of isolates of wheat stem rust disease based on generation time

- 1 = Fast isolates that sporulated 7 to 10 days after inoculation
- 2 = Normal isolates that sporulated 11 to 14 days after inoculation
- 3 = Slow isolates that sporulated 15 to 17 days after inoculation

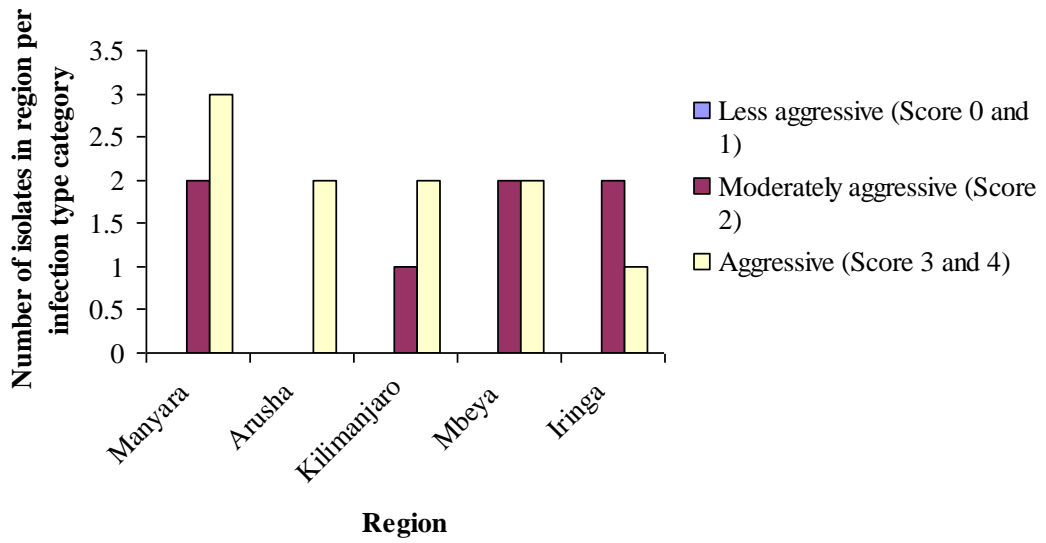


Figure 4.3: Groups of wheat stem rust isolates in Tanzania based on infection type

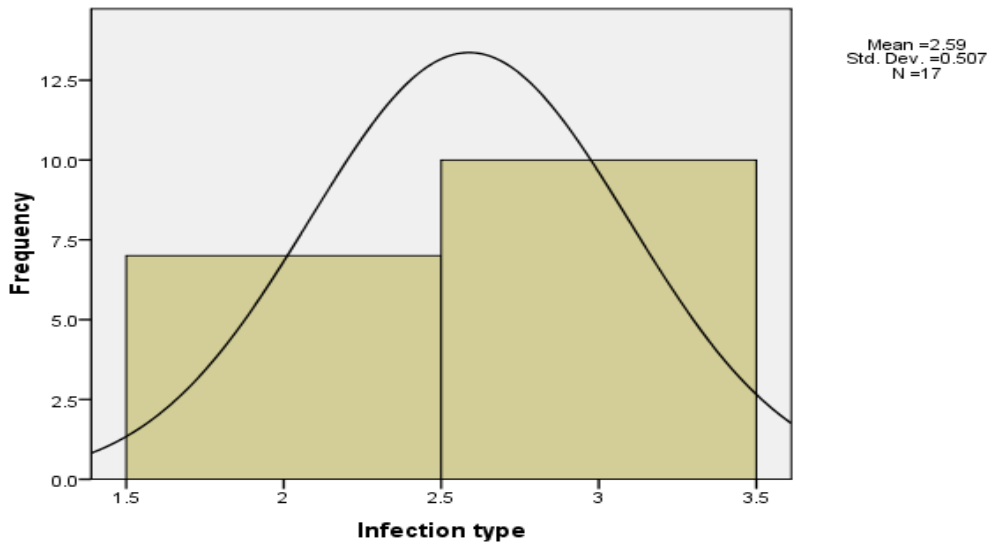


Figure 4.4: Groups of isolates of wheat stem rust disease based on infection type

- 2 = Moderately aggressive isolates group (Score 2 on 0 – 4 scale)
- 3 = Aggressive group of isolates (Score 3 and 4 on 0 – 4 scale)

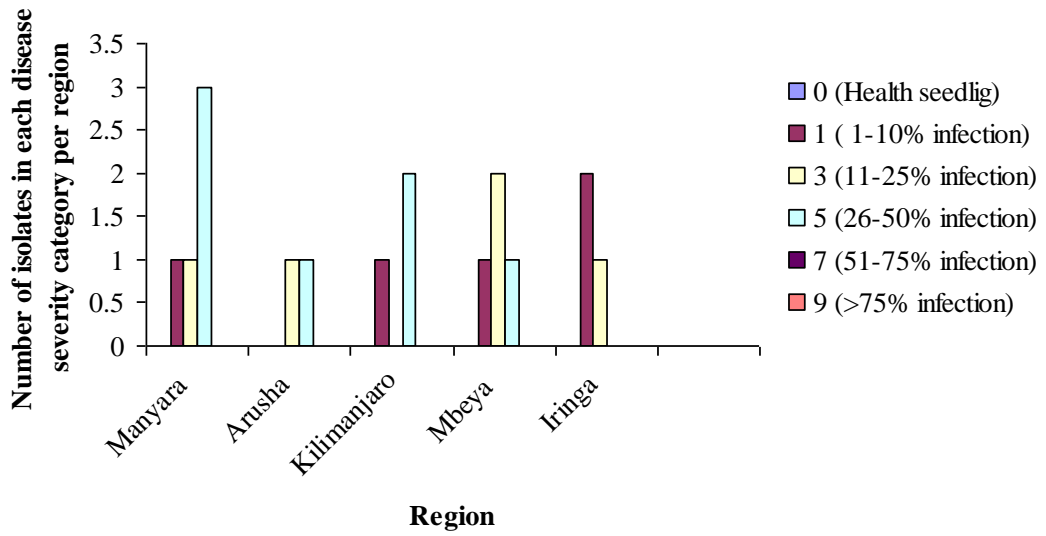


Figure 4.5: Groups of wheat stem rust isolates in Tanzania based on disease severity categories

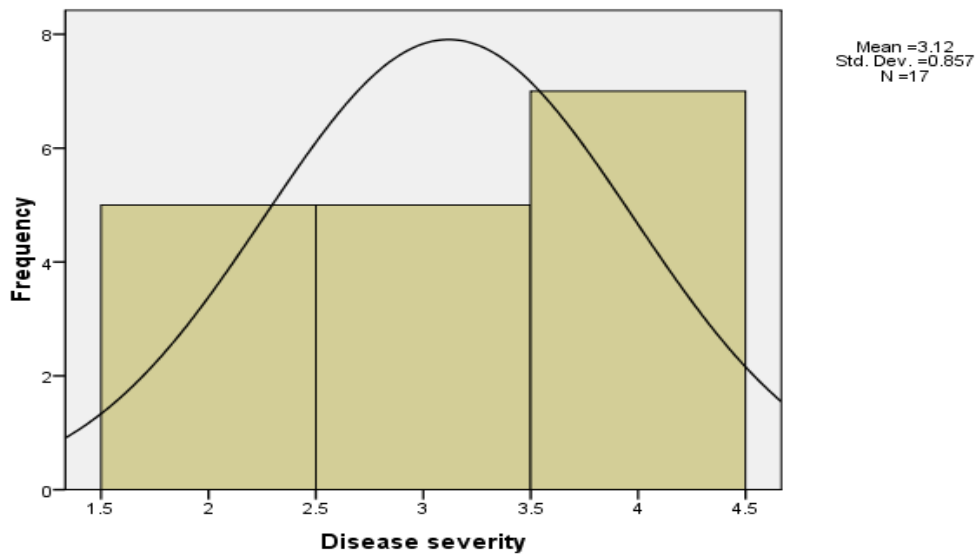


Figure 4.6: Groups of isolates of wheat stem rust disease based on disease severity

- 4 = Isolates that affected 1 – 10% of seedling area
- 5 = Isolates which affected 11 – 25% of seedling area
- 6 = Isolates which affected 26 – 50% of seedling area

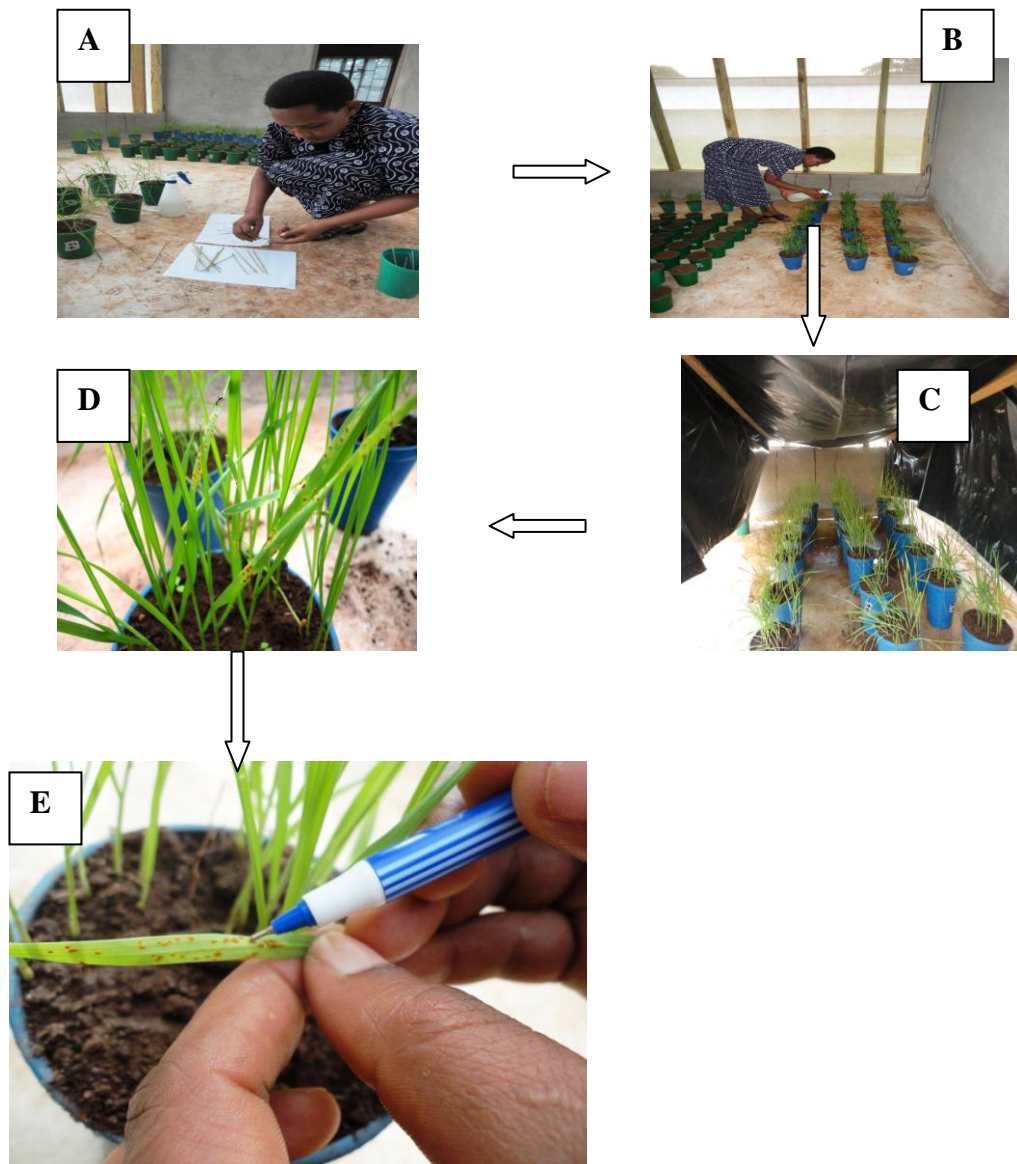


Plate 4.1: Scrapping of inoculum (A), inoculation of the seedlings (B), incubation of seedlings (C), disease development and scoring of infection types (E)

Note: Wheat stem rust disease infects both leaf and stem (Agrios, 2004) while wheat leaf rust disease infects leaf only, stem rust disease symptoms begin on leaf at seedlings stage and prolong to stem as plant mature into true stem (Roelfs *et al.*, 1992; CIMMYT, 2005). Since inocula were collected from infected wheat stems the causative pathogen in this case should be *Puccinia graminis* f. sp. *tritici*.

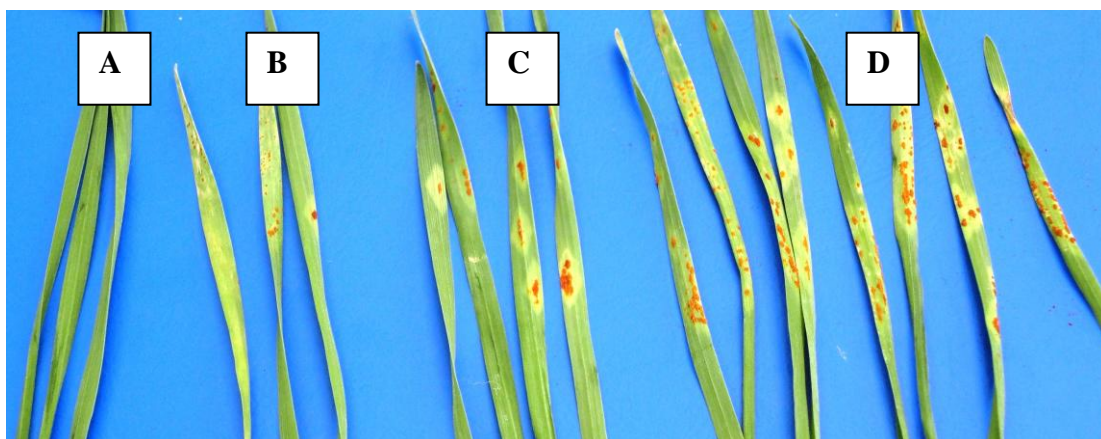


Plate 4.2: Various degrees of infection symptoms observed on some varieties

A = Sifa wheat variety

B = Azimio 87, Juhudi, and Ngamia

C = Kariega, Mbayuwayu, Riziki – C2 and Rv 593

D = Lumbesa, Kweche, Njombe 7, Chiriku, Joli, Tausi, Mamba and Viri

CHAPTER FIVE

5.0 REACTIONS OF WHEAT VARIETIES IN ASSOCIATION WITH THE MOST VIRULENT STEM RUST ISOLATES IN TANZANIA

5.1 Abstract

Eighteen wheat varieties were planted in screen house in 1 L plastic pots and inoculated by using five wheat stem rust inocula isolates collected from Manyara, Arusha, Kilimanjaro, Mbeya and Iringa regions. Fourteen to seventeen days after inoculation variety reaction was scored on a 0 – 9 scale. Reactions of wheat varieties with specific isolate from each region varied from highly susceptible (HS) to resistant (R). Of all varieties evaluated 27.78% exhibited HS reaction, 44.44% susceptible (S), 22.22% moderately resistant (MR) and only one variety (5.56%) was R to the isolates of wheat stem rust disease. In fact farmers from the northern highlands mostly grow Mamba, Mbayuwayu, Chiriku and Riziki while Sifa and Juhudi are commonly grown varieties in southern highlands. Similarly under artificially inoculation conditions Mamba, Viri, Mbayuwayu, Njombe 7 and Juhudi exhibited susceptible reaction to wheat stem rust pathogen whereas Sifa was resistant. Ngamia and Kariega which used to be resistant were generally susceptible; however validity of a variety being resistant or susceptible may be isolate and location specific. Information obtained from disease incidence and severity supported those of variety reaction in which varieties that expressed highly susceptible to susceptible reaction were severely infected by wheat stem rust disease. Sifa that was identified as resistant variety to the most virulent wheat stem rust isolates is hence recommended to be used as one of disease management strategies to reduce economic yield losses of wheat in Tanzania.

5.2 Introduction

Resistant varieties provide the most efficient control of wheat stem rust disease. Resistance of a cultivated variety to an isolate is a genetic character (Agrios, 2004). Therefore, a variety never loses its resistance to that isolate. With variations in temperatures, inoculum densities, light intensities, host nutrition levels, host growth stages or tissues, the resistance may be ineffective or not expressed, but the resistance gene remains. A variety may be resistant to one isolate and susceptible to another, and conversely an isolate may be virulent on one variety and avirulent on another (Joseph *et al.*, 2007). According to Ravi *et al.* (2011) resistance reaction is expressed as (i) a reduction in number of lesions and (ii) a reduction in the size of the sporulating area which can then be rated on a scale as described by Peterson *et al.* (1948). Some of the resistances function throughout the life span of the variety and still others only under at certain environmental conditions (Agrios, 2004).

Early maturing varieties escape much of the damage caused by wheat stem rust disease by avoiding the growth period of the fungus (Agrios, 2004). The use of resistant cultivars worldwide has reduced the disease as a significant factor in production. However, changes in pathogen virulence have rendered these strategies by making some resistances ineffective (Usha and Parimal, 2008).

Evaluation of plant reaction to the pathogen isolates is essential to gather information on the status of the cultivar at a particular time (Jain *et al.*, 2009). Generally, rust disease resistance can be scored using different rating scales, based on symptom severity. Two methods are commonly used to evaluate rust disease

reaction (Roelfs *et al.*, 1992). The first method is field assessment following natural infection. The second method is a controlled condition test by artificial inoculation of plants with rust spores (McNeal *et al.*, 1971).

Most varieties grown in Tanzania are considered to be susceptible to wheat stem rust disease (Ndoni *et al.*, 2001). These varieties were released between 1973 and 1980's except Chiriku, Sifa, Lumbesa, Riziki-C1, Riziki-C2 and Rv 593 which were released in between 2002 and 2006. Yield losses through reduced tillers, low grain weight and quality are estimated to be 30 to 60% (Ndoni *et al.*, 2001). The objective of this study was to determine level of susceptibility of each variety to wheat stem rust disease.

5.3 Materials and Methods

5.3.1 Location and description of the experimental site

Screen house experiments were conducted at Sokoine University of Agriculture (SUA) at the Department of Crop Science and Production (6° 45" S, 37° 40" E, 525 m.a.s.l). Experiments were carried out between May and June, 2012.

5.3.2 Collection of inocula of wheat stem rust from the infected wheat fields and inocula preparation

Plant stems bearing wheat stem rust pustules (urediniospores) were collected from two, five, three, four, and three wheat fields in Arusha, Manyara, Kilimanjaro, Mbeya and Iringa regions respectively. The infected stems were used as source of inocula. In screen house urediniospores from wheat stems per field were rubbed and dissolved in 100 ml sterile distilled water to make spore suspension.

5.3.3 An experiment to identify the most virulent wheat stem rust isolates

A pathogenicity test experiment was laid out in a screen house in a randomized complete block design (RCBD) with three replicates. Spore suspension of seventeen inocula samples (Table 5.1) was prepared by using sterile distilled water and sprayed over ten seedlings of Viri (susceptible wheat variety) which were eight days old maintained per pot. Plants were incubated for 24 h by covering using a polyethylene sheet. Thereafter seedlings were removed and placed on screen house bench for disease development.

5.3.4 An experiment to identify wheat resistant varieties to wheat stem rust disease

A split plot design experiment with three replications was laid down in screen house. Eighteen varieties (Table 5.3) were planted in 1 L plastic pots and ten plants were maintained per pot. The seedlings were irrigated every after other day and after eight days were inoculated by using five rust inocula (Table 5.2) of the most virulent isolates identified during pathogenicity experiment.

5.4 Data Collection and Analysis

5.4.1 An experiment to identify the most virulent wheat stem rust isolates

The generation time was observed and recorded (Jin, 2007b) whereby sporulation duration of 7 – 10 , 11 – 14 and 15 – 17 days after inoculation were designated to fast, normal and slow isolate groups respectively. Infection types in a scale of 0 to 4 in which 0 and 1 implied less aggressive, 2 = moderately aggressive and 3 and 4 aggressive nature of pathogen isolates were characterized as described by Stakman

et al. (1962). Averages of 10 seedlings were assessed for disease incidence and severity. The number of plants within each pot showing symptoms of wheat stem rust was recorded. The disease incidence was determined by taking the number of infected plants in each pot as a percentage of the total number of plants sampled in that pot. Average disease incidence was then calculated for isolates from each field in a region. Disease severity was rated using the Cobb scale on a 0 – 9 scale (McNeal *et al.*, 1971), where 0 = healthy seedling, 1 = 1 to 10% seedling area with rust urediniospores, 3 = 11 to 25%, 5 = 26 to 50%, 7 = 51 to 75%, and 9 = > 75% of seedling area affected.

Before analysis of disease severity data were transformed by calculating the square root value of each score to normalize and make variance relatively independent of the mean. Results of disease severity and infection type were subjected to analysis of variance (ANOVA) of complete randomized block design model and means were separated using the Bonferroni's test ($p \leq 0.05$). Gen Stat software V.13 statistical package (VSN International, UK) was used to facilitate the analysis with the following statistical CRBD models:-

$$Y_{ij} = \mu + T_i + B_j + e_{ij};$$

Where: μ = overall experimental mean,

T_i = effect of the respective isolate amongst the isolates

B_j = effect due to blocks

e_{ij} = random error effect.

Isolates were therefore, classified based on the generation time, infection type and disease severity of which the most aggressive isolates from each region were identified based on the means of virulence.

5.4.2 An experiment to identify wheat resistant varieties to wheat stem rust disease

Fourteen to seventeen days after inoculation variety reaction for each entry was evaluated. Disease reaction was scored on a 0 – 9 scale (Peterson *et al.*, 1948; Ngugi *et al.*, 2002), where 0 = No infection of stem rust disease = Immune, 1 – 2 = 1 to 4% area of leaf affected by stem rust spores = Highly Resistant (HR), 3 - 4 = 5 to 9% = Resistant (R), 5 – 6 = 10 to 19% = Moderately Resistant (MR), 7 – 8 = 20 to 29% = Susceptible (S) and 9 = > 75% of leaf area affected by spores of stem rust disease = Highly Susceptible (HS). Disease severity was rated using the modified Cobb scale on a 0 – 9 scale (McNeal *et al.*, 1971).

The disease incidence was determined by taking the number of infected plants in each pot as a percentage of five plants sampled per pot as shown below:-

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants in a pot}}{\text{Total number of plants sampled in a pot}} \times 100$$

Analysis of variance for disease incidence and severity and mean separation test was conducted using the Bonferroni's test at $p \leq 0.05$. Gen Stat software V.13 statistical package (VSN International, UK) was used to facilitate the analysis with the following experimental design model:-

$$Y_{ijk} = X + L_i + B_j + d_{ij} + V_k + (LV)_{ik} + E_{ijk};$$

Where: X = general mean common to all observations,

L_i = main plot treatment effect (source of Inocula),

B_j = the block effect,

d_{ij} = the main plot error (error a),

V_k = the sub plot treatment effect (wheat varieties),

$(LV)_{ik}$ = the main plot and subplot interaction effect of a respective inocula region with given wheat variety,

E_{ijk} = subplot error (error b).

Resistant variety was identified based on their infective reaction and symptom development by the isolates.

5.5 Results

5.5.1 Identification of the most virulent wheat stem rust isolates

Results of the pathogenicity test for 17 wheat stem rust isolates on Viri wheat variety indicated a mean generation time of approximately 7 days, infection type of 2.18 and disease severity of 20.88%. Mean separation indicated that generation time between isolates significantly differed for approximately 8 to 17 days. Isolates from Manyara and Arusha were the fastest in sporulation while the slowest isolates that sporulated about 17 days after inoculation was collected from Iringa region. Based on infection type scale described by Agrios (2004) and Stakman *et al.* (1962), isolates collected from field numbered 1, 2, 3, 6, 7, 11, 12 and 19 caused aggressive (score 3 and 4) type of infection whereas isolates from field numbers 4, 5, 8, 9, 10, 13, 14, 16 and 18 caused moderately aggressive (2 scoring) infection type as indicated by the disease severity scores in field (Table 5.1). Disease severity also significantly varied at $p \leq 0.05$ with isolates in the range of 10 to 37.50%. Isolates collected from field numbers 2 and 7 infected Viri severely (37.50%) while less severity of 10.00% was caused by isolate collected from field number 18 (Table

5.2). The rest of the isolates infected Viri variety in the following order; isolates from farm numbers 4 and 9 (27.50%), 6 (25.00%), 2, 3, 9 and 12 (22.50%), 11, 13 and 14 (15.00), 5, 10, 16 and 19 (12.50%). Based on generation time, infection type and disease severity the most virulent isolates from each region were identified as those obtained from field number 1, 7, 8, 12, and 19 of Manyara, Arusha, Kilimanjaro, Mbeya and Iringa regions respectively (Table 5.2). Of these five isolates obtained in field number 2 and 7 infected Viri aggressively thus considered the most virulent.

5.5.2 Reaction of varieties against the most virulent isolates of wheat stem rust pathogen

The reaction status of wheat varieties with specific wheat stem rust isolates from each region which varied from highly susceptible (HS) to resistant (R) was as presented in Table 5.3. Most varieties exhibited susceptible reaction at various levels to the most virulent isolates with the exception of Sifa which exhibited no leaf discoloration implying that there was no infection (Plate 5.1). Of all the varieties evaluated 27.78% exhibited highly susceptible (HS) reaction, 44.44% susceptible (S), 22.22% moderately resistant (MR) and only one variety (Sifa) (5.56%) was resistant to all isolates of wheat stem rust disease (Fig. 5.1).

5.6 Discussion

Vulnerability trend of wheat varieties to wheat stem rust disease was high to the extent that apart from Sifa most of the varieties cultivated were susceptible to the prevailing isolates in Tanzania. It was observed that currently grown wheat varieties

are susceptible to wheat stem rust disease except Sifa. Farmers from the northern highlands mostly grow Mamba, Mbayuwayu, Chiriku and Riziki while Sifa and Juhudi are the commonly grown varieties in southern highlands.

Similarly, under artificially inoculated and conducive conditions Mamba, Viri, Mbayuwayu, Njombe 7 and Juhudi exhibited susceptible reaction to wheat stem rust disease whereas Sifa was resistant. Ngamia and Karioga which were claimed to be resistant are generally susceptible; however validity of the claim may be isolate and location specific. For example, Ngamia exhibited moderately resistant reaction to isolates from Manyara and Kilimanjaro fields while the same variety (Ngamia) was susceptible to isolates from Mbeya and Iringa. This is again an indication that prevailing wheat stem rust isolates in Tanzania are different. At this point it is therefore essentially important to insist that recommendation for particular pest control measure including disease resistant varieties should be area and isolate specific (Joseph *et al.*, 2007).

Disease incidence and severity differed significantly ($p \leq 0.05$) with variety (Table 5.4). Information obtained from disease incidence and severity supported those of variety reaction in which varieties that expressed highly susceptible to susceptible reaction were severely infected by stem rust disease. Sifa wheat variety which exhibited resistant reaction on average had less number of seedlings infection with minimum to none disease severity as compared to other varieties. On the other hand varieties such as Viri, Mamba, Juhudi, Njombe 7, Chiriku, Mbayuwayu, Tausi, Joli, Kware and Lumbesa were adversely affected by wheat stem rust isolates. Most of

these varieties are old (released between 1973 and 1980's) and with time they turn out to be susceptible to pathogens (Agrios, 2004). With the emergence of new isolates and some of the present isolates becoming more aggressive mainly because of climate change (Usha and Parimal, 2008) cultivated varieties that were previously resistant break resistance to plant pathogens.

5.7 Conclusion and Recommendations

Present study has revealed that most of wheat varieties grown in Tanzania are susceptible with the exception of Sifa wheat variety. The following recommendations are made out of this study:-

- i. Sifa is recommended for use as one of the disease management strategies to decrease economic losses caused by wheat stem rust disease in Tanzania.
- ii. Specific varieties that exhibited resistant reaction in a specific location are recommended as one of disease management options in a given region.
- iii. Breeding research is recommended to replace susceptible varieties e.g. Viri.

Table 5.1: Inocula used for the identification of the most virulent isolates

Inocula location			Region	Isolate name (District)	Field identity	Disease severity (%)
4°28'33''S	35°00'14''E	1773.94m.a.s.l	Manyara	Hanang 1	1	20
4°26'32''S	35°12'25''E	1776.07m.a.s.l		Hanang 2	2	60
4°37'15''S	35°12'25''E	1764.79m.a.s.l		Hanang 3	3	15
4°23'24''S	35°36'21''E	1398.06m.a.s.l		Babati 1	4	45
4°53'58''S	35°28'33''E	1781.62m.a.s.l		Katesh 1	5	50
3°21'37''S	35°33'12''E	1534.17m.a.s.l	Arusha	Karatu 1	6	40
3°32'42''S	36°10'56''E	1369.98m.a.s.l	Kilimanjaro	Monduli 1	7	60
3°55'09''S	37°43'32''E	1343.59m.a.s.l		Hai 1	8	30
3°24'11''S	37°19'36''E	1482.61m.a.s.l		Hai 2	9	35
3°06'18''S	37°51'31''E	1398.27m.a.s.l	Mbeya	Hai 3	10	15
5°54'29''S	36°22'07''E	1756.79m.a.s.l		Mbeya rural 1	11	75
5°56'46''S	36°53'51''E	1784.67m.a.s.l		Mbeya urban 1	12	50
5°14'27''S	36°12'53''E	1801.43m.a.s.l	Iringa	Mbeya urban 2	13	30
8°01'03''S	34°53'47''E	1234.51m.a.s.l		Mbeya urban 3	14	5
9°33'47''S	34°47'31''E	1581.54m.a.s.l		Njombe 1	16	25
9°59'58''S	34°12'08''E	1965.32m.a.s.l	Iringa	Njombe 2	18	15
9°19'10''S	34°43'10''E	2943.33m.a.s.l		Makete 1	19	25

Table 5.2: Inocula used in varietal evaluation against wheat stem rust isolates

Inocula location			Source of inocula			Mean Disease severity (%)
			Field num ber	Isolate name (District)	Region	
4°28'33''S	35°00'14''E	1773.94 m.a.s.l	2	Hanang 1	Manyara	37.50a
3°32'42' S	36°10.56'E	1369.98 m.a.s.l	7	Monduli 1	Arusha	37.50a
3°24'11''S	37°19'36''E	1482.61 m.a.s.l	9	Hai 2	Kilimanjaro	27.50ab
5°56'46''S	36°53'51''E	1784.67 m.a.s.l	12	Mbeyaurban 1	Mbeya	22.50ab
9°19'10''S	34°43'10''E	2943.33 m.a.s.l	19	Makete 1	Iringa	12.50c

Table 5.3: Wheat varieties reaction to the most virulent isolates from each region and average reaction in Tanzania

Varieties	Average variety reaction grading per region						Reaction
	Manyara	Arusha	Kilimanjaro	Mbeya	Iringa	Mean ¹	
Mamba	8.10	7.20	7.50	7.90	6.70	7.48	HS
Kweche	4.40	5.20	4.80	3.60	2.20	4.04	MR
Joli	7.50	8.30	7.70	7.20	5.40	7.22	HS
Viri	8.80	7.20	6.50	6.00	6.90	7.08	HS
Mbayuwayu	7.00	6.20	5.50	6.30	6.70	6.34	S
Azimio 87	5.40	3.20	4.00	3.80	5.40	4.36	MR
Juhudi	6.10	6.40	7.40	6.00	6.20	6.42	S
Njombe 7	6.30	6.80	6.50	5.00	6.30	6.18	S
Kware	7.10	6.20	8.50	7.40	6.10	7.06	HS
Chiriku	6.40	5.90	7.60	5.30	6.50	6.34	S
Sifa	3.00	3.20	1.50	2.00	1.70	2.28	R
Riziki C1	4.10	3.20	2.70	3.10	3.70	3.36	MR
Riziki C2	5.30	4.90	3.70	4.00	3.40	4.26	MR
Tausi	8.70	7.90	6.60	6.30	7.50	7.40	HS
RV 593	5.00	4.80	5.20	4.30	4.20	4.70	S
Lumbesa	5.10	7.20	6.50	4.00	6.30	5.82	S
Kariega	4.80	5.30	5.67	6.00	5.90	5.53	S
Ngamia	3.90	4.30	3.58	5.69	5.44	4.58	S

¹Variety reaction in average grading under a scale of 0 – 9 (Peterson *et al.*, 1948) where 0 = Immune, 1 = Highly Resistant (HR), 2 = Resistant (R), 3 & 4 = Moderately Resistant (MR), 5 & 6 = Susceptible (S) and 7, 8 & 9 = Highly Susceptible (HS).

Table 5.4: Differences in mean disease incidence and severity caused by wheat stem rust on different wheat varieties in Tanzania

Variety	Mean disease incidence (%) ¹	Mean disease severity ¹
Sifa	8.00a	3.00a
Ngamia	14.00ab	12.00ab
Kweche	20.00abc	14.00abc
Kariega	22.00abc	12.00ab
Riziki-C2	24.00abc	25.00bcde
Chiriku	28.00abcd	22.00bcde
Mbayuwayu	28.00abcd	28.00cdef
Riziki-C1	30.00bcd	21.00bcde
Joli	31.00bcd	23.00bcde
Azimio 87	32.00bcd	18.00abcd
RV 593	32.00bcd	25.00bcde
Kware	34.00bcd	31.00def
Juhudi	38.00cd	36.00ef
Lumbesa	38.00cd	21.00bcde
Tausi	38.00cd	26.00bcdef
Mamba	40.00cd	32.00def
Njombe 7	40.00cd	28.00cdef
Viri	46.00d	41.00e
s.e	6.41	1.89
CV (%)	7.00	27.80

¹Means in the same column followed by the same letter do not differ significantly according to Bonferroni test at $p \leq 0.05$.

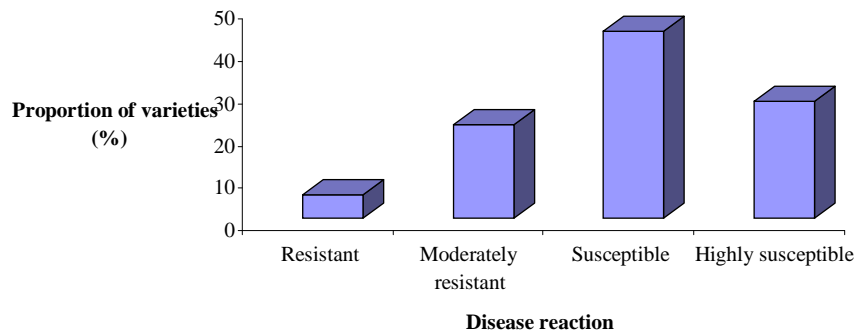


Figure 5.1: Status of wheat varieties against wheat stem rust disease in Tanzania

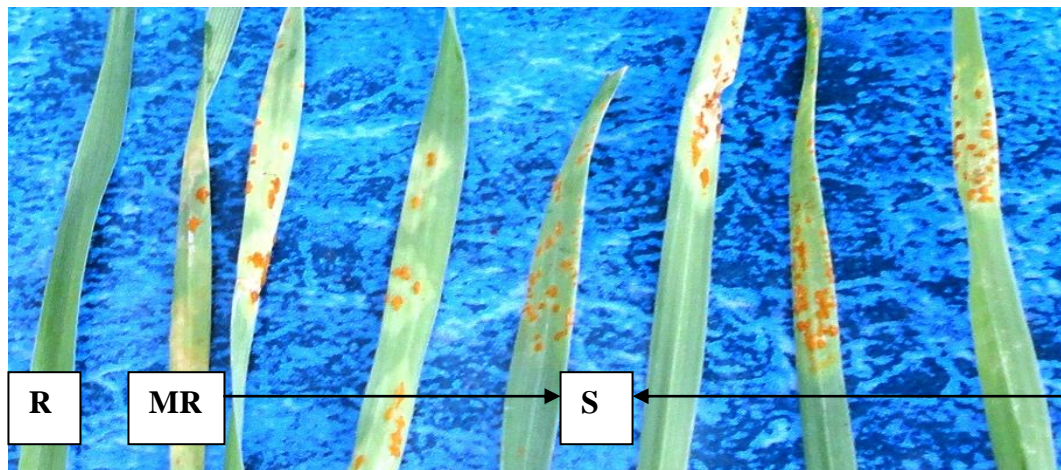


Plate 5.1: Some variety reactions following inoculation with the most virulent wheat stem rust isolate from selected regions

- R = Resistant reaction exhibited by Sifa as a response to the most virulent isolates from Manyara, Arusha, Kilimanjaro, Mbeya and Iringa
- MR = Moderately resistant reaction by Azimio 87, isolate from Manyara
- S = Susceptible reaction by Viri, Chiriku, Tausi, Mamba, Joli and Juhudi to the most virulent isolates from Manyara, Arusha, Kilimanjaro, Mbeya and Iringa.

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

This manuscript dissertation was produced after carrying out research activities which were interconnected. From field survey, wheat stem rust disease status in the fields was determined, agronomic, weather and crop management data were gathered. It was during this survey also wheat varieties and inocula used in the research were collected. Using results obtained from field survey isolates were ranked based on the severity of infection in the field and most aggressive isolates were identified from each of the surveyed region.

Isolates identified as most virulent were then inoculated into seedlings of the collected varieties to evaluate the way they respond to the isolates i.e. screening for possible differential varieties. Varieties segregated their responses to isolates and the most susceptible variety was also identified out of this experiment. A pathogenicity experiment was conducted by using the most susceptible variety previous pointed out in the previous experiment for differential varieties. The most susceptible variety was inoculated with inocula of isolates collected from all fields surveyed to find out the most virulent isolate in each region. Finally, isolates identified as most virulent were subjected to the collected wheat varieties to evaluate reactions of varieties against these isolates.

The collected isolates of wheat stem rust disease were categorized as fast, normal or slow in sporulating. There were isolates that caused moderately aggressive to aggressive type of infections and consequently caused severe seedlings infection. It was discovered that there was a relationship among virulence traits assessed whereby reduced generation time of isolates caused aggressive infection type that resulted into maximum disease incidence and severity both in the field and on seedlings in the screen house. The investigated isolates of wheat stem rust disease varied geographically as well.

6.2 Conclusions and Recommendations

Most isolates which sporulated fast, caused aggressive type of infection and infected wheat seedlings severely were mostly those obtained in northern Tanzania. Northern Tanzania produces more than half of the wheat produced in Tanzania and thus there is a need to pay more attention on this part as wheat stem rust disease is concerned.

Technology transfer is important to emphasize on merits of using improved varieties to replace old varieties which were more susceptible to pests and low yielding. Extension services should underline on importance of managing crop pests and good agricultural practices in general. Leaving wheat stem rust disease unchecked for example, not only affected wheat yield but also accelerated inocula build up and spread. It was apparent during the field survey whereby tools such as gum boots assisted rust dissemination across fields. The use of certified seeds should be encouraged as most fields were found not agronomically sound due to seed admixtures (off types).

Naming of isolates collected during this study based on districts where they were collected. Future research is recommended to name detected isolates based on binomial system of nomenclature after gene characterization.

The case of other pests and in particular wheat stripe rust disease and oxalis weed species as reported in this study should not be overlooked. Further research is essential to address farmers concern about severe infestation of oxalis weed species and birds in Mbeya and Manyara wheat fields respectively.

Information obtained during this study is recommended to be used by plant protectionists, breeders, farmers and other stakeholders to improve wheat production in Tanzania.

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