VIRULENCE SPECTRUM OF STEM RUST (*Puccinia graminis* f.sp. *tritici)* AND REACTIONS OF WHEAT VARIETIES TO DOMINANT RACES IN TIGRAY REGION, NORTHERN ETHIOPIA

M.Sc. THESIS

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Virulence Spectrum of Stem Rust (*Puccinia graminis* f.sp. *tritici)* and Reactions of Wheat Varieties to Dominant Races in Tigray Region, Northern Ethiopia

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A Thesis

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Jimma, Ethiopia

DEDICATION

This thesis work is dedicated to my father Hirpa Regasa, my mother Megartu Dinika, my brother Dawit Hirpa and my beloved friend Melat Girma.

STATEMENT OF AUTHOR

I declare that this thesis is my own work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in plant pathology at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and is deposited at the University Library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of an academic degree, diploma, or certificate.

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BIOGRAPHIC SKETCH

The Author, Gizachew Hirpa Regasa was born in November 1991, to his father Hirpa Regasa and his mother Megartu Dinika in Abuna Gindeberet District, West Shoa zone, Oromia Regional state. He attended his elementary education at Gago Osole Elementary School from 1998-2005. Then, he attended his high school and preparatory education at Gindeberet from 2006-2009, where he also took the Ethiopian Higher Education Entrance Qualification Certificate (EHEEQC) in June 2010. In November 2010, he joined Debre Markos University, where he obtained B.Sc. degree in plant science in July 2013.

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ABBREVIATIONS AND ACRONYMS

AARC	Ambo Agricultural Research Center
APR	Adult Plant Resistance
CSA	Central Statistical Authority
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agricultural Organization
f.sp.	forma specialis
GIS	Geographical Information System
HARC	Holeta Agricultural Research Center
IT	Infection Type
KARC	Kulumsa Agricultural Research Center
m.a.s.l	meters above sea level
MARC	Mekelle Agricultural Research Center
MhARC	Mehoni Agricultural Research Center
PA	Peasant Association
Pgt	*Puccinia graminis* f.sp. *tritici*
RH	Relative Humidity
R	Resistant
SARC	Sinana Agricultural Research Center
Sr	Stem rust
TARI	Tigray Agricultural Research Institute
Ug99	Uganda 1999 race

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Virulence Spectrum of Stem Rust (*Puccinia graminis* f.sp. *tritici)* and Reactions of Wheat Varieties to Dominant Races in Tigray Region, Northern Ethiopia

ABSTRACT

Wheat (Triticum spp.) is one of the most important staple cereal crops cultivated in the world particularly, Ethiopia. However, wheat stem rust (black rust) is one of the most important airborne diseases of wheat caused by Puccinia graminis f.sp. tritici remains a constraint to wheat production. It is an obligate biotrophic fungus only grow and multiply on living hosts. Since 1975, multiple wheat stem rust epidemics that caused significant losses have been occurred in different wheat growing regions of Ethiopia. Therefore, this study was aimed to assess the current distribution of wheat stem rust, identification of physiological race and reaction of bread wheat varieties against dominant races. The purposive multi-stage sampling procedure was used to select major wheat growing zones, districts and peasant associations. The current distribution of wheat stem rust was intensively assessed and examined in 95 sample wheat farms from 7 districts across the major wheat growing zones of Tigray region. The study showed that wheat stem rust was prevalent with significantly ($p < 0.01$) varied in incidence and severity of damage among fields, districts, and zones. The mean prevalence of wheat stem rust was 85.55% in Southern, 62.22% in Eastern and 53.33% in Southeast zones. The highest disease incidence of 78.67%, 66.50%, and 47.00% was recorded in Kilte Awulaelo, Raya Azebo, and Ofla districts, with corresponding severity of 43.67%, 35% and 20.67%, respectively which was higher than the previous reports from the region indicated the present distribution of the disease is remarkably on increasing trend. Race identification through inoculation of stem rust isolates, multiplication of single-pustule of the pathogen and race designation by inoculating on a set of wheat differential lines were done in the greenhouse. Forty-seven stem rust isolates were analyzed on the twenty stem rust differentials and resulted in the identification of six races namely; TTTTF, TKTTF, TRTTF, TTRTF, RRTTF and TKPTF. In this study, race TTRTF was detected for the first time in Ethiopia and throughout the world. Out of the six races identified, TTTTF was detected from 25 (53.19%) isolates and TKTTF from 15 (31.91%) isolates. The most virulent race that made 18 stem rust resistant genes non-effective was TTTTF, which virulent on 90% of stem rust resistance genes. Differential hosts carrying Sr24 and Sr31 were effective genes which confers resistance to all of the races identified. Hence, the stem rust resistance gene Sr24 and Sr31 can be used as sources of resistance in the wheat breeding program. Evaluation of bread wheat varieties was done by artificial inoculation at seedling stage against dominant stem rust races in the greenhouse. Thirty-nine bread wheat varieties evaluated for their reactions against the dominant races TTTTF, TKTTF, TTKSK, TRTTF, RRTTF and JRCQC. Seven varieties, namely Honqolo, Huluka, Millennium, ETBW-9017, ETBW-9042, Dilfiker and Wabe showed resistance reactions (fleck (;) to 2). However, to come up with a precise and reliable recommendation, the experiment should be done at adult stage resistance. Therefore, further research should be conducted with the same varieties against those dominant races at adult stage resistance.

Keywords: Gene, Incidence, Prevalence, Resistant, Seedling Stage, Severity

1. INTRODUCTION

Wheat (*Triticum* spp.) is one of the most important and major cereal crops in the world in terms of production and nutritional value. Worldwide, wheat is the leading source of cereal proteins and primary staple food (Figueroa *et al*., 2017). It is the second most important crop in the world next to rice in area of production (Ambika and Meenakshi, 2018). Wheat is a source of food and livelihoods for over 1 billion people in developing countries (FAO, 2017b). The global demand for wheat continues to increase at an annual rate of 1.6% and some estimates indicate that 60% more wheat will be needed by 2050 (Shiferaw *et al.*, 2013). Significant yield gains in wheat production over the past 40 years have resulted in a steady balance of supply versus demand. However, the predicted global population growth rates and dietary changes mean that substantial yield gains over the next several decades will be needed to meet this escalating demand (Figueroa *et al*., 2017; Chen *et al*., 2018).

Globally, wheat is cultivated on over 244 million hectares of land with a production of about 881 million metric tonnes. It is one of the key staple crops for global food security and a strategic cereal crop in Africa as an essential element of food security, yet African countries spend more on importing wheat every year. North Africa is the largest wheat producing part of Africa; of which, Egypt is the leading producer followed by Ethiopia (Shiferaw *et al.*, 2013; FAO, 2017a).

In Ethiopia, wheat is one of the most important cereal crops cultivated and it is one of the strategic food security crops, which is largely grown in the highlands (Beyene *et al*., 2016; Hill and Fuje, 2017). It ranks 4th after teff, maize, and sorghum in area coverage and 3rd in total production after maize and teff (CSA, 2018). It is the main staple food for about 36% of the Ethiopian population. It is cultivated on 1.70 million hectares of land with total production of 4.64 million tonnes which makes the country the largest wheat producer in Sub-Saharan Africa (CSA, 2018). Ethiopia is the only country in Sub-Saharan Africa where smallholder wheat production meets more than 70% of the national consumption demand (Shiferaw *et al*., 2011). Tigray is one of the major wheat producing regions of Ethiopia. In this region wheat is the 3rd important cereal crop both in area coverage and production. The total wheat area and

production in 2017 cropping season in the Tigray region was 107,929.86 ha and 214,003.14 tonnes, respectively (CSA, 2018). However, the national average yield of wheat in Ethiopia is estimated at 2.74 t/ha while it is only 1.98 t/ha in Tigray region (CSA, 2018), which is lower than the country's and world's average yield of 3.65 t/ha (FAO, 2017a).

The continual drive to match yield and quality increases is not without its challenges and a variety of unpredictable abiotic and biotic factors continually pose threats to wheat production locally and globally (Figueroa *et al*., 2017). The low productivity is attributed to a number of factors including abiotic like; poor soil fertility, waterlogging, decreasing the availability of suitable farmland, climate change (moisture stress, extreme temperature) and low adoption of new agricultural technologies and biotic (diseases, insect pest, and weed competition). The decline in the genetic diversity of wheat, in the pursuit of elite high-performing cultivars has also contributed to a perfect storm in pathogen emergence to the point at which diseases threaten global wheat supplies. Among those biotic factors, wheat is attacked by a number of diseases that cause great losses to the quality, quantity of produce and significant role in yield reduction (Gebremariam *et al*., 2011).

In Ethiopia, more than 30 fungal diseases of wheat have been identified (Admassu *et al*., 2004). Among the fungal diseases, the three rusts, stem rust (*Puccinia graminis* Pers. f.sp. *tritici* Ericks and Henn*)*, stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici)* and leaf rust (*Puccinia triticina* Eriks) are some of the major factors leading to serious economic losses (Sharma *et al*., 2013). Wheat is very susceptible to rust fungal pathogen (*Puccinia* spp.) and it is highly destructive that cause high yield losses (Singh *et al*., 2006). Rusts can cause up to 60% of yield loss for leaf or stripe (yellow) rust and stem rust has been reduced yields markedly in wheat growing areas worldwide by as much as 100% in susceptible varieties (Park *et al*., 2007; Njau *et al*., 2010; Hodson, 2013).

Since time immemorial, wheat stem rust caused by the fungus *Puccinia graminis* f.sp. *tritici* has been one of the major threats to wheat cultivation all over the world (Ambika and Meenakshi, 2018). I t is a macrocyclic with five spore stages and obligate biotrophic fungus only grow and multiply on living hosts. Stem rust or black rust of wheat is one of the most

potentially destructive wheat crops historically and becoming the most feared disease in wheat growing countries all over the world seriously threatening the production (Pardey *et al*., 2013). It is estimated that global annual losses to wheat rust pathogens range between US$ 4.30 to 5.00 billion and specifically, stem rust estimated that global losses would average 6.20 million metric tonnes per year or higher under severe epidemics (Pardey *et al*., 2013). In Ethiopia, yield losses of 70.70%, 60.50% and 60.00% were reported in Arsi, Bale and West Shoa zones of Oromia region, respectively (Hei *et al*., 2017).

According to Singh *et al*. (2006), the highland of Ethiopia is considered as a hot spot for the development of stem rust diversity. Several wheat stem rust epidemics have been recorded in different parts of Ethiopia in recent history that have caused great losses; epidemics in 1975 on variety Laketch, in 1992/93 on variety Enkoy, in 1994 on variety Kubsa and recently following the identification and spread of the Ug99 race group, a new race TKTTF in 2013 on variety Digalu outbreak (Temesgen, 1995; Badebo, 2002; Singh *et al*., 2008, Singh *et al*., 2015; Olivera *et al*., 2015; Olivera *et al*., 2017).

The high virulence diversity and evolution rate of the pathogen makes a considerable proportion of wheat germplasm at risk in the country (Admassu *et al*., 2009). The persistence of stem rust as a significant disease in wheat can be attributed to specific characteristics which have a capacity to produce a large number of spores, disseminated by wind over long distances and the ability to change genetically, thereby producing new races with increased aggressiveness on resistant wheat cultivars (Roelfs *et al*., 1992; Singh *et al*., 2015).

Population variability of *Puccinia graminis* f.sp. *tritici (pgt)* is very high in Ethiopia *(*Admassu and Fekadu, 2005). The mobility of stem rust coupled with their inherent ability to change through mutation, genetic recombination and new introductions from other countries makes continual monitoring (Park, 2007). Furthermore, some studies that were carried out in Ethiopia showed that most previously identified races were virulent on most of the varieties grown in the country and they are among the most virulent in the world (Admassu *et al*., 2009). Keeping this in mind, continuous and exhaustive surveys need to be carried out to give a clear picture of the virulence pattern of *Pgt* in Ethiopia, particularly in Tigray region. The

survey of races helps to generate information regarding virulence of races, their frequency and distribution patterns across the zones of Tigray region and to detect the evolution of new races and forecasting the virulence shifts in a population. This is the greatest significance in developing wheat cultivars with durable stem rust resistance (Admassu *et al*., 2009).

Eventually, stem rust is becoming the major problem of wheat production in the Tigray region. It is highly aggressive that apparently healthy looking crops three weeks prior to harvesting can be severely devastated the wheat crop if sufficient inoculum arrives and build-up in the areas. In the study area farmers grown a single cultivar over a large area, continuous cultivation of the crop without crop rotation and extensive cultivation of CIMMYT originated bread wheat genotypes with similar genetic background which might contributed to the development of new virulent race (Abebe *et al*., 2012; Hailu *et al*., 2015; Singh *et al*., 2015).

The intensity of wheat stem rust changes from year to year and from place to place depending on the type of variety grown and climatic conditions. Hence, timely detection of stem rust races is very important to resistance breeding as information on virulence profiles of new races would enable wheat geneticists and breeders to utilize effective resistance genes in their breeding programs. Therefore, knowing the pathogens their ecology, distribution, virulence pattern, and variability is very important in the management strategy of the pathogen.

General objective

- To assess the distribution, identification of physiological races and virulence spectrum of wheat stem rust (*Puccinia graminis* f.sp. *tritici*) as well as their reactions to bread wheat varieties against the major virulent (dominant) races in Tigray region

Specific objectives

- To assess the prevalence, incidence and severity of wheat stem rust in Tigray region
- To determine the physiological races and virulence spectrum of *Puccinia graminis* f.sp. *tritici* in Tigray region
- To evaluate the reactions of bread wheat varieties to the major virulent races of *Puccinia graminis* f.sp. *tritici* at seedling stage under greenhouse conditions

2. LITERATURE REVIEW

2.1. Global Importance of Wheat

Wheat is a source of food and livelihoods for about two billion people or 40% of the world population (FAO, 2017b). Wheat provides a substantial proportion of the calories and proteins consumed by humans, but further production increases are necessary to feed a growing human population (Zhang *et al*., 2017). Wheat is the most traded food crop internationally and is used as an emergency food in aid for developing countries. It is a staple food used to make cakes, pasta, steamed bread, cookies, noodles and for fermentation to make alcoholic beverages such as beer and liquors (Tsegaye and Berg, 2007). Additionally, wheat straw is commonly used as a roof thatching material and as animal feed (Kumar *et al*., 2011).

Wheat is one of the key staple crops for global food security, providing more than 35% of the cereal calorie intake in the developing world, 74% in the developed world and 41% globally from direct consumption. It also contributes about one-fifth of the total calories and proteins of the total daily dietary intake. In addition, about 20% of the total demand is used as feed for livestock while 2-3% is used in industrial processing (Shiferaw *et al.,* 2013).

Wheat provides more nutrients to the world than any other single food source, it has high nutritive value (>10% protein, 2.4% lipid, and 79% carbohydrates). It is reported that more than 75% of the world's population consumes wheat as part of their daily diet. Demand for wheat in developing countries is increasing due to extensive urbanization and high population growth (Dreisigacker, 2004).

The demand for wheat in the developing world is projected to increase significantly in the coming decades. Meeting this demand will require concerted efforts in research and innovation to develop and deploy solutions to existing and emerging challenges. Hence, increasing wheat yield potential remains a high priority issue and it is paramount to produce more wheat while ensuring the safety of wheat crop (Shiferaw *et al.*, 2013; Chen *et al*., 2018).

2.2. Wheat Production Constraints

Wheat production is constrained by a number of abiotic and biotic factors (Nelson, 2013). The major abiotic factors include; drought (moisture stress), nutrient deficiencies (poor soil fertility), waterlogging, climate change (extreme temperature) and low adoption of new agricultural technologies are the threats that role a significant yield reduction. Among the abiotic factors; soil fertility and moisture stress are the principal wheat production limiting factors in Ethiopia (Haile *et al*., 2012). The major biotic factors that pose a serious and constant threat to wheat production and cause major annual losses in the country are diseases, insect pests and weeds (Abebe *et al*., 2012; Yami, 2012).

Diseases are among biotic factors that are the most important yield-limiting constraints in wheat production and it is an outcome of the long-term interaction between plants and pathogens. Plants have evolved defense mechanisms while pathogens have evolved effectors to overcome plant defenses (Stukenbrock and McDonald, 2009). Changes in cropping systems and in climate are likely to maintain the plant-pathogen interactions and are assumed to play a key role in the emergence of infectious plant diseases (Gregory *et al*., 2009). Emerging disease refers to a recent disease on a new host and/or on a new area, or it is a disease that has recently become important due to an increase in virulence. The pathogenic fungi represent a significant constraint to wheat production worldwide (Giraud *et al*., 2010).

However, environmental conditions, amount of inoculum, host susceptibility, host physiological growth stage and timing of the epidemic are all factor that affects the degree of damage significantly (Duveiller, 2007). In Ethiopia, more than 30 fungal diseases of wheat have been identified (Admassu *et al*., 2004). Among those fungal diseases like rusts (stem rust (*Puccinia graminis* f.sp. *tritici),* stripe rust (*Puccinia striiformis* f.sp. *tritici)* and leaf rust (*Puccinia triticina*)), Fusarium head blight (*Fusarium graminearum),* Septoria leaf blotches (*Septoria tritici*), tan spot (*Pyrenophora tritici repentis*), smut (*Ustilago tritici*) and powdery mildew (*Erysiphe graminis* f.sp. *tritici*) are the dominant ones that were reported over time. Reducing yield losses caused by fungal pathogens can contribute to feeding a growing human population worldwide (Zhang *et al*., 2017).

Globally, prominent and very 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/scab are those contribute to significant yield losses. The biotrophs are highly specialized and significant variation exists in the pathogen population for virulence to a particular variety and threaten grain production (Figueroa *et al*., 2017).

With increased globalization, accidental exotic incursions of plant pathogens like rusts are increasing (Hodson, 2010). Rusts are the major disease of wheat since no other wheat disease could result in greater loss over a large area in a given year. Northern and Eastern Africa, the near East and West, Central and South Asia which are all vulnerable to rust diseases alone account for some 37% of global wheat production (FAO, 2017b). The major wheat production areas worldwide are suitable environments for disease development and stem rust can occur where wheat is grown and prone to severe losses (Pretorius *et al*., 2007; Singh *et al*., 2015).

Moreover, wheat, barley, triticale and a few related species are the primary hosts for *Puccinia graminis* f.sp. *tritici.* Identification of races of *Puccina graminis* f.sp. *tritici* on commercial wheat cultivars was a clear signal that tracking rust pathogen races and monitoring disease status on a global basis is a high priority issue today (Njau *et al*., 2010). The strategy to ensure the safety of the world's wheat crop is also to protect it from devastating fungal diseases like stem rust (United Nations, 2011).

2.3. Historical Background of Cereal Rusts

Wheat rust is an ancient disease. During the Neolithic period, wheat rusts were used as signatures of religious beliefs, greatly influenced human civilization. Stem rust of wheat has been a catastrophic disease since Biblical times. It is referenced in the Bible as one of the cereal rusts and smuts that affected the crops of the Israelites as punishment for their sins (Leonard and Szabo, 2005). The occurrence or emergence of cereal rusts (*Puccinia* spp.) has a long history and has been a source of trouble on mankind since ancient time (antiquity) when the first cereal crops were grown in the Fertile Crescent (Roelfs *et al*., 1992). Among the first records of the disease come from Roman times when losses to the disease were so serious that

the Gods, Robigo, and Robigus, were worshipped or offered sacrifices in hopes of limiting the unpredictable devastation of rusts in their wheat crops (Schumann and Leornard, 2000).

However, Aristotle and Theophrastus discovered that cereal rusts developed in the presence of warm and wet weather (Leornard and Szabo, 2005). The cereal rusts have caused heavy disease epidemics since time immemorial. High yield losses ranging from 40-50% were reported in the 1950. Even after we understood the nature and conditions that led to stem rust, yield losses continued to plague wheat production, occasionally leading to devastating losses. Since then, concerted efforts to combat the stem rust have been aimed at the use of host resistance and eradicating barberry; the alternate host of stem rust (Voegele *et al*., 2009).

According to Saari and Prescott (1985), stem rust was historically a major problem of Africa, Middle East, all of Asia except Central Asia, Australia, New Zealand, Europe and America (both North and South). Epidemics of wheat stem rust occurred in USA, Europe, the Indian subcontinent, Australia and Africa (Knott, 1989). Over the past 150 years, several devastating rust epidemics have resulted in major famines in Asia and grain losses on a massive scale in North America (Roelfs, 1986).

Although, the last major stem rust epidemic occurred in Ethiopia during 1993/94 (Badebo, 2002) when a popular wheat variety 'Enkoy' fell out of production, the rest of the world has practically remained unhurt from stem rust for over three decades (Singh *et al*. 2008) until the appearance of a new virulent race named Ug99 that overcomes the previously effective stem rust resistance gene Sr31. Even if Ug99 in Ethiopia first occurred in 2003 in a few locations, it has now become the dominant race across all regions (Admassu *et al*. 2009). Three additional variants of Ug99 have been detected in Ethiopia are TTTSK, PTKSK and PTKST races (Singh *et al*., 2015). Again, in 2013/14 and 2014/15 severe localized stem rust epidemics occurred on variety Digalu, caused by race TKTTF. Ug99 (race TTKSK) was first confirmed in 2003 and continued to be the predominant pathotypes until 2014 when TKTTF (Digalu) race became dominant (Haile, 2013; Olivera *et al*., 2015).

Wheat stem rust is found wherever wheat is grown and has been an ever-present threat to civilization and also several hotspot areas exist worldwide where stem rust is severe. In the USA, the outbreak of wheat stem rust epidemics in the early 1900s was attributed to the wide occurrence of the susceptible alternate host barberry in areas where wheat is widely cultivated (Kurt, 2001). Wheat stem rust spread rapidly over long distances by wind and if not detected and treated on time, they can turn a healthy looking crop only weeks away from harvest into a tangle of yellow leaves, black stems and shriveled grains (FAO, 2017b).

2.4. Taxonomy of Rust Fungi

The rust fungi called *Puccinia* spp. has about 7000 species in more than 100 genera (Webb and Fellers, 2006). The rust fungi derive their name from the rust coloured masses of urediniospores that are clonally produced on plant hosts (Kolmer *et al*., 2009). Wheat stem rust is characterized by pustules resulting from uredial development and belongs to; kingdom Fungi, phylum Basidiomycota, class Uredioniomycetes, order Uredinales, family Pucciniaceae, genus *Puccinia*. It contains 17 genera and approximately 4121 species, of which the majority are in the genus *Puccinia.* The stem rust genus attacks around 365 species of cereals and grasses in 54 genera and exhibits high genetic diversity (Groth *et al*., 1995; Leonard and Szabo, 2005).

Wheat stem rust has infectious structures with limited secretory activity. They also have carbohydrate and protein-rich layers which secrete the fungal and host plasma membranes (Bolton *et al*., 2009). The presence of haustoria enhances their feeding ability leading to prolonged host defense suppression. Haustoria are structures essential not only to acquire nutrients but to deliver effectors into the plant cell, allowing the suppression of plant defenses and cell reprogramming to accommodate fungal growth (Leornard and Szabo, 2005; Garnica *et al*., 2014; Ramachandran *et al*., 2016).

2.5. Life Cycle of Wheat Stem Rust

Wheat stem rust is obligate biotrophic organisms that are completely dependent on nutritional resources obtained from living host cells for growth and reproduction (Duplessis *et al*., 2012).

It is heteroecious, requiring two phylogenetically distinct or unrelated host plants to complete the life cycle. It has a macrocyclic life cycle involving five spore stages; basidiospores, pycniospores (spermatia), aeciospores, urediniospores (uredospores), and teliospore (Kolmer *et al*., 2009). The five spore stages differ from each other in appearance, ploidy, pathogenicity, virulence, structures formed, and mechanism of penetration (Staples, 2000).

The fungus has both sexual and asexual reproduction systems implying the presence of more phenotypes with more distribution. Spores occur during asexual reproduction on wheat or other *Poaceae* hosts and during sexual reproduction on common barberry (*Berberis vulgaris* L.) and Mahonia spp. or an alternate host Berberidaceae species (Leonard & Szabo, 2005). The devastating asexual reproductive phase is driven by urediniospores, which mediate infection through multiple developmental stages, such as haustoria formation (Staples & Macko, 2004; Singh *et al*., 2008).

Through asexual reproduction, billions of identical spores are released onto mature wheat. It is an obligate parasite has no resting stage in its cycle and this complicates the management of the stem rust epidemics. The full stem rust lifecycle begins with an infected plant, with elongated blister-like pustules, full of loose brownish red urediniospores found on the leaf sheaths, awns, glumes, stem tissue and leaves. Pustules typically form on the lower side of the leaf, but may occasionally penetrate the upper surface of the leaf (Singh *et al*., 2008).

The macrocyclic life cycle of wheat stem rust starts with teliospores, which are the overwintering structure for stem rust (Figure 1). As the growing season progresses and the infected plant matures, the uredinia convert into telia and start producing teliospores as part of the sexual stage of the life cycle. Teliospores are black in color and give forth the name black rust. Teliospores are firmly attached to the plant tissue and are commonly left in the field on the crop residue to serve as specialized survival structures to survive the winter (Leonard, 2005). During the dormant period, the first steps in sexual recombination occur. Each teliospore contains two nuclei per cell, and each nucleus has one set of chromosomes. The teliospore then begins to germinate, and the four haploid nuclei migrate to one of four

developing basidiospores. The four nuclei then divide to produce two haploid nuclei per basidiospore (Leonard, 2005).

The basidiospores infect the alternative host through the stomatal cell walls to produce haploid spores. When the basidiospores reach maturity, they are forcibly ejected and carried by air currents to infect the alternate host. Young leaves of common barberry are infected the most, as barberry leaves become resistant as the plant matures. This occurs when the leaf surface develops thick cuticles as the plant ages, thereby not allowing the penetration peg of the basidiospore germ tube to penetrate the surface of the leaf. When the basidiospore penetrates the cuticle, pycnia are formed on the upper leaf surface (Leonard and Szabo, 2005).

Within the pycnium, pycniospores containing a single haploid nucleus are produced in sugary nectar to function as male gametes, and monokaryotic hyphae are produced to function as the female gamete. Each gamete is either a + or a – mating type to prevent self-fertilization, as the + mating type can only fuse with the – mating type. When a pycniospore finds a receptive hypha fusion occurs, allowing for the pycniospore migrating through the hypha to the base of the pycnium. Nuclear division with paired + and – mating type nuclei causes the cells to change to a dikaryotic state to form an aecium (Leonard, 2005).

An aecium will develop on the underside of the barberry leaf directly underneath the pycnium, with single-celled dikaryotic aeciospores rupturing the epidermis of the leaf. Aeciospores can infect the Poaceae host, but not the Berberidaceae alternate host (Leonard and Szabo, 2005). Genetic recombination is a very important aspect of the stem rust life cycle, as mentioned previously it can lead to new virulent races being formed. Without recombination, the pathogen would have to rely on rare mutations to form new virulent races. When aeciospores are disseminated to a Poaceae host, the spore germinates to form a dense mass of hyphae below the leaf epidermis. Single-celled urediniospores are produced to form a uredinium, and the full life cycle is completed (Ward, 2012).

At this stage, the urediniospores can continue to infect the *Poaceae* host and can be disseminated long distances to infect other grassy hosts. However, at this stage, freestanding

moisture is essential for urediniospores to infect. Without 6-8 hours of dew or moisture from rain, germination cannot take place (Singh *et al*., 2008). In the presence of free moisture, urediniospores are very successful in causing infections. Uredinia produce the summer rust spores and telia produce the overwintering spores. Urediniospores are also very efficient at traveling long distances by air currents to infect other *Poaceae* hosts (Singh *et al*., 2011).

Hence, there can be multiple generations of inoculums produced during a single growing season. One uredinium can produce at least 100,000 urediospores leading to explosive epidemics during favorable environment and stem rust pustule can produce 10,000 urediniospores per day. Disease severity can increase extremely rapidly once the crop is uniformly infected (Roelfs *et al*., 1992; Beard *et al.*, 2004). Though most spores are deposited within the crop canopy and in close proximity to the infected plant, a number of spores can become airborne and reach heights of up to 3000 m. These spores can be relatively long-lived, as they can survive being away from host plants for a period of several weeks (Ward, 2012).

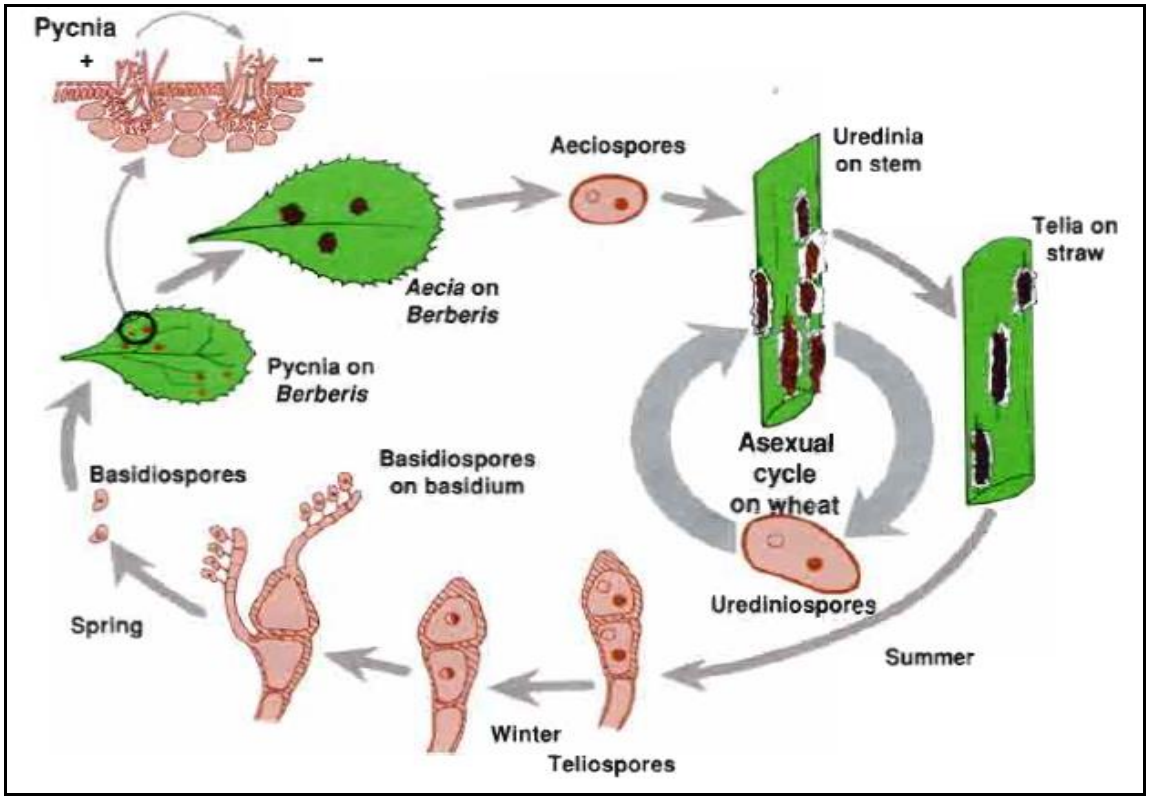

Figure 1: Life cycle of wheat stem rust (*Puccinia graminis* f.sp. *tritici*)

Source: **(**Roelfs *et al*., 1992)

2.6. Symptoms and Signs of Wheat Stem Rust

Stem rust can be distinguished from other rust diseases of wheat using several diagnostic features. When severe, stem rust can be differentiated from leaf rust or stripe rust by the parts of the plant that are infected. Stem rust appears as elongated or oval-shaped blister-like pustules or uredinia, most frequently on the leaf sheaths of a wheat plant but also on true stem tissues, leaves, and occasionally infects parts of the head including glumes and awns (De Wolf *et al*., 2011). Stem rust pustules on leaves develop mostly on the lower side but may penetrate and produce limited sporulation on the upper side (Leonard and Szabo, 2000). On the leaf sheath and glumes, pustules rupture the epidermis and give a ragged appearance. When the disease is less severe and only a few lesions can be found, it is important to focus on specific characteristics of the lesions. The orange-red spores of the fungus burst through outer layers of the plant tissue giving the margins of the lesion a tattered appearance and tearing is visible without magnification (Sheikh *et al*., 2017).

Compared to stem rust, the lesions of leaf rust are smaller, tend to be more rounds and cause less tearing of the outer plant tissue. In other words, stem rust lesions tend to be larger and longer than those caused by leaf rust. On a plant that is susceptible, urediniospores will develop. This visible manifestation of the fungus at the uredinial spore stage gives the pathogen its name. Primary infection by means of uredospores of wheat while as secondary infection by means of aeciospores of barberry. Urediniospores are rather resistant to atmospheric conditions if their moisture content is moderate (20-30%) (Sheikh *et al*., 2017). Another diagnostic sign for stem rust is the shiny black teliospores that are produced at the end of the growing season. These black spores are the reason for stem rust's alternate name, black rust (Leonard & Szabo, 2005).

2.7. Evolution of New Pathogen Races of Stem Rust

Wheat stem rust has the potential to cause the most damage when an epidemic occurs (Dean *et al.*, 2012). The resurgence of stem rust has received high attention from pathologists and breeders in wheat breeding programs due to the yield losses incurred worldwide (Singh *et al*., 2011). According to Haile and Roder (2013) evolution and occurrence of Ug99 variants have

further broken down most of widely deployed stem rust resistance genes existing in commercial cultivars and the introduction of new cultivars could contribute to the evolution of new stem rust races. New pathogen races evolve through mutations, genetic drift, gene flow, reproduction and selection (McDonald and Linde, 2002).

Mutations cause changes in the DNA sequences of individual genes forming new alleles of pathogen populations which later turn into virulent races which erode the available genetic resistance (Rajender *et al*., 2004; Bariana *et al*., 2007). With regard to genetic drift, the use of small host populations leads to loss of valuable alleles (Leonova *et al*., 2002). Through gene flow, virulent mutant alleles of pathogens when moved across different field populations increase their effective population size within a specific region as is the case with cereal rusts. Given the efficiency of sexual, asexual and para-sexual recombination, a pathogen population recombines new virulent alleles well ahead of the breeders thus overcoming the available resistance (Rosewarne *et al*., 2008). Finally, the selection is a great force influencing pathogen evolution especially with the practice of wheat monoculture (Webbs and Fellers, 2006).

In the 1920s, the barberry (*Berberis vulgaris*) eradication in the USA reduced the adverse effect of the *Puccinia graminis* f.sp. *tritici* on wheat production. This implied that the emergence of new races of the fungus through sexual stages was interrupted. Thus, mutation remained the most probable cause of evolution enabling wheat breeders to combine race-specific resistance genes. Research done on host resistance breeding of stem rust incidences were suppressed for many years by efforts of Dr. Norman Borlaug. This meant that research efforts were geared towards other constraints (Smith *et al*., 2009).

In Ethiopia, stem rust of wheat is the most destructive disease causing heavy damages. A stem rust epidemic caused a yield loss of up to 100% in some major wheat producing regions of the country, mainly due to the susceptibility of the most popular cultivar named “Enkoy” suffered severe yield loss (Temesgen *et al*., 1995; FAO, 2017b). Moreover, to minimize the threat of future epidemics, it is important to characterize the race composition of stem rust and detection of appearance of new races in Ethiopia (Woldeab *et al*., 2017).

Following the identification and spread of the Ug99 race group, a new race TKTTF (not a member of the Ug99 lineage) outbreak caused a wheat stem rust epidemic with an estimated 20,000 to 40,000 ha cultivated wheat variety "Digalu" which is introduced to provide protection against the Ug99 race group in Southern Ethiopia during 2013-2014 (Olivera *et al*., 2015, 2017). It breakdown the resistance gene SrTmp in the widely planted wheat variety Digalu. Similarly, in 2014 Kenya experienced significant losses in fields of the wheat variety "Robin," which also carries Sr*Tmp*, in this case caused by a new race in the Ug99 lineage (Singh *et al*., 2015; Patpour *et al*., 2016). Currently, it has been confirmed in 11 countries, and given the rapid and destructive nature of race TKTTF, close monitoring of this race is advised in countries which have cultivars carrying the SrTmp resistance gene (Xu *et al*., 2017).

2.8. Emergence, Evolution, and Spread of the Ug99 Races

The Ug99 strain was first emerged in East Africa around 1999, has the potential to infect most of the world's wheat production (Singh *et al*., 2011). East Africa is known to be a hotspot for the origin of new virulent races of stem rust fungus (Singh *et al.,* 2006). With the current mutants of race Ug99 being reported, breeders were not sure how this race is distributed in East Africa. There is continued fear that race Ug99 is still mutating as previously resistant materials are increasingly losing their resistance (Pretorius *et al.*, 2010). Realizing the threat posed by the Ug99 race group, over 400,000 wheat lines that included accessions from germplasm collections to breeding materials from wheat breeding programs throughout the world were screened for resistance to Ug99 in Kenya and Ethiopia. About 85-95% of wheat lines grown globally are susceptible to the Ug99 races (Singh *et al*., 2011).

Virulence for Ug99 was first detected in Uganda in 1999 (Pretorius *et al.,* 2000). Although it is not well studied, this race is expected to be widely distributed in most East African countries, particularly in Ethiopia and Kenya. In Ethiopia, the occurrence of new races of stem rust can be attributed to airborne spread from other regions within the epidemiological zone and mutation, followed by selection (Singh *et al*., 2006). This race overcame the long-term protection that had been provided by the stem rust resistance gene Sr*31,* this gene has been widely employed in CIMMYT wheat germplasm, which is widely cultivated in different parts of the world (Singh *et al*., 2006). Stem rust isolates with virulence on Sr*31* are already

widespread in the Eastern African highlands and pose an immediate danger to wheat production in the region (Wanyera *et al*., 2006). It was designated as TTKS based on North American stem rust nomenclature (Xu *et al*., 2009) and later TTKSK after the fifth set of differentials was added following further characterization (Singh *et al*., 2011).

The Ug99 race also has shown virulence to Sr*38* stem rust resistance gene transferred from *Triticum ventricosum* and continued to evolve into more virulent forms like TTKST and TTTSK showing virulence to stem rust resistant genes Sr*24* and Sr*36,* respectively and up to date these races most notably have virulence for Sr genes Sr*24,* Sr*31,* Sr*36,* and Sr*38* (Singh *et al*., 2015). The highly potent Ug99 is now present in 13 countries and is continuing to move and evolve. It has spread over vast areas causing epidemics in a northward trend from East Africa to the Middle East and has the potential to affect many wheat varieties grown worldwide as it keeps producing new variants (FAO, 2017b).

Ug99 has broad virulence, mutates and spreads quickly. Currently, there are 13 variants of Ug99 races have been found that are derivatives from the original race TTKSK (FAO, 2017b) and rendering additional resistance genes ineffective having an almost identical DNA pattern, only differing in their avirulence/virulence formula. The Ug99 race group has been found to be virulent on a total of 34 Sr genes, however, there are still 39 resistance genes that confer moderate or full resistance. About 35 of the 73 described Sr resistance genes were derived from cultivated bread wheat but only a few are effective against races in the Ug99 lineage (Singh *et al*., 2015). Due to the constant evolution and mutation of *Pgt* races, many resistance genes are rendered ineffective within a relatively short period of time (Rahmatov *et al*., 2016).

TTKSK (Ug99) is causing alarm for three reasons. First, this race has broad virulence to currently deployed Sr genes (Singh *et al*., 2006; Jin *et al*., 2007). Jin and Singh (2006) found that among North American cultivars, only 16% of hard red spring wheat, 48% of hard red winter wheat, and 28% of soft winter wheat had resistance to race TTKSK. Second, race TTKSK (Ug99) has continued to evolve. In 2006–2007, variants of TTKSK designated TTKST and TTTSK with added virulence to Sr*24* and Sr*36,* respectively were detected in Kenya (Jin *et al*., 2008; 2009). The third concern was the rapid movement of TTKSK from

Africa. A predicted path for dispersal of TTKSK from Eastern Africa to the Arabian Peninsula and ultimately to the Indian subcontinent was proposed by Singh *et al*. (2006). Race TTKSK has closely followed this path, appearing in Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014) (Nazari *et al*., 2009; Singh *et al*., 2011, 2015).

The steady geographical spread and rapid evolution of the Ug99 race group poses a significant threat globally. This threat has led to a major worldwide investment in improving genetic resistance to wheat stem rust through identifying and incorporating new sources of res istance effective against this race group (Singh *et al*., 2011; Steffenson *et al*., 2013). Studies using microsatellite markers showed that most of these pathotypes have identical fingerprints, consistent with them having arisen from a common ancestor via single-step mutation. Within East and Southern Africa, available data indicate that members of the *Ug99* lineage are now the predominant stem rust pathotypes throughout the entire region (Pretorius *et al*., 2010).

2.9. Dispersal Mode of Wheat Stem Rust

The urediniospores are dispersed by wind and water and can spread over vast distances. In addition to natural dispersal mechanisms, accidental transfer by means of farm implements, contaminated clothing or goods may also contribute to the spread of spores. There are three modes of transportation that the urediniospores can spread. One of them is long-distance dispersal by a single event as well as assisted dispersal, the second one being stepwise range expansion, and the third being extinction and recolonization. All of these modes of transportations have been observed before, although some of them are more common than others (Singh *et al*., 2008).

Dispersal by a single event is mostly rare of all other modes. This type of mode includes the movement of the urediniospores across whole continents. Although it has been noted that this type of transportation is extremely rare, it has been recorded before. Brown and Hovmoller (2002) report that stem rust spores have moved up to 8000 km from the south of Africa all the way to Australia. Although these events are rare and the ability of spores to withstand a high range of environmental pressures make this large distance travels completely possible.

Another type of dispersal by a single event is assisted dispersal. Unlike the previous example, humans mostly cause assisted dispersal. In assisted dispersal, the spores mostly travel on clothing as well as through the trade of infected wheat (Singh *et al*., 2008).

The second mode of event travels is in the smaller distance as well as it takes more time for the spores to travel. Stepwise range expansion does not expand across continents like single event dispersal. This type of transportation mostly spreads at the slightly smaller scale of countries and regions. Out of all the transportation modes, this is the most common one. The current expansion of the stem rust strain Ug99 is an example of stepwise range expansion. The strain first originated in Uganda in 1999, hence getting its name, then migrated into the Middle East, and now has made its way into all continents like Asia. Although the effects that Ug99 has left in its path are devastating, its slow expansion, taking it 10 years to spread to Asia, is giving scientists time to try and come up with a wheat resistant strain against Ug99 before it reaches India (Singh *et al*., 2004b).

The third mode of dispersal is extinction and recolonization. Although it is generally believed to be a different mode of dispersal, it is much similar to the stepwise range mode of expansion. Both of these modes expand through smaller distances, as well as move much slower than the single event mode. The only difference is that this type of dispersal happens on land that is too stressful for the spores to survive (Singh *et al*., 2008). The "*Puccinia* pathway" of North America, where spores are transferred by wind from south to north, exemplifies the extinction and recolonization mode since the disease eventually ends once the wheat season is over (Schumann and Leonard, 2000).

2.10. Factors Affecting Epidemics of Wheat Stem Rust

Plant disease epidemiology is the study of disease dynamics in both the temporal (time) and spatial (space) changes of epidemics caused by populations of pathogens in populations of plants (Milgroom, 2003). The three factors are necessary for disease development; a favourable environment, a susceptible host and presence of inoculum. If one of this prerequisite is not met, there will be no or limited damage of the disease. The temporal aspects of plant disease epidemiology are reflected by disease progress curves and

investigations as to whether the pathogen studied is monocyclic or polycyclic. On the spatial scale, patterns of inoculum dispersal and disease development are studied, both quantitatively and qualitatively. In addition, analysis of dynamic changes integrating both temporal and spatial aspects can be included (Milgroom, 2003). There are generally two basic sources of inoculum for the cereal hosts, the uredospores and the aeciospores. Urediniospores and aeciospores are wind borne but teliospores remain with the straw (Peterson, 2001).

Wheat stem rust is strictly obligate pathogens of living tissue and thus requires a host as a "green bridge" in order to survive until the next growing season (Staples, 2003). More favourable conditions like growing of wheat in different agro-ecological zones and the staggered cropping system ensures the availability of "green bridges" for rust spores (inoculum) throughout the year (Singh *et al*., 2008) and a significant amount of air-borne urediniospores that initiate early epidemics (Wanyera *et al*., 2009).

2.10.1. Environment

Environmental factors play an important role in the stem rust epidemiology. The physical environment influences the development of an epidemic through effects on various phases of the pathogen's life cycle as it interacts with specific phases in the development of the host plant. The main cause of the epidemic may be the favorable environmental conditions. Under a suitable environment, the chances of disease incidence are increased. Stem rust is more important late in the growing period than in early stages, on late-sown and maturing wheat cultivars, and at lower altitudes than at higher elevations (Singh and Tewari, 2001).

Much research is expended on capturing meteorological information and relating disease outbreaks to weather conditions. The rate of *Puccinia graminis* f.sp *tritici* infection is heavily influenced by cultivar susceptibility, the virulence of the rust race as well as favourable environmental conditions such as temperature and humidity. Temperature and moisture conditions in most wheat producing regions vary significantly from year to year and are the major limiting factors for the development of stem rust epidemics and wheat stem rust is a serious disease occurring frequently in warm and moist environments (Wanyera *et al*., 2010).

2.10.1.1. Temperature

Temperature is the environmental variable most often correlated with biological responses and it is measured almost universally in studies on plant disease epidemics. Due to changes in temperature and precipitation regimes, climate change may alter the host growth stage, development rate and pathogenicity of infectious agents, the physiology and resistance of the host plant. Moreover, temperature influences the germination, infection and survival of the uredospores as well as sporulation and host resistance (Charkraborty and Datta, 2003).

The uredial stage of *Puccinia graminis* f.sp. *tritici* is favored by hot days (25-30ºC) and mild nights (15-20ºC) and wet leaves from rain or dew. The minimum, optimum and maximum temperatures for spore germination are 2, 15 to 24 and 30ºC, respectively (Table 1) and for sporulation 15, 30 and 40ºC, respectively (Rowell, 1984; Roelfs *et al*., 1992). Worldwide, stem rust is mostly found in regions with a continental climate where summer temperatures regularly exceed 25 (Singh *et al.*, 2015). Stem rust will able to adapt an increased temperature associated with climate change. Recent studies suggest that rust pathogens can adapt to different optimal temperatures (Mboup *et al*., 2012).

Table 1: Environmental conditions required for different stages of stem rust development

Stage (process)	Temperature (^{0}c)			Light	Free water (moisture)
	Minimum	Optimum	Maximum		
Germination	2	15-24	30	Low	Necessary
Germ tube		20		Low	Necessary
Appressorium		16-27		None	Necessary
Penetration	15	29	35	High	Necessary
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None

Source: Roelfs *et al*. (1992)

2.10.1.2. Sunlight

Light is an important factor for the development of penetration pegs from the appressorium, but it is seldom a limiting factor in the field as dew often occurs in the morning. However, little infection results when evening dew and/or rain are followed by winds causing a dry off prior to sunrise (Rowell, 1984). Ultraviolet radiation also plays an important role in the natural regulation of diseases. Sunlight affects pathogens due to the accumulation of phytoalexins or protective pigments in host tissue. Atmospheric and soil moisture can have profound effects on the development of plant pathogens, vectors, host plants and thus on epidemics of plant disease (Yanez *et al.*, 2012).

2.10.1.3. Relative humidity and free moisture

The most important factor affecting the rate of stem rust development, ultimate severity and ultimate damage caused by the disease is the frequency of days with six or more hours of moisture at 10ºC or higher, favorable period for infection prior to dough stage of plant growth (Peterson, 2001). The life cycle of most plant pathogens contains one or more phases that are affected by the states, forms and energy of environmental water. Both aeciospores and urediospores require free water for germination. Moisture directly affects the uredospore germination, infection and survival. The six hours of uninterrupted moisture availability are needed for uredospore germination and infection to occur along with other necessary environmental conditions such as temperature and light (Chen, 2005; Mideksa *et al*., 2018).

The spores require relative humidity near 100% saturation and the hydration of the spores before inoculation increases germination (Line, 2002). On the contrary, free moisture also negatively affects the viability of the uredospores, decreasing overall survival of the spores. This occurs due to the absence of the inhibition mechanism of fungal growth, otherwise known as fungistasis which causes the death of the fungus (Chen, 2005). Relative humidity plays an important role in the penetration of haustorium of fungus as it makes the leaf tender due to moisture content. There is also a strong association between severity and hydric variables such as precipitation and relative humidity (Mideksa *et al*., 2018).

2.10.1.4. Wind

Air movement influences the dispersal and development of plant disease epidemics. Wind can transport inoculum of plant pathogens and vectors from one location to another within and among crop canopies, among fields, states, countries and even continents. It is also important in modifying the temperature and moisture available at the leaf surface within the crop canopy and to a more limited extent in the soil. Movement of plant parts by wind (hail or storm) also causes abrasions or wounds to plant surfaces that may serve as entry sites for pathogens. In addition to this, wind decreases the moisture content of inoculum inhibiting the spore germination which reduces the rates of infection. Wind also simultaneously influences the viability of the inoculum because of decreased moisture content that inhibits the immediate germination of the uredospores (Chen, 2005).

2.11. Management of Wheat Stem Rust

There are various management options available for combating wheat stem rust. Attempts have been made to minimize or manage stem rust through; cultural methods, resistant varieties, chemical control measures and Integrated disease management. Overall, it is essential to understand the epidemiology of stem rust to start any management measures (Singh *et al*., 2002). Effective management of stem rust requires a coordinated effort, including race monitoring, collection and characterization of sources of resistance and resistance breeding (Boshoff *et al*., 2000).

2.11.1. Cultural practices

Cultural practices provide other options for at least partial management of wheat stem rust epidemics. Cultural methods for controlling and preventing stem rust infection include planting date, irrigation, fertilization application, early maturing cultivars, crop rotation and removal of wheat debris. Planting as early as possible and planting early maturing cultivars would help to reduce the time of exposure of the crop to the pathogen, reduces the time frame for establishment of urediniospores and ultimately limits the growth period of the fungus and, hence, reduce yield loss (Fetch *et al*., 2011).

Other cultural control methods helpful in the fight against stem rust that growers need to take notice is fertilization and water application (irrigation). Extra moisture on leaves and an excess of nitrogen can lead to a more severe stem rust infection because the fungus thrives in wet environments with lush foliage (Schumann and Leonard, 2000). In order to limit a potential infection from those factors, farmers need to ensure that their fields have proper row spacing, a properly timed irrigation and fertilization schedule that does not correspond to the fungus prime infection period. The removal of wheat debris used to be an essential practice for stem rust control due to the fact that this is where the fungus produces its overwintering structures, known as teliospores (Leonard and Szabo, 2005).

Of course, single practice is not effective under all conditions, but using a series of cultural practices greatly enhances the existing resistances. Crop rotation helps to limit the genetic diversity of the pathogen population and to minimize the number of urediniospores produced. The success of implementing this method depends on sufficient knowledge of the epidemiology of stem rust fungus in a particular area. It is only feasible where inoculum is exogenous and arrives well into the cropping season (Bariana *et al*., 2007).

2.11.2. Host plant resistance

The principal mechanism of control of the stem rusts has been using host resistant cultivars (Mamuluk *et al.,* 2000). Host resistance is the ability of the host to limit the growth or development of the pathogen. Host resistance to pathogens may become more effective because of increased static and dynamic defenses from changes in physiology, nutritional status and water availability. Singh *et al.* (2002) reported that when adequate genetic resistance to stem rust is achieved, no other control methods may be required but achieving and maintaining adequate resistance may not be easy. However, achievement of resistance to wheat stem rust requires monitoring of the pathogen population to track newly evolved physiological races (Singh *et al*., 2006).

In Ethiopia, the use of resistant cultivars has been also the major management strategy for stem rust. The wheat stem rust resistance genes were designated after their actual locations on the chromosome arms were established (Leornard and Szabo, 2005). More than 150 wheat

rust resistance genes have been genetically defined in wheat or wild relatives, most conferring race-specific resistance (McIntosh *et al*., 2013). Globally, about 73 stem rust resistance genes have been identified and characterized against different races of stem rust (Singh *et al*., 2015).

Generally, the most effective and environmentally sound, foreseeable and feasible best option method to control these diseases is through the deployment of resistant cultivars or resistance breeding to control wheat stem rust is economic, effective and protective of the environment, and has been proved to be the best control method by repeated practice (Goutam *et al*., 2015). There are two primary classes of genetic resistance that are used: seedling plant resistance (race-specific or qualitative resistance) and adult plant resistance (race non-specific or quantitative resistance) (Singh *et al*., 2008; Periyannan *et al*., 2017).

2.11.2.1. Seedling plant resistance

Seedling resistance is a type of resistance that is controlled by one gene that confers resistance against a particular race of a pathogen. It is also known as race-specific resistance, vertical resistance and major gene resistance. Seedling resistance can be responsible for a large amount of the resistance to a particular race of a pathogen in their action and effective through all plant growth stages. This means that it functions against certain rust races or biotypes but not against others. This is because of the gene-for-gene interaction that occurs when the pathogen is detected in the plant (Babiker *et al*., 2009; Sheikh *et al*., 2017).

The gene for gene concept implies that with each host plant resistant gene (R gene); a corresponding gene locus (race-specific effectors) is present in the pathogen with alternate alleles conditioning avirulence (Avr) gene and virulence. When there is a reaction between the resistance gene of the plant and the avirulence gene of the pathogen, resistance is triggered, but if just one of those genes is present, in either the plant or the pathogen, resistance will not be triggered (Flor, 1971).

Filamentous plant pathogens use molecules called effectors that modify host defense-related signaling, cell structure, metabolism and function to effect successful infection (Giraldo and Valent, 2013). The stem rust fungi produce elicitor (effector) molecules detected by receptor

molecules in wheat. The effectors contain many chemical compounds like oligosaccharides, lipids, peptides and proteins. Race-specific effectors are produced only when specific avirulence genes are present in particular pathotypes of the pathogen. When the plant's receptors detect the pathogen's elicitors, a host defense mechanism is stimulated. This is followed by the death of the infected cells and the pathogen growth is hindered (Flor, 1971).

Most of these resistance genes result into hypersensitive responses; the rapid death of the infected cells which aims to restrict the spread of the pathogen to other parts of the plant (Singh *et al.,* 2008; Lowe *et al*., 2011). This leads to the collapse and death of the infected host cells preventing a compatible host-pathogen interaction (Leornard and Szabo, 2005). Any breakdown in resistance leads to the absence of the defense mechanism. This implies that changes in the elicitor lead to the non-recognition by the receptors of host plant thus increasing the frequency of the pathogenic races which eventually cause rust infection and reproduction; a compatible host-pathogen response mechanism (McDonald and Linde, 2002).

Seedling resistance can be very powerful and can sometimes offer the plant near immunity against a specific race of the pathogen. The reason why this type of resistance has been used for years and is frequently very successful, but in almost all cases is overcome eventually. This is because once a seedling gene is discovered it is often deployed over a broad area, which exposes the gene to incredible amounts of inoculum. There is no obvious way to predict the durability of these genes; for now, only time will tell if they are long-lasting (Hulbert and Pumphrey, 2014). Since this type of resistance is race-specific and in most cases only one seedling gene is used, the pathogen will eventually overcome it. This is because of the ability of the pathogen to change by sexual recombination and mutation (Ayliffe *et al*., 2008). Sexual recombination can occur in areas where the alternate host is present. Once a pathogen overcomes a seedling gene, use of the gene often becomes futile (Keane, 2012).

Defeated seedling genes can still prove useful if combined with other resistance genes but at a reduced effect. This is the primary issue with seedling resistance that breeders are struggling with today. In order to reduce the probability of a pathogen overcoming this type of resistance, combining multiple effective seedling genes has the potential to achieve durable

resistance, but this is difficult due to the lack of effective R genes available. Many other R genes have virulence detected in other races, linkage with undesirable traits, low levels of resistance in high disease pressure or a lack of testing against Ug99 in field trials (Singh *et al*., 2011). The most useful seedling resistance genes that are still agronomically viable are genes Sr*22*, Sr*25*, Sr*26*, Sr*33*, Sr*35*, Sr*45*, and Sr*50* (Singh *et al*., 2015).

2.11.2.2. Adult plant resistance

Adult plant resistance (APR) is also known as race-nonspecific resistance, horizontal resistance and minor gene resistance has great potential as a durable resistance strategy. As its name indicates resistance is usually functional only in adult plants. For these types of genes, the effects are often small individually, but when combined with other APR genes, an additive effect could be observed. This is where the concept of pyramiding genes has proven beneficial. One or two APR genes may increase resistance slightly but combining three, four or five could have the potential for even more significant resistance (Sheikh *et al*., 2017).

Moreover, for APR gene there is no gene-for-gene interaction, which means there is no host and pathogen effector recognition. Without this interaction, the pathogen cannot easily overcome the host's defenses making this type of resistance more durable (Vanderplank, 1984). APR is also theoretically effective against all races of a pathogen but does not start to become effective until the plant reaches maturity in its growth. Although this type of resistance offers many benefits to growers and breeders, in the same case there are some drawbacks. APR genes frequently have small effects on their own which makes them difficult for researchers to properly identify and locate (Keane, 2012).

Another issue with APR gene is that even after locating and combining the genes, they may not achieve the complete immunity that seedling resistance offers. Often times there is an intermediate resistance response, which is still very beneficial but is not usually as notable as seedling resistance (Vanderplank, 1984). Combining multiple APR genes though, (four or more) has the potential to give the resistance response that is desired and should dramatically improve the health of a plant that would have normally been infected.

Two particularly important adult plant resistance genes are Lr*34/Yr18/Pm38/*Sr*57* and Sr*2/*Yr*30*. There have not been many specific APR genes cataloged, but these genes are particularly important because Lr*34* has been cloned and Sr*2* is a well-known, effective APR gene that confers resistance against stem rust. The broad-spectrum wheat stem rust resistance gene Sr2 confers adult plant resistance to stem rust and is located on chromosome arm 3BS. Another added benefit to APR genes is that some have the ability to confer resistance against all races of the pathogen and the ability to control all three types of rust and some additional pathogens (Xu *et al*., 2017).

2.11.3. Chemical control

Currently, the majority of bread wheat varieties are susceptible to wheat stem rust (Tadesse *et al*., 2010). In the absence of the option to grow resistant varieties, the use of fungicides becomes necessary or mandatory. Adequate rust management could be attained by application of fungicides before the onset of stem rust and frequent application thereafter throughout the growing season (Peterson, 2001). A number of fungicides are highly effective against stem rust and have been used to successfully manage the disease in Ethiopia. These include triadimefon (Baylaton and Noble) 25% WP (Wettable powders) at 0.5 l/ha, propiconazole (Tilt and Bumper) 250 EC (Emulsifiable Concentrates) at 0.5 l/ha and Epoxiconazole plus Thiophanatemethy (Rex Duo) (Hundie, 2003).

Chemical control is usually considered only where heavy losses are expected and are cost effective. Repeated applications of fungicides are necessary under heavy epidemic conditions, but increasing costs further. Lack of knowledge and awareness about appropriate fungicides and unavailability of the chemicals is also the main limitation, particularly to the small-scale farmers. Early disease detection and immediate application of fungicides should be considered in the management of stem rust with fungicides. It has been reported that stem rust becomes too difficult to control as it progresses. This is because fungicides would reduce subsequent rust severities on plant parts that are slightly infected at the time of fungicide application, but they are not effective on plant parts that are heavily infected (Beard *et al.*, 2004). Fungicides can help to limit damage caused by wheat stem rust but early detection and rapid action are crucial, so integrated management strategies in the long run (FAO, 2017b).

2.11.4. Integrated disease management

Economical and sustainable disease control can be obtained through the establishment of an integrated disease management system. The most effective strategy for long-term management would be to consider all possible options in an integrated manner. Integrated disease management practices include several and all possible control measures include cultivar resistance, seed health, cultural practices and fungicides. As a rule, an integrated disease management practice must always be eco-friendly. To combat wheat stem rust diseases effectively, integrated management approaches are essential due to the complex nature of the pathogen. For long term prevention, single control methods are not adequate but rather integrated strategies and practices (FAO, 2014).

Planting resistant varieties of wheat is the most effective means of preventing wheat stem rust diseases but contingency plans should also be developed to control possible outbreaks with proper use of fungicides and cultural practices. Fungicides may be biologically effective, but for stem rusts they are not economically and practically feasible, especially for smallholder producers in low-yielding environments. Fungicides are recommended in the absence of resistant cultivars as an emergency control measure in case of sudden epidemics until resistant wheat varieties are again available. There is also a high risk that pathogens may develop resistance to fungicides rendering them ineffective. Cultural practices, such as changing planting dates, destroying volunteer (green bridges between crop cycles) and alternate host plants, appropriate irrigation and fertilization, employing early maturing varieties and using multiline or varietal mixtures, are also recommended because they are effective in reducing the levels of inoculum and hence the disease pressure (Fetch *et al*., 2011; FAO, 2014).

3. MATERIALS AND METHODS

3.1. Description of the Survey Areas

Wheat stem rust survey and sample collection were conducted in the Southern, Southeast and Eastern zones of Tigray regional state in Ethiopia during 2017 main cropping season. The survey was carried out in the major wheat growing districts of the Southern Tigray zone; Ofla, Enda-Mekoni, and Raya-Azebo, Eastern Tigray zone; Saesia Tseadaemba, Kilte Awulaelo and Ganta Afeshum and Southeast Tigray zone; Enderta.

Table 2: Description of the survey areas

Zones	Altitude ranges (m)	Latitude and Longitude	Annual average RF (mm)	Temperature (°C)	
				Mean Max.	Mean Min.
Southern Ofla, R/Azebo and E/Mehoni	930-3925	12° 25' 50" N - 39° 30' 0" E	692.20	12.17	25.51
Eastern K/Awulaelo, G/Afeshum and S/Tsaedaemba	600-2700	14° 06' 55" N - 39° 35' 13" E	574.40	10.45	25.50
Southeast Enderta	1970-2589	13° 5' 0" N - 39° 48' 12" E	440.50	11.78	24.86

R/Azebo - Raya Azebo, E/Mehoni - Enda Mehoni, K/Awulaelo - Kilte Awulaelo, G/Afeshum - Ganta Afeshum, S/Tsaedaemba - Sasia Tsaedaemba, RF - Rainfall, Max. - Maximum and Min. - Minimum. The annual average rainfall and temperature data of the zones were collected during 2017 (from January to December) cropping season.

Source: (Mekelle Meteorological Agency Office, unpublished; Gebrekristos *et al*., 2016).

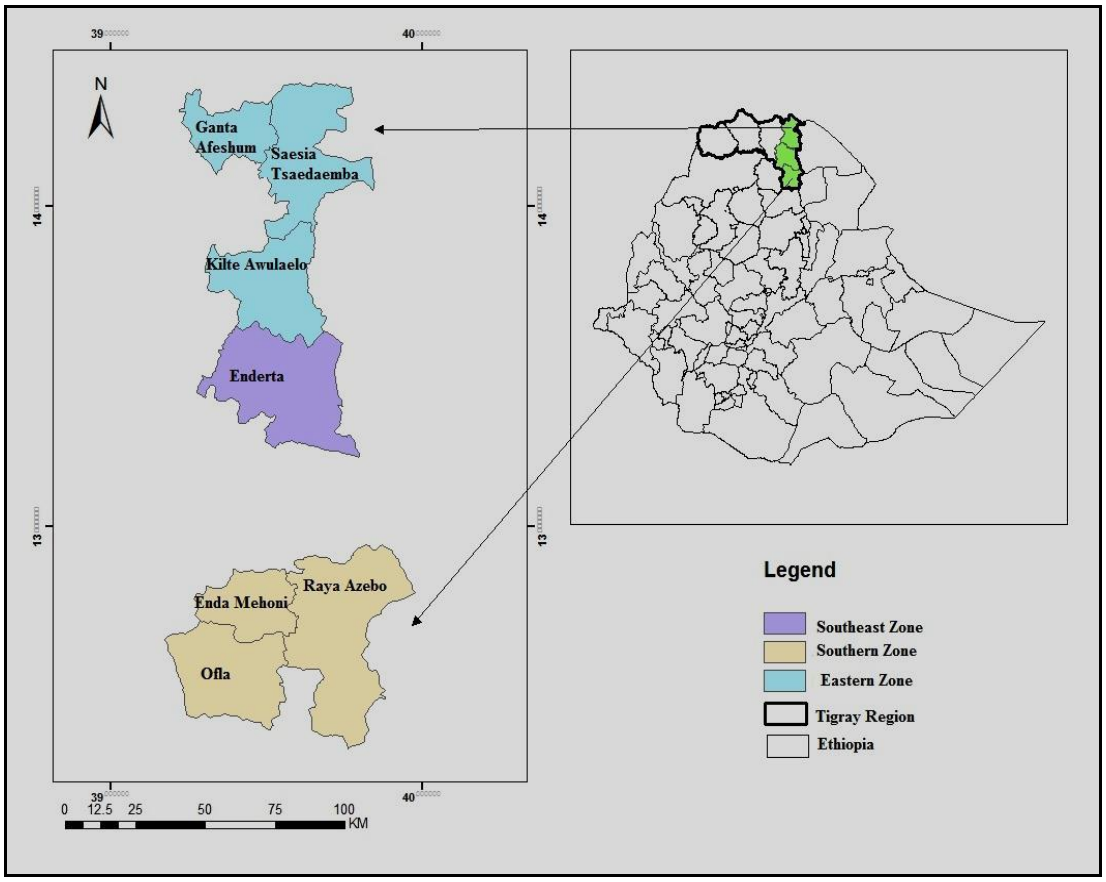

Figure 2: Geographical locations of the different stem rust survey zones of Tigray region

3.2. Sampling Method and Strategy

The study was conducted from milk to hard dough growth stages of the crop using purposive multi-stage sampling techniques and based on wheat area coverage. The three zones were selected purposively from the region and three districts were selected based on wheat area coverage for each except the Southeast zone where only one district was considered. From each district three peasant associations were assessed, except from Raya Azebo and Saesia Tseadaemba districts where two peasant associations were considered. Of each peasant association, five farms were assessed at 5-10 km interval followed by systematic sampling along the main and feeder (accessible) roadsides on pre-planned routes in areas where wheat is predominantly grown. The survey included farmer's fields, Farmer's Training Center (FTC) and research stations. Finally, one sample per field was collected during the survey.

3.3. Assessment of Wheat Stem Rust

Stem rust assessments were made at five points along the two diagonals (in an ''X'' pattern) of the field using 0.5m x 0.5m ($0.25m^2$) quadrate and used to calculate average values. In each field, wheat plants within the quadrate were counted and recorded as diseased/infected and healthy/non-infected and disease incidence was calculated. The incidence of stem rust was calculated using the number of infected plants and expressed as a percentage of the total number of plants assessed.

$$\textbf{\textit{Disease Incidence}}\ (\%) = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ in\ quadrant}\ x100$$

The disease severity was measured as a percentage of stem/leaf area covered by rust disease according to Modified Cobb's scale as developed by Peterson *et al.* (1948). According to this scale, at 100% disease severity, the actual leaf/stem area covered by rust pustules is 37% (Figure 3). The severity of the disease was examined randomly by selecting five plants from a single quadrate and five quadrates were used for the estimation of disease severity from a single wheat field.

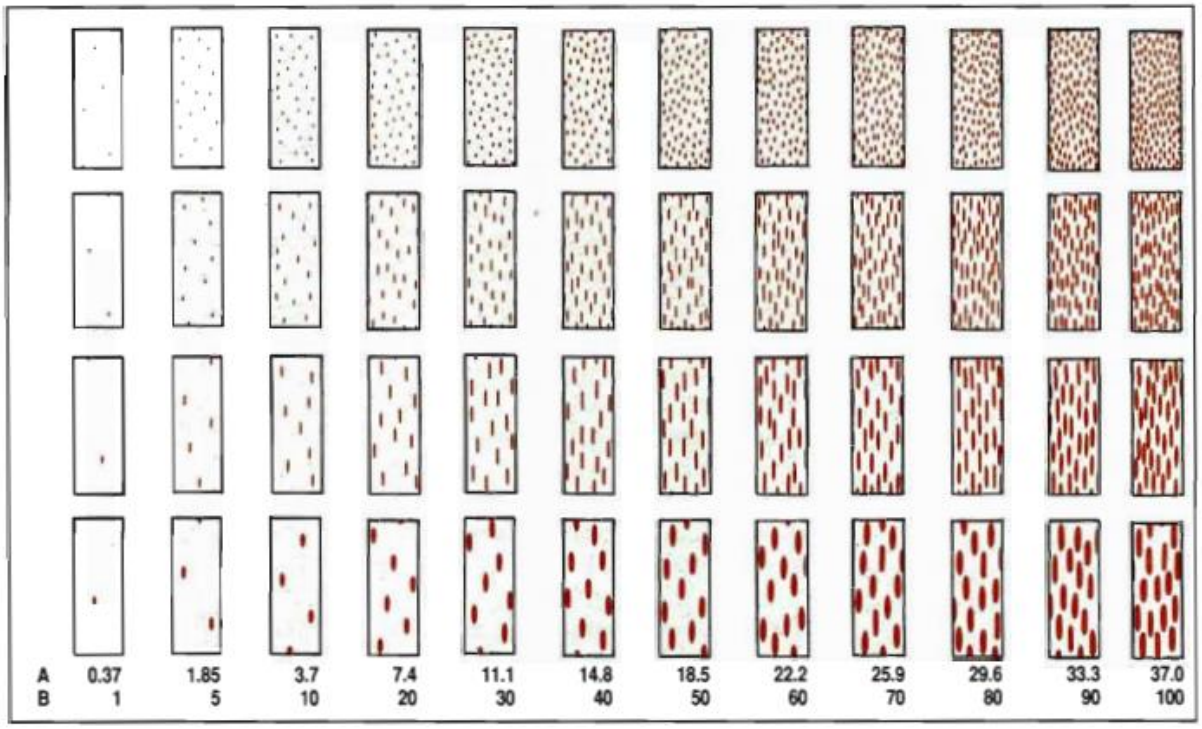

Figure 3: A diagrammatic scale for estimating rust severity on leaves and stem of cereal crops A, actual percentage occupied by rust uredinia; B, rust severities of the modified Cobb scale after Peterson *et al*. (1948).

Source: (Roelfs *et al.*, 1992).

The prevalence of the disease was measured by using the number of fields affected divided by a total number of fields assessed and expressed in percentage.

$$\textbf{\textit{Disease Prevalence}}\ (\%) = \frac{Number\ of\ infected\ fields}{Total\ number\ of\ fields\ assessed}\ x100$$

Data collected

Disease prevalence, incidence and severity of stem rust data were recorded during the survey. Growth stages of the crop were recorded according to Zadoks cereal growth stage key. Type of infection (plant response) data was recorded using the description of Roelfs *et al*. (1992). Variety grown and weed infestations level per field were also recorded during the survey time. Data on geographical information such as latitude, longitude and elevation of each field

were recorded using GPS. The Meteorological/weather data like; temperature and rainfall were recorded to correlate with disease intensity (Appendix: 5 and 6).

3.4. Identification of Physiological Races of *Puccinia graminis* f.sp. *tritici*

3.4.1. Sample collection

Stems and/or leaf sheath of wheat plants infected with stem rust were cut into small pieces of 5-10 cm in length using scissors and placed in paper bags after the leaf sheath was separated from the stem in order to keep stem and/or leaf sheath dry. This technique was used in order to prevent spores germination before processing in the greenhouse. The collected samples were labeled with the name of the zone, district, variety and date of collection and kept in a refrigerator at 4^{o}C until the surveys in all districts completed and used for the virulence analysis. Once sample collection has completed the samples were kept in the icebox and transported to Ambo Agricultural Research Center's (AARC) laboratory for race analysis and it was done in the greenhouse of Ambo Agricultural Research Center.

3.4.2. Isolation of the pathogen

Seedlings of variety "McNair", which does not carry known stem rust resistance gene were raised in 5 cm diameter pots for inoculation in the growth chamber. For raising seedlings, sterilized soil composed of three different materials; soil, sand and farmyard manure mixed at the ratio of 2:1:1 by volume were used. Seedlings were raised by spreading the seeds on filter paper in Petri dishes, moisten with water and close the lid to pre-germinate seeds. On the third day, the seeds germinate and the radicles were seen. Then, these germinated seeds were planted in pots using forceps. Inoculation in the greenhouse was done to revitalize the spores collected from the field, to multiply the isolates and to inoculate the differential lines sets for race identification. Greenhouse inoculations was done using the methods and procedures developed by Stakman *et al. (*1962).

Leaves of seven-day-old McNair seedlings or seedlings with fully expanded primary leaves and second leaves beginning to grow were rubbed gently with clean (disinfected with 70% alcohol) moistened (with distilled water) fingers to remove the waxy layer from the surface of

the leaves that hinders the penetration of the spores. Spores from the stem rust infected sample was collected using atomized spore collector/vacuum pump in the cubicle, then suspended in lightweight mineral oil (Soltrol 170) and then sprayed onto seven-day-old seedlings of McNair (Roelfs *et al*., 1992). Inoculation of the susceptible check McNair was done late in the afternoon when the ambient temperature is low and cool. Cool temperatures help moisture to stay longer on the leaves, thus facilitating the germination of spores resulting in infection (Woldeab *et al*., 2017).

The inoculated seedlings were placed on a table for 30 minutes until the Soltrol evaporates and leaves have dried out. Following this, the seedlings were moistened with fine droplets of distilled water produced with an atomizer and placed in the incubation chamber for 18 hours in a dark at 18-22°C followed by exposure to light for 3-4 hours to provide a condition for infection and seedlings were allowed to dry their dew for about 1-2 hours. The incubation chamber has light, dew chambers and humidifiers. The incubation chamber was wooden boxes covered with the white polyethylene sheet and again the black polyethylene sheet was coved white polyethylene sheet in order to create darkness in the wooden box. The humidifier (Model V5100NS, China) on for about 1:30 hours, so the seedlings have enough moisture for the whole dark period, this condition facilitates the initial infection process successful.

Then after, the seedlings were transferred from the dew chamber to glass compartments in the greenhouse where conditions are regulated at 12 hours photoperiod, at a temperature of 18-25°C and relative humidity of 60-70%. The remaining rust spore samples were kept in the refrigerator (Model 5819, USA) at 4°C, in order to substitute for samples that failed to produce infection on McNair in the greenhouse. After seven to ten days of inoculation (when the flecks/symptoms are clearly visible) leaves containing a single fleck that produces a single pustule were selected from the base of the leaves and the remaining seedlings within the pots were removed using scissors. Leaves with a single pustule were separately covered with cellophane bags (145×235 mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004).

3.4.3. Multiplication of single-pustules

After two weeks of inoculation (when a pustule is well developed), spores from each pustule were collected using power-operated vacuum aspirator and stored separately in gelatine capsules (Model 1000/PK, 1560 Industry Road, Hatfield, PA). A suspension, prepared by mixing urediospores with lightweight mineral oil (Soltrol), was inoculated on seven-day-old seedlings of the susceptible variety 'McNair' for multiplication purpose for each of the single pustules on separate pots. Immediately after inoculation, the seedlings were placed in an incubation chamber in dark condition at 18-22ºC for 18 hours and light for 3-4 hours, after which they were transferred to a greenhouse where the temperature varied between 18-25°C and RH of 60-70% following the procedures mentioned earlier. Then, 14 days after inoculation, the spores of a single pustule were collected in separate test tubes and stored at 4°C until they were inoculated on the standard differential sets. The spore multiplication procedure was repeated again until sufficient spores are produced to inoculate the set of stem rust differential hosts (Figure 5).

3.4.4. Inoculation of wheat stem rust differential host lines

Five seeds of the twenty wheat stem rust differentials (Table 3) with known resistance genes and one susceptible variety McNair were grown in 5 cm diameter pots separately in the greenhouse. Each rust isolate derived from a single pustule was suspended in Soltrol 170. The suspension was adjusted to 4-5 mg spores per 1ml lightweight mineral oil suspension and inoculated onto seedlings of the differentials using spore inoculators. After inoculation, plants were moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 18 hrs dark period at 18-22°C and 98-100% relative humidity.

Upon the seedling removed from the incubation chamber, plants were exposed to 3-4 hrs of light to facilitate the infection process and seedlings were allowed to dry/remove their dew for about 1-2 hrs. Inoculated plants were placed in separate glass compartments in the greenhouse to avoid contamination and produce infection. Greenhouse temperature was maintained between 18°C and 25°C. Natural daylight was supplemented with an additional 4 hrs/day that emitted by cool white fluorescent tubes arranged directly above plants (Hailu *et al*., 2015).

Table 3: Wheat stem rust differential lines with their corresponding stem rust resistant gene used for the present study

Differential host lines	Stem rust genes	Pedigree
LcSr24Ag	24	Little Club/Agent (Cl 13523)
W2691SrTt-1	36	Cl12632 *T. timopheevii*
ISr7b-Ra	7b	Hope/Chinese Spring
ISr8a-Ra	8a	Rieti/Wilhelmina//Akagomughi
CnSSrTmp	Tmp	Triumph 64(Cl 13679)/ Chinese Spring
Sr31(Benno)/6*LMPG	31	Kavkaz
CnS-T-.mono-deriv	21	Einkorn Cl 2433
Trident	38	Spear*4/VPM (Pl519303)
ISr9a-Ra	9a	Red Egyptian/Chinese Spring
ISr9d-Ra	9d	Hope/Chinese Spring
Combination VII	17	Esp 518/9
ISr5-Ra	5	Thatcher/Chinese Spring
ISr6-Ra	6	Red Egyptian/Chinese Spring
W2691Sr9b	9b	Kenya 117A
Vernsteine	9e	Little Club//3*Gabo/2*
W2691Sr10	10	Marquis*4/Egypt NA95/2/2*W2691
BtSr30Wst	30	Festival/Uruguay C10837
CnsSr9g	9g	Selection from Kubanka (Cl1516)
ISr11-Ra	11	Kenya C6402/Pusa4/Dundee
McNair 701	McN	Cl 15288

Source: Roelfs and Martens (1988) and Jin *et al*. (2008).

3.5. Determination of Stem Rust Races

Seedling infection types (ITs) were scored 14 days after inoculation using 0 to 4 scoring scale described by Stakman *et al*. (1962). The IT readings of 3 (medium-size uredia with/without chlorosis) and 4 (large uredia without chlorosis or necrosis) were regarded as susceptible. Other readings, i.e. 0 (immune or fleck), 1 (small uredia with necrosis) and 2 (small to medium uredia with chlorosis or necrosis) were resistant (Figure 4). The variations were refined by modifying characters as follows: -, uredinia somewhat smaller than normal for the infection type; +, uredinia somewhat larger than normal for the infection type. Hence, ITs were then grouped into low (“0”, “;”, “;1”, “1”, “1+”, “2-”, “2”, “2+”) and high (“3-”, “3”, “3+”, and “4”) infection types (Stackman *et al*., 1962).

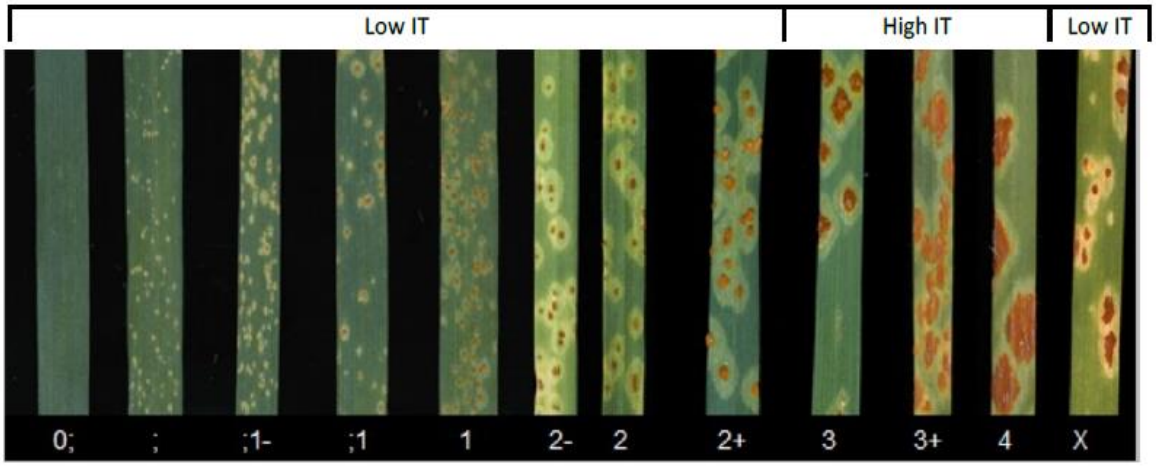

Figure 4: Pictorial infection types of *Puccinia graminis* f.sp. *tritici* of stem rust and host response

Source: Stackman *et al*. (1962)

Race identification was done using the North American nomenclature system for *Puccinia graminis* f.sp. *tritici* (Roelfs and Martens 1988; Jin *et al*., 2008). Races were identified based on their reaction on differential hosts. Race designation was done by grouping the differential lines into five subsets as indicated in Table 4. Each isolate was assigned using a combination of a three letter code of Roelfs and Martens (1988) and an additional two letter race code by Jin *et al*. (2008) which finally give a five letter of designation based on its reaction on the differential lines (Fetch and Dunsmore, 2004).

For instance, low infection type (IT) on all four hosts in a set was assigned the letter B, while high IT on the four hosts assigned T. Hence, an isolate produces low IT (resistant reaction) on each of the 20 differential lines, the race was designated with a five letter race code BBBBB. In the same way, an isolate that produces a high IT (susceptible reaction) on the 20 differential lines had a race code TTTTT. If an isolate produces a low IT on Sr*31* and Sr*24*, but a high infection type on the remaining 18 differential lines, the race was designated as TTTTF. The frequency of each race identified was also recorded.

Table 4: North American nomenclature of *Puccinia graminis* f.sp. *tritici* based on 20 differential wheat lines

Wheat *Pgt* gene differential sets and infection phenotype of pathogen coding				
Set	Differential lines identified by *Pgt* resistance gene			
Set1	5	21	9e	7b
Set2	11	6	8a	9g
Set3	36	9b	30	17
Set4	9a	9d	10	Tmp
Set5	24	31	38	McN
Pgt-code	Infection phenotype; High = virulent reaction (susceptible) and low = avirulent reaction (resistant)			
B	Low	Low	Low	Low
C	Low	Low	Low	High
D	Low	Low	High	Low
F	Low	Low	High	High
G	Low	High	Low	Low
H	Low	High	Low	High
J	Low	High	High	Low
K	Low	High	High	High
L	High	Low	Low	Low
M	High	Low	Low	High
N	High	Low	High	Low
P	High	Low	High	High
Q	High	High	Low	Low
R	High	High	Low	High
S	High	High	High	Low
T	High	High	High	High

*Low/Resistant infection type (0 to 2+), High/ Susceptible infection type (3- to 4).

Source: Roelfs and Martens (1988) and Jin *et al*. (2008).

Depending on the above illustration: race TTTTF where;

Set 1 = T (No genes in set are effective)

Set 2 = T (No genes in set are effective)

Set 3 = T (No genes in set are effective)

Set 4 = T (No genes in set are effective)

Set 5 = F (Only genes Sr24 and Sr31 are effective)

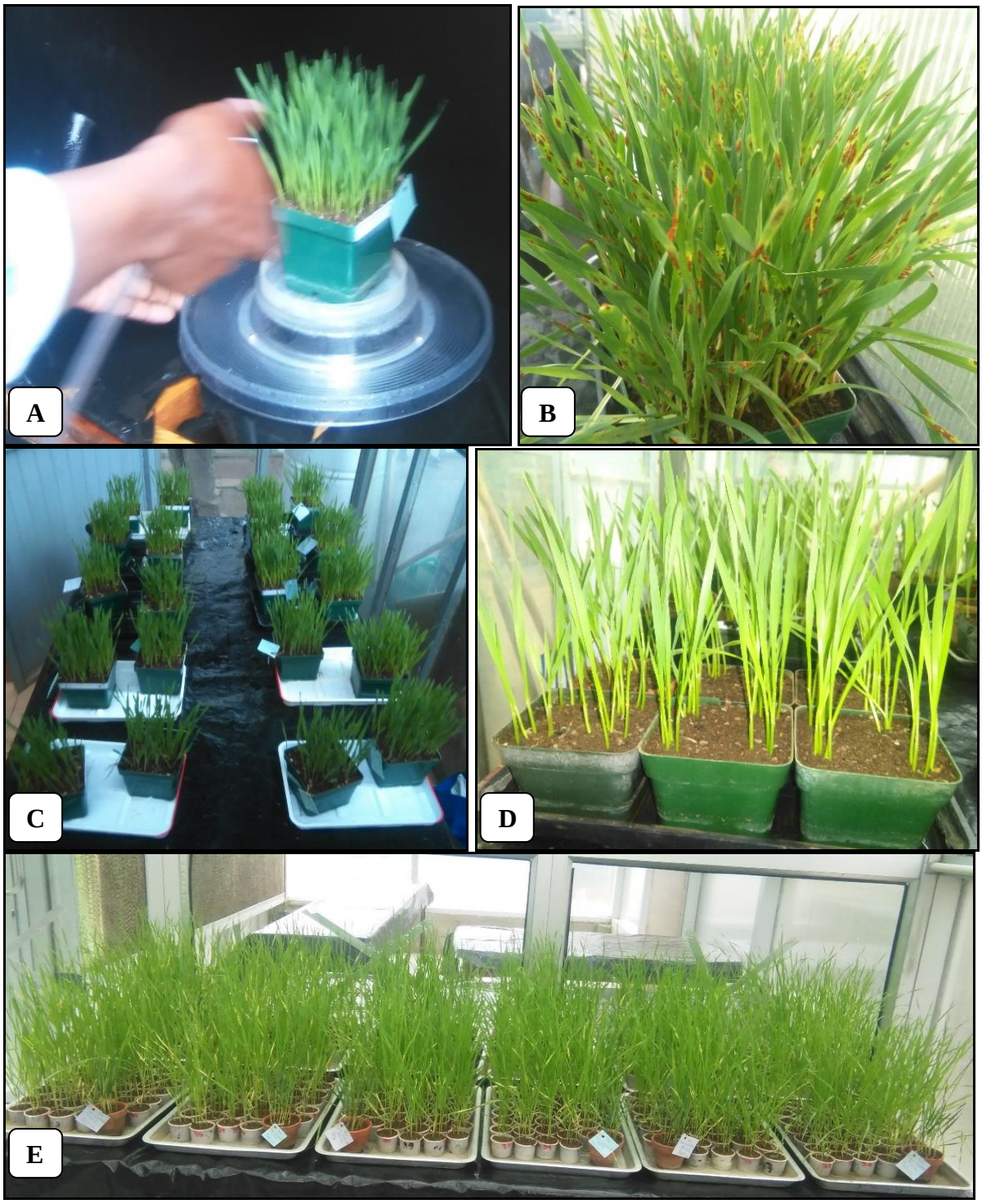

Figure 5: Schematic overview of the protocols for inoculation of spores on McNair to variety evaluation in the greenhouse at APPRC, Ethiopia; A. Inoculation of field collected spores on McNair for multiplication, B. Development of wheat stem rust on McNair after 14 days, C. Development of a single pustule on McNair and D. Inoculated a single pustule on differentials, E. Stem rust development on selected (tested) varieties in the greenhouse

3.6. Evaluation of Bread Wheat Varieties against Dominant Stem Rust Races at Seedling Stage in the Greenhouse

The evaluations of bread wheat varieties to the dominant races were done in Ambo Agricultural Research Center. The spores of dominant stem rust races previously identified in Ethiopia and those detected in the present race analysis study were multiplied on the McNair and collected in separate test tubes for inoculation of the wheat varieties. Thirty-nine bread wheat varieties were selected, including those commonly grown in the Tigray region and the susceptible variety McNair as a check (Table 5) and evaluated against the selected virulent stem rust races. The stem rust races used for seedling evaluation were TTTTF, TKTTF, TTKSK, JRCQC, RRTTF and TRTTF. The susceptible variety was used to determine the viability of spores inoculated on the wheat varieties. The evaluations were done by using a Complete Randomized Design with three replications in the greenhouse.

Five seeds of each wheat variety and a susceptible check were planted in 5 cm diameter plastic pots separately. Seven-day-old seedlings (the first leaf is fully expanded and the second leaf is just emerged to grow), were inoculated with spores of virulent races (approximately 4-5 mg spores per 1ml lightweight mineral oil suspension). For incubation, inoculated plants were moistened with fine droplets of distilled water by using atomizer after 30 minutes of inoculation. Seedlings placed in a dew chamber for 18 hrs dark period at 18-22^{0}C and 98-100% relative humidity (RH) followed by exposure to light at least for 3-4 hrs to provide a favorable condition for stem rust infection. Seedlings were then allowed to dry/remove their dew/moisture for about 1-2 hrs. Following this, the seedlings were transferred from dew chamber to glass compartments in the greenhouse where conditions are regulated at 12 hrs photoperiod, and a temperature range of 18- 25^{0}C and RH of 60-70%.

Table 5: List of bread wheat varieties used for evaluation against dominant stem rust races

Variety Name	Year of Released	Breeder/ Maintainer	Pedigree
Shorima	2011	KARC/EIAR	UTQE96/3/PYN/BAU//Milan
Hidassie	2012	KARC/EIAR	YANAC/3/PRL/SARA//TSI/VEE#5/4/CROC 1/AE.SQUAROSA(224)//OPATTA
Mekelle I	2011	MARC/TARI	PARULA[2800]
Mekelle II	2011	MARC/TARI	HIND162/BOBWHITE/CPAN2099
Mekelle III	2013	MARC/TARI	NA
Mekelle IV	2012	MARC/TARI	NA
Kingbird	2015	KARC/EIAR	TAM-200/TUI/6/PABON-F-76//CARIANCA 422/ANAH
Danda'a	2010	KARC/EIAR	UAC75/5/BOBWHITE/CROW//BUCKBU CK/ PAVON-F Kiritati//2*PBW65/2*Seri.1B
Kakaba	2010	KARC/EIAR	Kititati//Seri/Rayon
Laketch	1967	KARC/EIAR	PJ"S"/GB55
Kubsa	1995	KARC/EIAR	NDG9144//KAL/BB/YAC"S"/4VEE#5"S"
Dashen	1984	KARC/EIAR	VEE 17/KUZ/BUHO"S"//KAL/BB
Shehan	NA	NA	NA
Hoggana	2011	KARC/EIAR	PYN/BAU//MILAN
Digalu	2005	KARC/EIAR	Sha 7 / Kauz
Meraro	2005	KARC/EIAR	11-6-24
Huluka	2011	KARC/EIAR	UTQE96/3/PYN/BAU//Milan
Deresel ign	1974	KARC/EIAR	CI 8154//2*FR
Enkoy	1974	KARC/EIAR	(HEBRARDSel/WIS245XSUP51)X FRFN/YA
Pavon-76	1982	KARC/EIAR	VCM//CNO"S"/7C/3/KAL/BB
Mitikie	1994	KARC/EIAR	(FSYR20.6/87BOW28) X (RBC (ET1297))
Wabe	1995	KARC/EIAR	MRL"S"-BUC"S"
Galema	1995	KARC/EIAR	4777(2)//FKN/GB/3/PVN"S"
Hawi	1999	KARC/EIAR	CHIL/PRL
Alidoro	2007	HARC/EIAR	HK-14-R251
Biqa	2014	NA	NA
Honqolo	2014	NA	NA
Galil	2010	Hazera genetics	NA
Millennium	2007	KARC/EIAR	ALD/CEP75630//CEP75234/PT7219/3/BUC/BIY/4/
KBG-01	2001	KARC/EIAR	(300/SM+501M)/HAR 1709
ETBW-9017	NA	KARC/EIAR	NA
ETBW-7956	NA	NA	NA
ETBW-9042	NA	NA	NA
ETBW-7638	NA	NA	NA
Dilfiker	NA	NA	NA
K6295-4A	1980	KARC/EIAR	Romany X GB-GAMENYA
ET-13A2	1981	KARC/EIAR	UQ 105 SEL. X ENKOY
Lemu	NA	NA	NA
Ogolcho	2012	KARC/EIAR	WORRAKATTA/2*PASTOR
Madawelabu	1999	SARC/OARI	TI/3/Fn/Th/Nar 59 *2/4/Bol'S'
McNair	NA	AARC/EIAR	NA

Source: Kulumsa Agricultural Research Center and Mekelle Agricultural Research Center
NA = Not Available

Data collected

In the greenhouse, infection types were recorded after 14 days of the inoculation using 0 to 4 scoring scale depending on reaction response of varieties. Infection types were then grouped into low ("0", ";", ";1"; "1", "1+", "2-", "2", "2+") which were regarded as resistant whereas high ("3-", "3", "3+", and "4") infection types were susceptible (Stackman *et al*., 1962).

3.7. Data Analysis

Disease incidence and severity were analyzed using three stage nested design with the model:

$$y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \gamma_{k(ij)} + \varepsilon_{l(ijk)}$$

Where: y_{ijk} is the wheat stem rust disease intensity whereas peasant association k is nested within district J nested within zone i, μ is the overall mean, τ_i is the effect of the i^{th} zone, $\beta_{j(i)}$ is the effect of the j^{th} district within the i^{th} zone, and $\gamma_{k(ij)}$ is the effect of the k^{th} peasant association within the j^{th} district and i^{th} zone, and $\varepsilon_{l(ijk)}$ is the error term.

Analysis of variance (ANOVA) was performed using SAS version 9.3 Software package (SAS, 2012). Means were separated using LSD test at the alpha level of 5%. The associations of disease incidence and severity with independent variables viz. altitude, variety growth stage, variety grown and weed management were computed using simple correlation analysis. Each of the independent variables were tested with incidence and severity of stem rust as the dependent variable. Linear regression analysis was done by plotting disease severity against altitude. Determination of regression intercept, slope and coefficient of determination were computed using Microsoft excel. Physiological race analysis and greenhouse evaluation of bread wheat varieties were computed on the Microsoft excel by using the descriptive statistical analysis.

4. RESULTS AND DISCUSSION

4.1. Distribution and Occurrence of Wheat Stem Rust

Wheat stem rust was observed in all surveyed zones of the region at variable levels. The results of the assessments revealed that the intensity of stem rust varied from slight to complete infection in wheat fields depending on the variety grown and agro-ecological divergence. During the assessment, the disease was observed on 67 (70.53%) of the 95 wheat fields inspected. It was prevalent in all assessed zones of the region. The number of fields assessed in the Southern, Eastern and Southeast zones were 40, 40 and 15, respectively. Of which, 34 (85%), 25 (62.50%) and 8 (53.33%) were affected by stem rust, respectively.

Wheat stem rust was prevalent in all assessed districts of the zones and its intensity among the districts was significantly different ($p < 0.01$). The disease was more prevalent at Ofla and Kilte Awulaelo districts with a similar prevalence value of 93.33% while the lowest disease prevalence 33.33% was recorded at Ganta Afeshum district (Table 6). Besides the locational variations in disease intensity, the field assessment results showed that there was a wide distribution of stem rust across the districts of the zones in the season.

Table 6: Prevalence of wheat stem rust across districts in 2017 main cropping season

Zones	Districts	Altitude ranges (m.a.s.l)	No of fields assessed	No of fields infected	Prevalence (%)
Southern	Ofla	2432-2497	15	14	93.33
	Raya Azebo	1567-1762	10	9	90.00
	Enda Mehoni	2303-2520	15	11	73.33
Subtotal/Mean		1567-2520	40	34	85.00
Eastern	Kilte Awulaelo	1950-2194	15	14	93.33
	Ganta Afeshum	2444-3008	15	5	33.33
	S/Tsaedaemba	2018-2584	10	6	60.00
Subtotal/Mean		1950-3008	40	25	62.50
Southeast	Enderta	1981-2332	15	8	53.33
Mean/range		1567-3008	95	67	70.53

The distribution and extent of damage caused by the wheat stem rust varied significantly ($p < 0.01$) among the zones. The overall mean incidence in the zones was 41.88% in the season. The highest disease incidence was recorded in Southern followed by Eastern zone with the mean values of 49.61% and 37.00%, respectively whereas the lowest disease incidence was recorded in Southeast zone (34.33%). Similarly, the highest disease severity was recorded in the Southern zone (24.22%) followed by Eastern zone (20.06%). The lowest was observed in Southeast with 16.67% disease severity (Figure 6). The overall mean disease severity of the zones was 21.36% in the season.

Crop rotation practice was used to minimize inoculum build-up, however, it was not practiced well in the study area. This might be contributed more chance of disease occurrence in the areas than where crop rotation practice was done. Crop rotation practice probably contributed to the low wheat stem rust disease incidence and severity. It also limit the genetic diversity of the pathogen population and to minimize the number of urediniospores produced. According to Joseph *et al.* (2007) crop rotation 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 reasons for more widespread of wheat stem rust disease in the Southern zone than in the Eastern and Southeast zones might be due the contribution of the agro-ecological divergence. In the Southern zone the altitude ranges from 1567-2520 m.a.s.l which was suitable or important for the pathogen development and warmer temperature compared to the others zones (Appendix: 5 and 6). According to this study, stem rust development varied from place to place depending on agroecological divergence and variety grown. This is probably due to the differences in relative humidity and temperature (Mideksa *et al*., 2018).

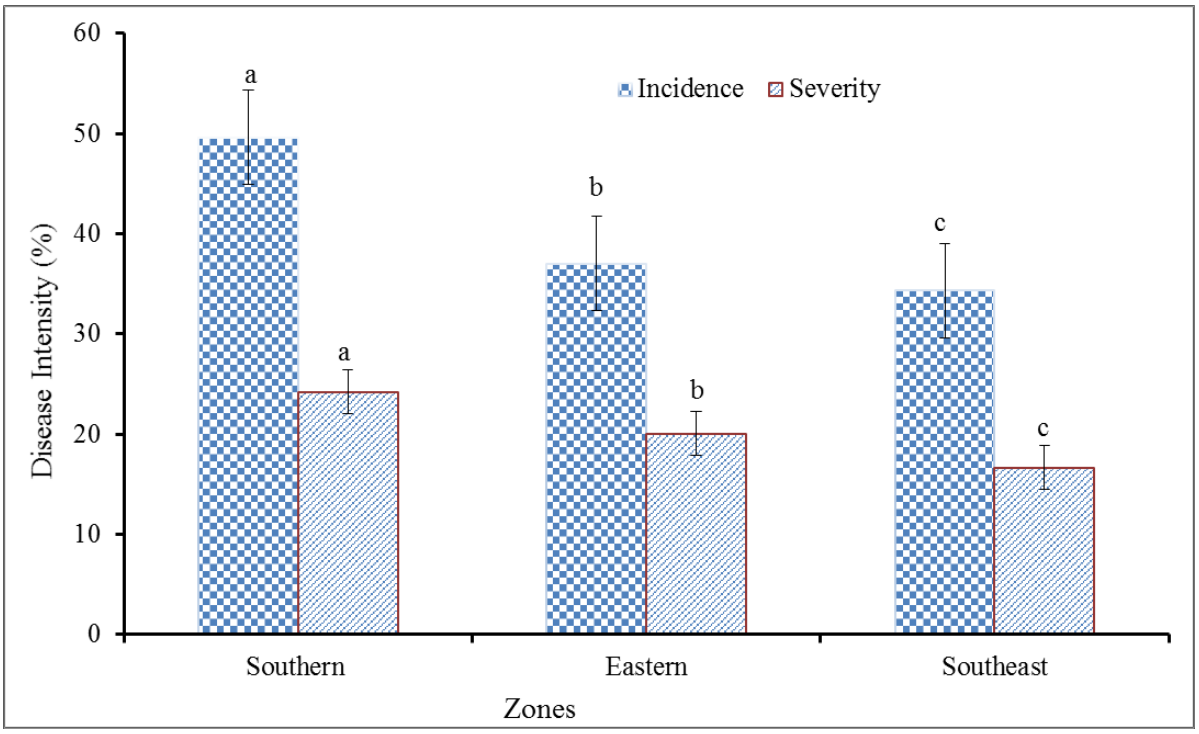

Figure 6: Stem rust distribution across the different zones of Tigray region in 2017 main cropping season

Bars with the same letter(s) are not significantly different at p<0.05.

There was a significant difference ($p < 0.01$) among districts in the incidence of wheat stem rust. The disease incidence ranged between 22 and 64% in Ofla, 62-71% in Raya Azebo, 16-46% in Enda Mehoni, 64-92% in Kilte Awulaelo, 0-26% in Ganta Afeshum, 10-32% in Saesia Tsaedaemba and 24-39% in Enderta districts. The mean disease incidence was ranged from minimum of 11.00% to maximum of 78.67% within the districts (Table 7). The highest wheat stem rust mean incidence was recorded in Kilte Awulaelo (78.67%) followed by Raya Azebo district (66.50%). However, the lowest disease incidence was recorded at Ganta Afeshum district (11.00%).

Table 7: Mean incidence and severity of wheat stem rust across the districts during 2017 main cropping season in the zones of Tigray

Zones	Districts	Disease incidence (%)		Disease severity (%)	
		Range	Mean	Range	Mean
Southern	Ofla	0-100	47.00^{c}	0-50	20.67^{c}
	Raya Azebo	0-100	66.50^{b}	0-60	35.00^{b}
	Enda Mehoni	0-100	35.00^{d}	0-50	17.00^{c}
Eastern	Kilte Awulaelo	0-100	78.67^{a}	0-90	43.67^{a}
	Ganta Afeshum	0-60	11.00^{f}	0-40	5.00^{e}
	Saesia Tsaedaemba	0-100	21.00^{e}	0-60	11.50^{d}
Southeast	Enderta	0-100	34.00^{d}	0-50	16.67^{cd}
Over all mean			41.88		21.36
LSD (0.05)			9.45		5.39
CV %			12.82		25.49

*Means with the same letter(s) within the column are not significantly different at p<0.05.

There was a significant ($p < 0.01$) difference among districts in terms of wheat stem rust severity. The disease severity ranged between 9 and 29% in Ofla, 32-38% in Raya Azebo, 7-25% in Enda Mehoni, 33-56% in Kilte Awulaelo, 0-12% in Ganta Afeshum, 5-18% in Saesia Tsaedaemba and 10-20% in Enderta districts. The highest mean disease severity was recorded in Kilte Awulaelo (43.67%) followed by Raya Azebo district were 35% severity was recorded. (Table 7). Enda Mehoni and Enderta districts had moderate levels of disease severities 20.67% and 17%, respectively and were not significantly different from each other. On the other hand, Saesia Tsaedaemba district had a mean disease severity of 11.50%. The lowest disease severity was recorded from Ganta Afeshum district (5.00%).

Abebe *et al*. (2012) reported that the highest mean incidence of 42.30% and 26.70% severity of stem rust was recorded in Raya-Azebo district from Southern zone during 2010 main cropping season. The highest severity of 80% was recorded where the highest incidence 100% was noted at Raya-Azebo district. The present study showed increased disease incidence of wheat stem rust in major wheat growing zones of Tigray region, with mean values of 49.61% in the Southern zone; 37% in Eastern zone and 34.33% in Southeast, as compared to results of previous surveys.

According to Abebe *et al*. (2012), the mean percent incidence of wheat stem rust was 15.60 in the Southern zone during 2010 cropping season. Correspondingly, mean incidence of 33% and 10.80% severity of wheat stem rust in the West and Southwest Shoa zones of Oromia region were reported by Hailu *et al*. (2015) during 2014 main cropping season. The increment in the intensity of wheat stem rust in this report emanated from the rapid expansion of new pathogen races such as TKTTF (Digalu race), TTTTF and extensive planting of the susceptible varieties.

The detection of TKTTF race was the first report of virulence to SrTmp in the country. This non-effective gene is present in the most popular and widely grown bread wheat variety Digalu (Hodson, 2015). Digalu variety was resistant to the Ug99 race group became susceptible to race TKTTF (not a member of the Ug99 lineage) after planting by many growers in the 2013/14 season (CSA, 2014, Olivera *et al*., 2015). The continuous wheat production and favorable microclimates in the major wheat production areas could be the main reasons for the rapid evolution of the pathogen. The recent climatic changes like warm temperature and moist (amount and duration of rainfall) have reasonably predisposed and favoured the wheat crop to be infected by stem rust pathogen (Admassu *et al*., 2009; Hailu *et al*., 2015; Singh *et al*., 2015). Similarly, sexual recombination may have also contributed to the high virulence diversity of *Puccinia graminis* f.sp. *tritici* because *Berberry holstii* is present in proximity to wheat production areas of Ethiopia and the pathogen is able to complete its life cycle in the country (Woldeab *et al.*, 2016).

4.1.1. Distribution and intensity of wheat stem rust across peasant associations

The within district comparison of wheat stem rust distribution indicated that, highest prevalence (100%) of the disease were recorded in the peasant associations of Ofla (Hashange and Menkere), Raya Azebo (Kara Adishabo), Kilte Awulaelo (Genfel and Aynalem) districts. However, these peasant associations recorded different mean percent of incidences and severities of stem rust. The disease was more important in Genfal, Agulae, Kara Adishabo, Aynalem, Hashange, and Wargiba localities with mean incidences of 92%, 80%, 71%, 64%, 64% and 62% in the order listed. The lowest disease incidence was recorded in Betahawaryat,

Guamse and Maichew peasant associations with their mean value of 8%, 10%, and 16%, respectively (Table 8).

Similarly, the severity of wheat stem rust in the surveyed peasant associations didn't show a similar trend to that of incidences. The highest mean disease severity of 56% was recorded in Genfel, followed by Aynalem, Kara Adishabo, Agulae and Wargiba with mean values of 42%, 38%, 33%, and 32% in the same order. However, the lowest severity was recorded in Bethawaryat (3%) followed by Guamse and Maichew with mean values of 5% and 7%, respectively. In the localities of Ganta Afeshum district, the disease severity was very low as compared to others districts. Stem rust disease was not recorded in any of wheat fields of Hager Selam locality in Ganta Afeshum district. The zero disease prevalence in this locality might be due to the fact that wheat stem rust is more important at mid-altitude than at high altitude and lower temperature at higher altitude in the area during the survey. The annual rainfall and temperature were low in the district of this locality comparing to other districts assessed in the season (Appendix: 5 and 6) and also the locality fall in the altitude range of 2965-3008 m.a.s.l. Dagnatchew (1967) reported that due to a lower temperature at higher altitude stem rust disease is not a threat to the wheat crop.

Peterson (2001) who reported that stem rust is favored by warm temperature with six or more hours of moisture and develops to devastating levels if such conditions prevail during early reproductive phase prior to dough stage of the crop growth stage. Mideksa *et al*. (2018) also reported that temperature (minimum, maximum and average); rainfall and relative humidity interacted simultaneously in nature and contributed collectively to make the environment favorable for the stem rust development, which showed the existence of multicollinearity among them. When the effects of these weather factors were assessed individually, it was found that there existed no significant positive impact between stem rust development and individual weather factors except with a few weather variables. If one of these weather factors was missed, the disease would not develop. However, if disease develops, its severity fluctuates with changing environmental conditions. Eventually, environmental conditions, amount of inoculum, host susceptibility, host physiological growth stage and timing of the epidemic are all factor that affects the degree of damage significantly (Duveiller, 2007).

Table 8: Prevalence and distribution of wheat stem rust across different peasant associations of the districts in 2017 main cropping season

Zones	Districts	Peasant Association	Field Assessed	Prevalence (%)	Incidence (%)	Severity (%)
Southern	Ofla	Hashenge	5	100	64^{c}	27de
		Adigolo	5	80	22^{gij}	9^{ij}
		Menkere	5	100	56^{cd}	29^{d}
	Raya Azebo	K/Adishabo	5	100	71^{bc}	38^{bc}
		Wargiba	5	80	62^{c}	32^{cd}
	Enda Mehoni	Machiew	5	60	16^{ijk}	7^{ijk}
		Mehan	5	80	46de	25^{def}
		H/T/haymanot	5	80	43de	19^{fg}
Eastern	Kilte Awulaelo	Genfal	5	100	92^{a}	56^{a}
		Aynalem	5	100	64^{c}	42^{b}
		Agulae	5	80	80^{ab}	33^{cd}
	G/Afeshum	B/hawaryat	5	40	8^{kl}	3^{jk}
		Sasun	5	60	26^{fghi}	12^{ghi}
		H/selam	5	0	0^{l}	0^{k}
	S/Tseadaemba	Guamse	5	60	10^{jkl}	5^{ijk}
		H/hiwot	5	60	32^{f}	18^{fgh}
Southeast	Enderta	Ilala	5	60	39^{efg}	20^{efg}
		Kelamino	5	40	40^{ef}	20^{efg}
		Kihen	5	60	24^{ghij}	10^{hij}
LSD					15.32	8.73

*Means with the same letter(s) within the column are not significantly different at $p<0.05$.

Where: K/Awulaelo - Kilte Awulaelo, G/Afeshum - Ganta Afeshum, S/Tseadaemba – Saesia Tseadaemba, H/T/haymanot - Hizba Teklahaymanot, H/hiwot - Hadush hiwot, B/hawaryat - Beta hawaryat and K/Adishabo - Kara Adishabo.

4.1.2. Distribution and prevalence of wheat stem rust across altitude ranges

Wheat stem rust survey was carried out at altitude ranges of 1567-3008 m.a.s.l particularly, 1567-2520 m.a.s.l in Southern, 1950-3008 m.a.s.l in Eastern and 1981-2332 m.a.s.l in Southeast zones of Tigray region. According to the traditional classification system of agro ecological zones of Ethiopia; 500-1500 m lowlands, 1500-2300 m midlands and 2300-3200 m highlands (Ferede *et al.*, 2013). Based on this altitude classification, from the total fields inspected, 41.05% of the fields assessed were fall in mid altitudes ranging from 1567 to 2300 m.a.s.l while the remaining 58.95% were fall in the high altitude ranged from 2301-3008

m.a.s.l (Table 9). There was a significant ($p < 0.01$) difference among two altitude classes in terms of incidence and severity of wheat stem rust. Out of 39 wheat fields inspected in the altitude ranges 1567-2300 m.a.s.l stem rust was observed in 32 (82.05%) wheat fields with 62.05% mean incidence and 33.59% mean severity.

Similarly, out of 56 wheat fields surveyed in the high altitudes, wheat stem rust was recorded in 35 (62.50%) wheat fields with mean incidence and severity of 27.77% and 12.50%, respectively. The highest prevalence (82.05%) of stem rust was recorded at the mid altitude (1567-2300 m.a.s.l) where as a prevalence of 62.50% was recorded at the high altitudes (2301-3008 m.a.s.l). However, disease prevalence was not recorded at above 2585 m.a.s.l during the assessment. This study showed that stem rust has been more important in the mid-altitude agroecologies.

Table 9. The intensity of wheat stem rust across altitude ranges in 2017 main cropping season

Altitude range	Class name	No of field Inspected	Prevalence (%)	Incidence (%)		Severity (%)	
				Range	Mean	Range	Mean
1567-2300	Mid-altitude	39	82.05	0-100	62.05[a]	0-90	33.59[a]
2301-3008	High-altitude	56	62.50	0-100	27.77[b]	0-50	12.50[b]
Range		95		0-100		0-90	
LSD (0.05)					14.39		7.53
CV %					4.72		3.25

*Means with the same letter(s) within the column are not significantly different at $p<0.05$.

The survey results revealed that mean incidence and severity of wheat stem rust decreased from mid-altitude to high-altitude and markedly very low at high altitude >2500 m.a.s.l. The maximum stem rust disease severity recorded at mid-altitude was 90% while the maximum disease severity at high altitude was 50% on the farms. The highest disease severity 90% was recorded at 1950 m.a.s.l from Kilte Awulaelo district, Genfel locality. Stem rust incidence of 100% was recorded both at mid and high-altitude ranges. According to Badebo *et al*. (2008), the highest level of stem rust infection has been reported in the altitude ranges from 1600 to

2500 m.a.s.l. Similarly, 50% disease severity and 100% disease incidence was recorded below 2473 m.a.s.l in the present study.

Abebe *et al*. (2012) also reported that the highest prevalence of 68% of stem rust was recorded at altitude range 1494-1800 m.a.s.l followed by 66.1% prevalence at 1801-2300 m.a.s.l and none in higher altitudes >2300 m.a.s.l during 2010 cropping season in Southern Tigray region. Dagnatchew (1967) also stated that stem rust of wheat disease was very important at an altitude below 2300 m.a.s.l. The reports were contradicted with the current results where stem rust was observed at altitudes above 2300 m.a.s.l. This indicated that although stem rust has been more important at low and mid-altitude, it is now occurring at higher elevations. This might be associated with climate change, widespread cultivation of susceptible commercial varieties and appearance of new virulent races.

4.1.3. Prevalence and distribution of wheat stem rust by wheat variety

The survey results indicated that all farmer in the study areas were engaged in bread wheat production. The farmers were growing both improved and/or local varieties while the proportion of improved varieties were 81.05% and the rest 18.95% grown local varieties. They obtained wheat seed from formal and/or informal sources including non-governmental organizations, governmental organizations and their own seeds (local varieties). The highest seed provider to farming communities were Agricultural Bureau Offices of the study area, Agricultural Transformation Agency, Mekelle Agricultural Research Center and Alamata Agricultural Research Center. The majority of wheat crop was solely cultivated on small-scale farms with size ranged from 0.01-2.5 ha coverage whereas farmers grown barley, teff and field pea in the previous cropping season.

Fourteen wheat varieties were grown by farmers in the study area namely; Kakaba, Shehan (Local), Dashen, Mekelle I, Hidassie, Mekelle III, Ares (local), Danda'a, Pavon-76, Kingbird, Mekelle II, Mekelle IV, Gambo and Fentale. Out of 95 wheat fields inspected, 20 (21.10%), 12 (12.60%), 11 (11.58%), 9 (9.50%), 9 (9.50%), 7 (7.37%), 6 (6.30%) and 5 (5.30%) fields were covered by Kakaba, Shehan (Local), Dashen, Mekelle I, Hidassie, Mekelle III, Ares (Local) and Danda'a, respectively. Pavon-76 and Kingbird were sown in four fields (4.20%)

each. Similarly, two varieties (Mekelle IV and Gambo) were planted in 2 (2.10%) of the fields assessed for each whereas Mekelle II and Fentale were sown in 3 (3.20%) and 1 (1.00%) farms, respectively.

However, wheat stem rust was observed in 4 (100%), 2 (100%), 1 (100%), 8 (89%), 10 (83%) and 4 (67%) of wheat fields covered by varieties Pavon-76, Gambo, Fentale, Mekelle I, Shehan local and Ares local, respectively (Figure 7). The lowest disease prevalence was recorded from Danda'a variety 1 (20%). However, zero disease prevalence of wheat stem rust was observed on Mekelle IV variety during the assessment.

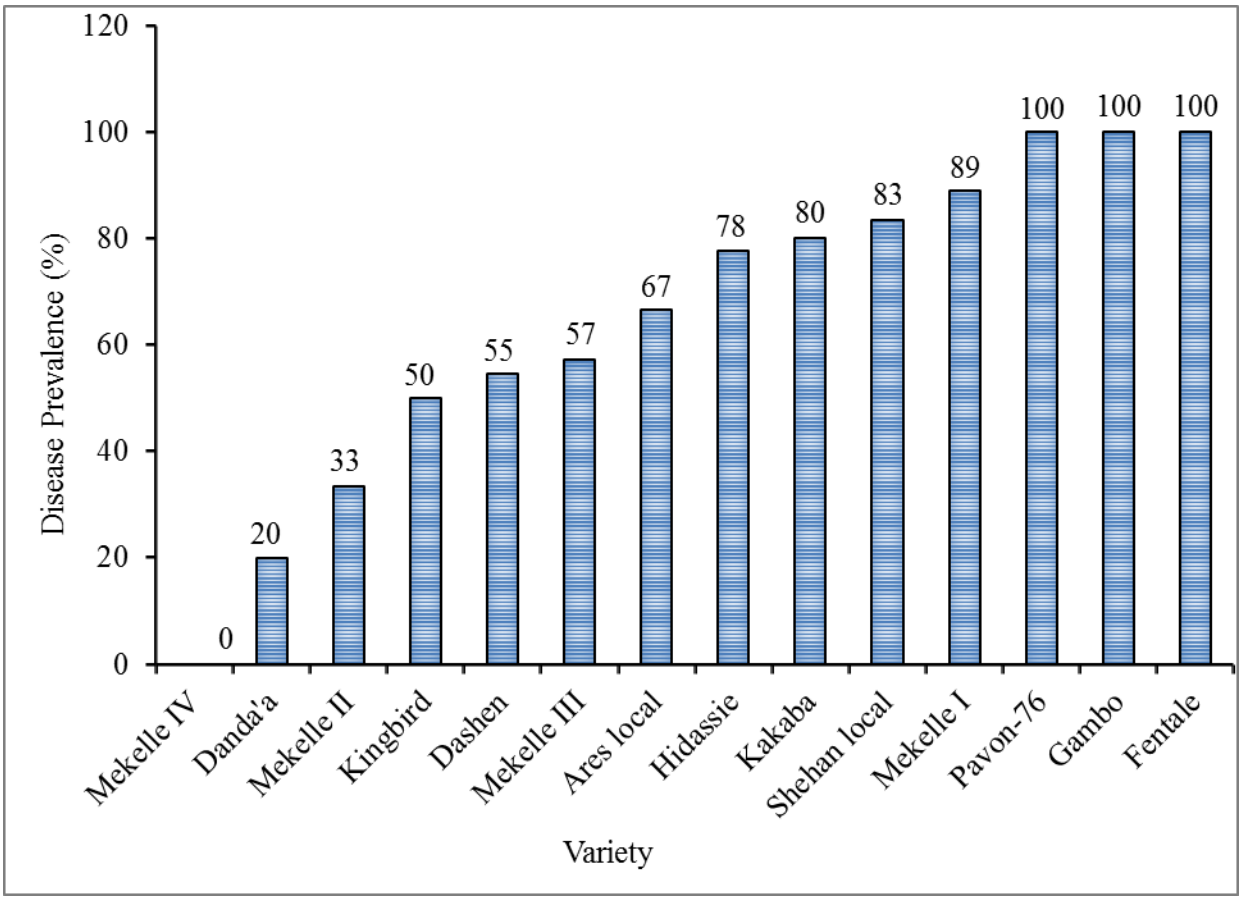

Figure 7: Disease prevalence across varieties in the zones of Tigray region during 2017 cropping season

The study indicated that, out of 67 (70.53%) infected wheat fields, 53 (79.10%) wheat fields were sown with improved wheat varieties whereas 14 (20.90%) fields were covered by the local varieties. Most improved wheat varieties have shown the susceptible type of reaction to

wheat stem rust disease in surveyed areas in 2017 main crop growing season. The host response to infection in the field was scored using 'R' to indicate resistance or miniature uredinia; 'MR' to indicate moderate resistance, expressed as small uredinia; 'MS' to indicate moderately susceptible, expressed as moderate sized uredinia somewhat smaller than the fully compatible type (Figure 8) and 'S' to indicate full susceptibility (Roelfs *et al*., 1992).

The most widely grown wheat variety was Kakaba and it covered 21.05% of surveyed wheat fields in the zones of Tigray region with 0 to 70% disease severity ranges in the fields. It showed susceptible to moderately resistant reactions with 52% mean incidence and 25.25% mean severity. The second commonly grown variety was Shehan (local) and this variety showed susceptible to moderately susceptible stem rust reaction with mean incidence and severity of 50.83% and 24.17%, respectively (Table 10).

There was a significant difference ($p < 0.01$) in disease incidence among the grown varieties. The highest mean disease incidence was recorded from Fentale variety (100%) followed by Gambo and Pavon-76 which recorded 80% and 75%, respectively. They were not significantly different with the highest disease incidence. The zero disease incidence was recorded from Mekelle IV variety followed by Danda'a variety which registered 2%.

Similarly, there was a significant difference (($p < 0.05$) among varieties grown in disease severity. The highest mean disease severity was recorded from Fentale variety (60%) followed by Gambo and Pavon-76 which recorded 40% for each and statistically par with the highest disease severity. The zero disease severity was recorded from Mekelle IV variety followed by Danda'a which recorded 1% and they were statistically similar (Table 10).

Table 10: Mean incidence and severity of wheat stem rust across varieties in zones of Tigray region in 2017 main cropping season

Varieties	Variety response at fields	Number of field assessed	Disease Incidence (%)	Disease Severity (%)
Kakaba	MR-MS-S	20	52.00^{abcd}	25.25^{bcd}
Shehan (local)	MS-S	12	50.83^{abcde}	24.17^{bcd}
Danda'a	MS	5	2.00^{de}	1.00^{d}
Hidassie	MS-S	9	49.44^{abcde}	24.44^{bcd}
Dashen	MR-MS-S	11	21.82^{cde}	11.36^{cd}
Mekelle I	MS-S	9	51.67^{abcd}	25.00^{bcd}
Mekelle II	MS-S	3	10.00^{cde}	6.67^{cd}
Mekelle III	MS-S	7	21.43^{cde}	10.00^{cd}
Mekelle IV	-	2	0.00^{e}	0.00^{d}
Gambo	S	2	80.00^{a}	40.00^{ab}
Fentale	S	1	100.00^{a}	60.00^{a}
Kinbird	MR-S	4	23.75^{bcde}	12.50^{bcd}
Pavon 76	MS-S	4	75.00^{ab}	40.00^{ab}
Ares (local)	MS-S	6	55.00^{abc}	33.33^{abc}
LSD (0.05)			51.43	28.16
CV%			4.94	3.51

*Means with the same letter(s) within the column are not significantly different at $p<0.05$.

Where: R- resistant, MR- moderately resistant, MS- moderately susceptible and S- susceptible

Generally, most varieties released recently in the country were succumbed to stem rust disease shortly after their introduction. The high susceptibility of the wheat varieties to stem rust might be due to the climatic change especially, warmer temperature and adaptation of the pathogen to the wider environmental conditions. In most cases, the failures have been due to the virulence present in the pathogen population and deployment of the qualitative type of resistance in a wide array of wheat cultivars (Hei *et al*., 2017). However, Mekelle IV variety was free from stem rust disease during the survey time and cultivated at higher altitudes (>2900 m.a.s.l). This could be due to the fact that the varieties became resistant to stem rust as rainfall decreased or the existence of weather conditions and higher elevation which was not conducive for the development of the pathogen (Appendix: 5 and 6).

In agreement with this result, Mideksa *et al*. (2018) reported that cultivars might be become resistant to disease as the amount of rainfall decreased or the prevailing weather conditions were not conducive for the development of stem rust epidemics. According to Jain *et al*. (2009), the differences in disease incidence and severity within the similar agro-ecological area when a similar variety grown are an indication of pathogen virulence variability. The typical symptom of wheat tem rust in the zones of the region was illustrated below (Figure 8).

Figure 8: Typical symptoms of stem rust during survey in the zones of Tigray region from Eastern zone, Kilte Awulaelo and Southern Zone, Raya Azebo districts

4.1.4. Occurrence of wheat stem rust by wheat growth stages

Whenever disease assessments are made, the growth stage of plants is essential for meaningful comparisons between varieties, locations and years. During the assessments, the crop growth sages ranged from milk to hard dough growth stages or GS73-GS87, according to Zadoks (1974) cereal growth stage guideline. Out of 95 fields inspected, 10 (10.50%), 45 (47.40%), 25 (26.30%) and 15 (15.80%) were at milk, early dough, soft dough and hard dough growth stages, respectively. In the same order, stem rust was observed on 2 (20%), 36 (80%), 15 (60%) and 14 (93.30%) of 10, 45, 25 and 15 wheat fields inspected in the mentioned growth stages (Table 11).

The results revealed that the intensity of wheat stem rust varied significantly among the growth stages of the wheat crop. The highest disease incidence (66.33%) was observed in the hard dough growth stage followed by early dough growth stage (47.89%) which was not significantly different. The highest disease severity was recorded from the hard dough stage (34.00%) followed by early dough growth stage (23.56%). The lowest disease intensity was recorded from milk growth stage which was 5% disease incidence and 2% disease severity. The variation in the levels of wheat stem rust infections depended on the growth stages.

Table 11: Occurrence of wheat stem rust by wheat growth stage during 2017 growing season

Growth stages	Number of fields	Incidence (%)		Severity (%)	
		Range	Mean	Range	Mean
Milk	10	0-30	5.00^{c}	0-10	2.00^{c}
Early dough	45	0-100	47.89^{ab}	0-90	23.56^{ab}
Soft dough	25	0-100	31.00^{b}	0-60	16.80^{b}
Hard dough	15	40-100	66.33^{a}	20-60	34.00^{a}
LSD (0.05)			23.58		13.00
CV (%)			5.05		3.61

*Means with the same letter(s) within the column are not significantly different at $p<0.05$.

In the study area, wheat was grown in different agro-ecological zones that have different planting dates. This staggered planting date provides a green crop most part of the season allowing stem rust spores to move by wind from one growth stage to another and that way

spreads to different farms nearby. According to Fetch *et al*. (2011) planting as early as possible and planting early maturing cultivars would help to reduce the time of exposure of the crop to the pathogen, reduces the time frame for establishment of urediniospores and ultimately limits the growth period of the fungus. Singh *et al*. (2008) also reported that wheat stem rust is more important late in the growing season, on late-sown and late maturing wheat cultivars. The highest disease incidence and severity in hard dough growth stage could be due to the fact that the pathogen is more important in the late growth stage.

The importance of wheat stem rust was increasing with mounting in the growth stage of the crop and therefore, the prevalence and intensity of the disease were highest during hard dough development growth stage. Roelfs *et al*. (1992) mentioned that the late growth stage of the crop is the important period to reach stem rust disease of wheat at its maximum severity level. The same results were also reported by Mandefro (2000). Bhavani *et al*. (2011) indicated that disease severity was as high as 80-100% at soft dough to hard dough growth stages.

4.1.5. Association between stem rust intensity with altitude, weed infestation levels and growth stages

The study showed that disease severity was linearly correlated with disease incidence, which means for higher disease incidence higher disease severity was recorded. There was highly significant ($P < 0.001$) and negative correlation between altitude and incidence ($r = -0.44$) and severity ($r = -0.50$) of wheat stem rust disease. The negative relationship between altitude and level of wheat stem rust is also reported by Abebe *et al*. (2012) and Hailu *et al*. (2015) who found that wheat stem rust pathogen was more important at mid altitudes than at high altitude (Table 12). Similarly, there was significant ($P < 0.05$) and positive correlation between wheat growth stages and disease incidence ($r = 0.26$) and severity ($r = 0.27$). The positive relationship between wheat growth stages and intensity of wheat stem rust reflected that as the wheat growth stages increase the development of the pathogen become more severe (Mandefro, 2000). In this study, there was also highly significant ($p < 0.001$) and strong positive correlation between weed infestation levels and disease incidence ($r = 0.74$) and severity ($r = 0.66$). This implies that the disease became more intense in the weedy farm (Joseph *et al*., 2007).

Table 12: Pearson's correlation coefficients between major factors, and incidence and severity of stem rust in 2017 main cropping season

Variables	DI	DS	ALT	GS	WIL
DI	1	0.94***	-0.44***	0.26*	0.74***
DS		1	-0.50***	0.27**	0.66***
ALT			1	-0.33**	-0.25*
GS				1	0.21*
WM					1

DI - Disease incidence, DS - Disease severity, ALT - Altitude, GS - Growth stage and WIL- Weed infestation level. *Significant level at $p<0.05$; **Significant level at $p<0.01$ and ***Significant level at 0.001.

The regression analysis between altitude ranges and severity revealed that a negative relationship was observed among them. This demonstrates that elevation increment in meter, stem rust disease severity decreased by about 0.03% (Figure 9). The negative relationship between altitude and disease severity implied that the pathogen was more important at lower and mid altitudes resulting in decreased intensity at higher altitudes (Badebo *et al*., 2008).

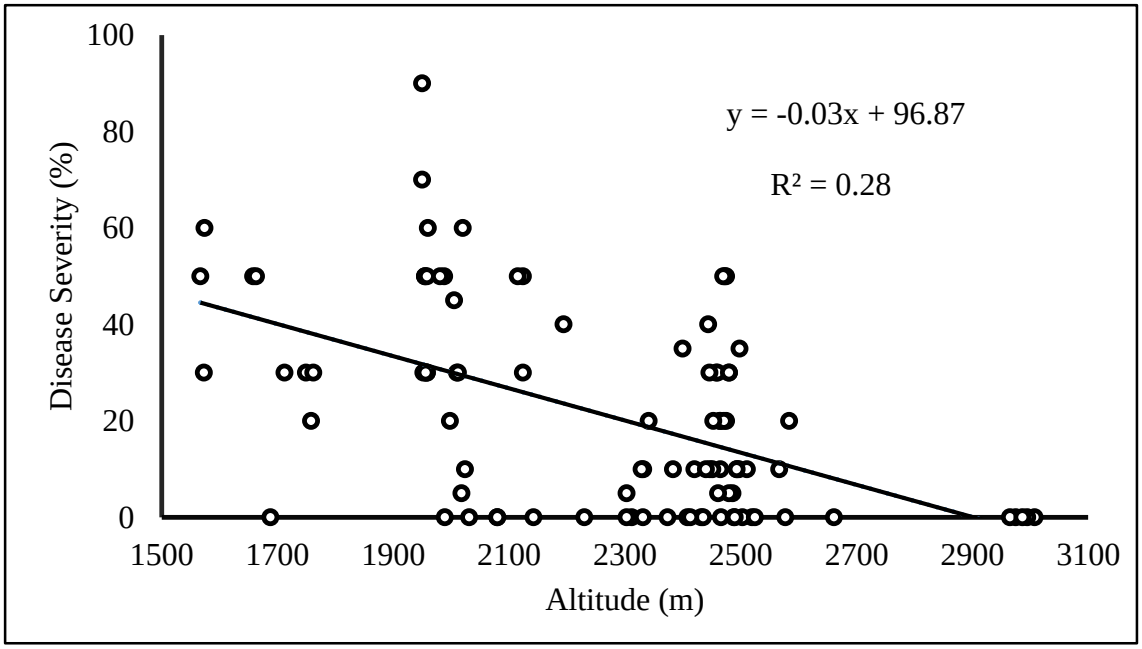

Figure 9: Regression relationship between disease severities (%) against altitude (meter)

4.2. Virulence Spectrum and Physiological Race Composition of Stem Rust Pathogen

Sixty-six infected wheat stem samples were collected from Southern, Eastern and Southeast zones of Tigray region. Of these, 19 did not yield viable isolates at the time of inoculation in the laboratory, hence, 47 isolates were used for the race spectrum analysis. Of these isolates, 6 races namely TTTTF, TKTTF, TRTTF, RRTTF, TTRTF and TKPTF were identified (Figure 10). All the 6 races were found in the Southern zone. Three races (TTTTF, TKTTF, and TKPTF) were identified from Southeast zone while 2 races TTTTF and TKTTF were detected in Eastern zone. Only 1 race TKTTF was detected in Saesia Tsaedaemba district while in the other districts 2 or more races were detected. TKTTF race was a common race detected in all the studied districts of the three zones and also TTTTF was detected from all districts except Saesia Tsaedaemba district.

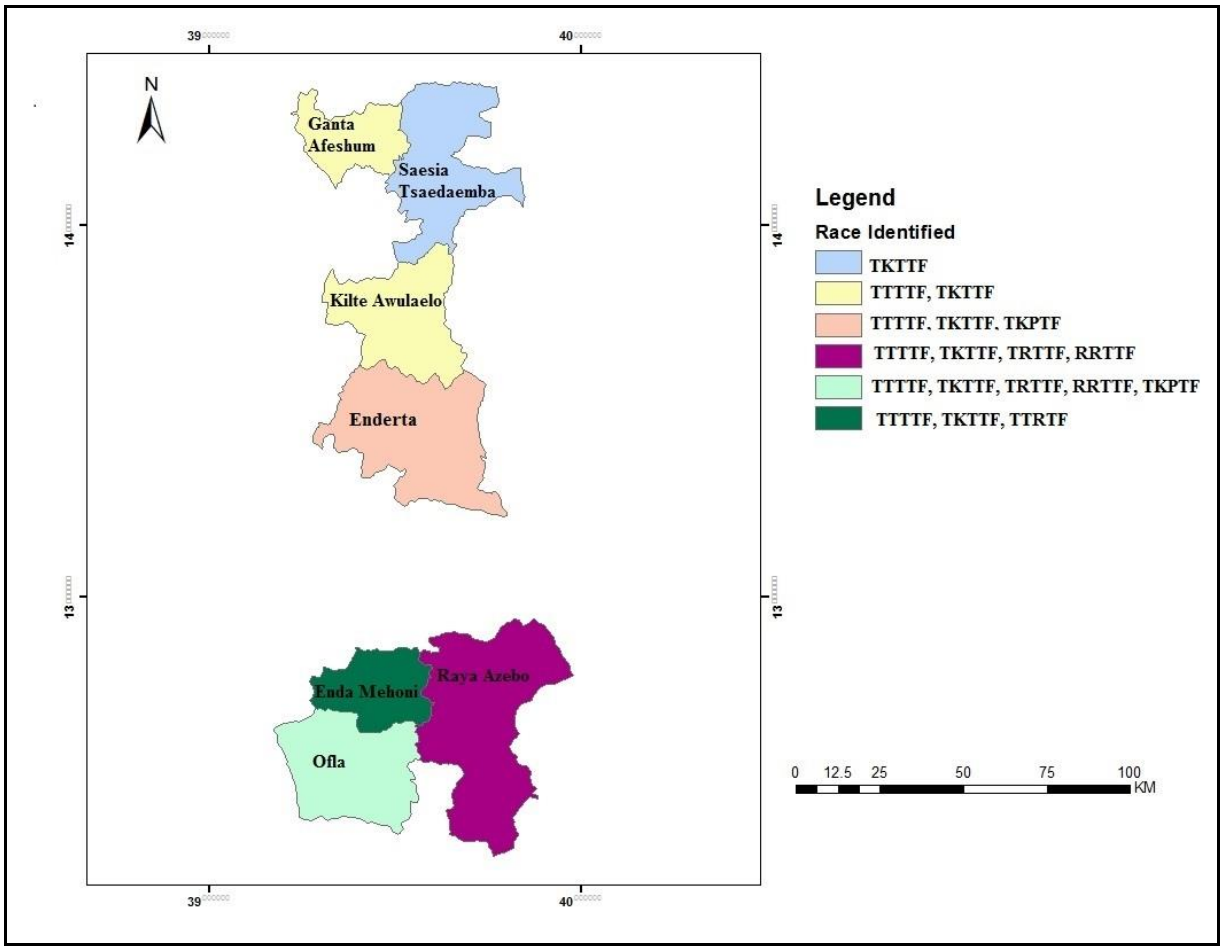

Figure 10: Distribution of *Puccinia graminis* f.sp. *tritici* races in the Southern, Eastern and Southeast zones of Tigray region

Five races were identified from Ofla district followed by Raya Azebo district that had 4 races both districts them were from the Southern zone of Tigray region. The races prevalent in the Southern zone may have a wider virulence spectrum compared to the races in Eastern zone and Southeast zones of Tigray region. Among the identified races, TTRTF was detected for the first time in Ethiopia, particularly in Southern zone of Tigray region, Enda Mehoni district of Maichew locality. Variation in race composition over location and time depend on the type of wheat varieties grown and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*., 1992).

Out of the 47 viable stem rust samples collected, TTTTF race was identified from 25 isolates while TKTTF detected from 15 isolates indicating that TTTTF and TKTTF were the most abundant races in the study areas in the season. TTTTF with virulence to Sr9e and Sr13 attacked thousands of hectares of wheat, resulting in the largest burst of wheat stem rust in the world and a large number of spores produced by it may continue the epidemic (Bhattacharya, 2017). This race has a virulence formula which is almost similar to TKTTF but is clearly different from stem rust race TTKSK (Ug99) as it has avirulence to Sr24 and Sr31. TTTTF race was first detected from samples collected in 2009 in Eastern Shoa zone of central Ethiopia at trace level (Lemma *et al*., 2014). It was also detected in Iran from samples collected during 2010-2014 (Patpour *et al.*, 2014; Afshari *et al*., 2015). TTTTF hit several thousands of hectares of durum wheat on the Italian Islands of Sicily in 2016, causing the largest stem rust outbreak that Europe has seen in decades (FAO, 2017b).

This study revealed that TKTTF race was the second most dominate stem rust population in the season. TKTTF was detected for the first time at a trace level in 2012 main cropping season, samples collected from Arsi and Bale zones of Oromia region and was found to be primary cause of the epidemics in the Southeastern parts of the country in 2013 and 2014 cropping seasons (Olivera *et al*., 2015; Hodson, 2015). The detection of this race in 2012 was the first report of virulence to SrTmp in the country. This non-effective gene is present in the most popular and widely grown bread wheat variety Digalu (Hodson, 2015). According to Mert *et al*. (2012) as cited by Singh *et al*. (2015) races similar to TKTTF occurred in Turkey in the 1990s and still are predominant races in country. TKTTF has been detected in Iran

(2010), Lebanon (2012), Egypt (2013), Azerbajan, Eriteria and Yemen (Olivera *et al*., 2015). It is also detected in Kenya from samples collected in 2014 and 2015. The presence of stem rust race with identical virulence profiles throughout this vast region implies that there are inoculum exchanges and the race is a serious threat to the wheat production to wider scale and needs monitoring (Hodson, 2016). Studies indicated that TKTTF does not belong to Ug99 lineage based on avirulence to Sr11 and Sr31 and molecular fingerprints (Olivera *et al*., 2015).

The frequency of each race was calculated as a percentage of the total number of isolates analyzed. The races identified from major wheat grown areas in the zone of the region had wide virulence spectra (Table 13). Out of the six races identified, the most frequently and predominantly occurred race was TTTTF with a frequency of 53.19%. The second most frequently detected race was TKTTF with a frequency of 31.91%. The remaining 14.91% fields infected by other races such as TRTTF (4.26%), RRTTF (4.26%), TKPTF (4.26%), and the least frequent registered race was TTRTF with 2.13%. This study confirmed the presence of wider virulence diversity within the *Puccinia graminis* f.sp. *tritici* population in the study area and in agreement with the previous studies conducted in the country (Admassu *et al*., 2009; Abebe *et al*., 2012; Hailu *et al*., 2015).

The most important virulent race TTTTF was isolated from 25 wheat fields grown with Kakaba, Shehan, Ares local, kingbird, Gambo, Fentale, Pavon-76, Mekelle II, Mekelle I, Dashen, and Hidassie of which 5 (50%), 6 (66.67%), 3 (75%), 2 (100%), 2 (100%), 1 (100%), 1 (100%), 1 (100%), 2 (50%) 1 (33.33%), and 1 (16.67%) were infected with this race, in the same orders. For instance, nine viable sampled wheat fields with Shehan local bread wheat variety, 6 (66.67%) were infected with TTTTF in all surveyed zones. Out of 10 samples taken from Kakaba variety, 5 (50%) of wheat fields were infected with TTTTF. This race was detected from an altitude range of 1567-2567 m.a.s.l which show that it is adapted to wider wheat growing agro-ecological zones.

TKTTF was the second most virulent race on bread wheat varieties and it was isolated from 15 field sampled with Mekelle III, Dashen, Hidassie, Mekelle I, Shehan, Kakaba, and Ares

local of which, 2 (100%), 1 (33.33%), 3 (50%), 3 (60%), 2 (22.22%), 3 (30%) and 1 (25%) were infected in the same orders. This race was distributed in the altitude range of 1663-2519 m.a.s.l. It is rapidly spreading to a wide altitude ranges, this might be due to favorable environmental conditions as well as cultivation of susceptible wheat varieties in those districts. On the other hand, TKPTF was detected from Mekelle II and Kakaba varieties. Similarly, new race TTRTF was detected only at a single location from Hidassie variety. RRTTF race was also obtained from Kakaba and Shehan local varieties. Lastly, TRTTF race was detected from Dashen and Hidassie variety from a single wheat field each. TKPTF was detected from elevations of 2124 and 2471 m.a.s.l and TTRTF was from 2493 m.a.s.l.

Table 13 *Puccinia graminis* f.sp. *tritici* races identified from samples collected and wheat varieties grown in varying altitude ranges of zones of Tigray region in 2017 main cropping season

Zone	District	No. of isolates	Identified races	Wheat varieties	Altitude (m)
Southern	Ofla	1	RRTTF	Kakaba	2465
		1	TKPTF	Mekelle II	2471
		1	TRTTF	Hidassie	2475
		3	TKTTF	Kakaba, Hidassie and Dashen	2460-2497
		4	TTTTF	Kakaba, Hidassie, Mekelle I and Shehan (local)	2458-2480
	Raya Azebo	6	TTTTF	Kingbird, Gambo, Fentale and Shehan	1567-1758
		1	TKTTF	Shehan local	1663
		1	RRTTF	Shehan local	1762
		1	TRTTF	Dashen	1650
	Enda Mehoni	3	TKTTF	Hidassie and Shehan	2400-2480
		5	TTTTF	Kakaba, Dashen and Shehan	2303-2511
		1	TTRTF	Hidassie	2493
Eastern	Kilte Awulaelo	4	TTTTF	Kakaba, Pavon-76, Ares local	1950-2194
		3	TKTTF	Kakaba, Mekelle I, Ares local	1950-1955
	Ganta Afeshum	3	TTTTF	Mekelle II Mekelle I, Kakaba	2451-2567
		2	TKTTF	Mekelle III	2444-2447
	S/Tsaedaemba	2	TKTTF	Kakaba	2024-2519
South east	Enderta	3	TTTTF	Ares local and Shehan local	1981-2332
		1	TKPTF	Kakaba	2124
		1	TKTTF	Mekelle I	1998
Total		47	6		1567-2567

The virulence spectra of detected stem rust races was varied between 16 and 18 stem rust resistance genes (Table 14). The broadest virulence spectrum was recorded for the race TTTTF that exhibited virulence on 18 stem rust resistance genes. Based on a set of twenty North American wheat differential lines, this race was virulent to all differential lines except Sr24 and Sr31. The most devastating stem rust race TTTTF was first detected in the U.S in 2000 (Jin, 2005; Jin *et al*., 2007) and had been spread to most of the wheat growing areas of our country now a day. Out of the races detected, it was the most virulent race that producing high infection types (ITs) on the majority of stem rust differential lines in the study. In agreement with this finding, the presence and potential of TTTTF race to wheat production in Ethiopia has been already reported by Lemma *et al*. (2015).

The second widest virulence spectrum was recorded from TKTTF, TRTTF and TTRTF races that showed virulence on 17 stem rust resistance genes. The new race TTRTF was recorded broadest virulence on 17 Sr resistance gene. Similarly, RRTTF and TKPTF races were virulence on the 16 stem rust resistance gene of differential lines. Abebe *et al*. (2012) also reported RRTTF race was a virulent spectrum on the 16 resistance gene of differential lines in the Southern zone of the Tigray region. The same to this previous report, RRTTF race was detected from Southern Tigray zone; Raya Azebo and Ofla districts.

About 16.67% of the races (TTTTF) identified showed virulence to 90% of the Sr genes and 50% (TRTTF, TKTTF, and TTRTF) of the races showed virulence on 85% Sr genes. The remaining 33.33% (TKPTF and RRTTF) of the races identified were virulent on 80% of the 20 Sr genes. The new race TTRTF defeated 85% of the Sr resistance genes in the wheat differential lines including Sr30, Sr11, Sr36, and Sr38. The virulence pattern observed in this study confirmed the presence of a wider range of virulence in the study area. Moreover, the detected races had a wider range of virulence in the study areas and high virulence diversity of stem rust races were reported by many authors earlier in Ethiopia (Admassu *et al*., 2009; Lemma *et al*., 2015). Co-evolution of *Puccinia graminis* f.sp. *tritici* along with wheat being the reason for high virulence diversity in Ethiopian *Puccinia graminis* f.sp. *tritici* populations. Virulence diversities within *Pgt* were also reported from abroad countries such as South Africa, Mexico, USA and Canada (Jin, 2005).

Table 14: Virulence/Avirulence spectrum and frequency of *Puccinia graminis* f.sp. *tritici* races collected from Southern, Eastern and Southeast zones of Tigray region

Race	Virulence spectrum (ineffective Sr gene)	Avirulence spectrum (effective Sr genes)	No of isolates	Races frequency (%)	Virulence of races on Sr gene (%)
TTTTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	24, 31	25	53.19	90
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	11, 24, 31	15	31.91	85
RRTTF	5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	9e, 8a, 24, 31	2	4.26	80
TKPTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 17, 9a, 9d, 10, Tmp, 38, McN	11, 9b, 24, 31	2	4.26	80
TRTTF	5, 21, 9e, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	8a, 24, 31	2	4.26	85
TTRTF	5, 21, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	24, 31, 9e	1	2.13	85
Total			47	100	

The stem rust virulence spectrum in Ethiopia was definitely different from other parts of the world. For instance, surveys in Canada, USA, Russia and South Africa detected fewer races such as 15, 5, 6 and 7, respectively (Fetch, 2004; Jin, 2005; Pretorius *et al*., 2010). However, more races were identified from Ethiopia; for example, 15, 40 and 88 races were reported in Bale, Arsi, Sidamo and Harargie (SPL, 1988) and 17, 22 and 20 races were detected from Arsi, Bale and Southern Tigray (Serbessa, 2003; Admassu *et al*., 2009; Abebe *et al*., 2012). However, the present study was dissimilar to the previous works conducted in Ethiopia as evident from the fact that only 6 races have been identified from three zones of Tigray region.

4.3. Virulence Frequency of *Puccinia graminis* f.sp. *tritici* Isolates on Stem Rust Resistant Genes

The study revealed that most of the races identified in the present study were virulent to many of the resistance genes. Fourteen differential lines carrying stem rust resistance gene Sr*5*, Sr*21*, Sr*7b*, Sr*6*, Sr*9g*, Sr*36*, Sr*30*, Sr*17*, Sr*9a*, Sr*9d*, Sr*10*, Sr*Tmp*, Sr*38* and Sr*McN* were found to be 100% ineffective to all races. Similarly, four stem rust differentials carrying resistance genes Sr*9b*, Sr*9e*, Sr*8a* and Sr*11* were found to be ineffective against most of the races detected, with virulence frequencies of 95.74, 93.62, 91.49 and 63.83%, respectively (Table 15).

Of the 20 stem rust resistance genes, the differential hosts carrying Sr*11*, Sr*8a*, Sr9e and Sr*9b* were resistant to 36.17%, 8.51%, 6.38% and 4.26% of races identified, respectively. Stem rust resistance gene Sr*24* was effective against all of the races. Likewise, Sr*31* also effective in this study due to the reason TTKSK (Ug99) was not detected in the present study. Admassu *et al.* (2010) also indicated that no virulent race was detected against Sr24 gene in Ethiopia. This study confirms the report of Abebe *et al*. (2012) which stated, the Sr24 stem rust resistance gene is amongst the effective genes to all stem rust collected from the Southern zone of Tigray region, which have an adequate and some immediate values to almost all races in the world. However, virulence to Sr24 gene was reported in Kenya in 2006. A variant of Ug99 group that added virulence on stem rust gene Sr*24* (Ug99+Sr*24* virulence, called TTKST) has further increased the vulnerability of wheat to stem rust worldwide (Jin *et al*., 2008).

TTTTF race was avirulent to Sr*24* and Sr*31* while TKTTF (Digalu race) was avirulent to Sr*11*, Sr*31*, and Sr*24*. The virulence of race TKTTF on the resistance gene SrTmp was considered as the main factor behind the complete susceptibility of the variety "Digalu" to this race. TTRTF was avirulent to Sr24, Sr31 and Sr9e while TKPTF was avirulent to Sr24, Sr31, Sr11 and Sr9b. The breakdown of the Sr*31* resistant gene in Ethiopia is reported by many authors previously which were evidences for the existence of Ug99 (TTKSK) (Admassu *et al*., 2009; Abebe *et al*., 2012; Hailu *et al*., 2015). In contrast, this study revealed that there was no TTKSK race and its variants detected in the study area during the 2017 cropping season. This finding disagreed with the previous report that Sr*31* were 100%

effective for the races detected. Similarly, surveys carried out by other colleagues nationally during 2017 main cropping season also indicated there was no race TTKSK and its variants in the season. This result might be due to unfavorable environmental condition and resistant varieties grown in the surveyed areas. In conclusion, Sr24 and Sr31 resistance gene were 100% effective for all stem rust races detected in the season. Therefore, use of these genes for breeding program is pertinent.

Table 15: Virulence frequency of *Puccinia graminis* f.sp. *tritici* isolates on the 20 stem rust resistance genes

Stem rust resistance gene (Sr gene)	Virulence frequency (%)	Stem rust resistance gene (Sr gene)	Virulence frequency (%)
5	100	30	100
21	100	17	100
9e	93.62	9a	100
7b	100	9d	100
11	63.83	10	100
6	100	Tmp	100
8a	91.49	24	0
9g	100	31	0
36	100	38	100
9b	95.74	McN	100

According to Admassu *et al*. (2009) and Abebe *et al*. (2012), most of the races in Ethiopia varied from one another by single-gene changes. Such single-step changes in virulence were reported to be the main process of evolutionary change in *Puccinia graminis* f.sp. *tritici* populations. In agreement with this previous finding, two races identified were varied by single gene changes. For instance, TKTTF was similar to TRTTF with virulence to Sr*11* and Sr8a, respectively. However, most of the identified races were not varied by single step changes. These might be due to other factors for race variation in the studied area like, parasexualism, migration, selection pressure and gene combination.

4.4. Evaluation of Bread Wheat Varieties against Dominant Races of *Puccinia graminis* f.sp. *tritici* at Seedling Stage in the Greenhouse

The greenhouse evaluation revealed that bread wheat varieties differed in their reaction to the dominant stem rust races; TTTTF, TTKSK, TKTTF, TRTTF, RRTTF and JRCQC. Out of 39 bread wheat varieties evaluated at the seedling stage, none showed complete resistance (zero IT) while 7 (Honqolo, Huluka, ETBW-9017, ETBW-9042, Dilfiker, Wabe and Millennium) had resistance infection types (IT = ";" or fleck to 2). The resistance reaction in these varieties implied the presence of seedling resistance gene towards these virulent races. Three varieties namely; Lemu, Hoggana and ETBW-7956 exhibited resistance to all virulent races but susceptible to TTKSK race (IT=3-).

Among the 39 bread wheat varieties evaluated, 9 (Danda'a, Kakaba, Kubsa, Shehan, Mekelle I, Mekelle II, Mekelle III, Mekelle IV and ETBW-7638) showed susceptible reaction (3- to 3+) to all selected virulent races (Table 16). Danda'a and Kakaba were stem rust resistant varieties released in 2010 and their popularity increased throughout the country featuring adult plant resistance or minor gene resistance to stem rust (Tolemariam *et al*., 2018). Even though, in the present study infection types, 3- to 3 were recorded on these varieties against all the virulent races tested. Hailu *et al.* (2015) also reported similar findings that Danda'a and Kakaba showed susceptibility to TTKSK and TKTTF races (3- to 3) in the greenhouse.

Two wheat varieties namely, Digalu and Hidassie showed resistance to TTKSK (Ug99) races but susceptible to all the other virulent races. Admassu *et al*. (2009) and Abebe *et al.* (2012) also reported that Digalu variety was resistant to TTKSK race at seedling stage and its resistant response to this particular race might be due to the presence of SrTmp gene in the variety. Digalu variety was introduced in 2005 to provide protection against the Ug99 (TTKSK) race (Singh *et al*., 2015).

However, Digalu (carrying SrTmp) was susceptible to race TKTTF that first detected in Ethiopia in 2013 (Hodson, 2015). According to Olivera *et al*. (2015), the stem rust resistance gene SrTmp became ineffective in Ethiopia due to the evolvement of this race and most

farmers who grow Digalu were highly affected in 2013 and 2014 cropping seasons. The susceptibility of currently high yielding bread wheat varieties such as Digalu, Danda'a, Kakaba and Kubsa to race TKTTF indicated the potential threat of TKTTF in wheat production in the country.

Seedlings of Enkoy, Mitikie, Dereselign, Alidoro, Galema and Medawelabu varieties were resistant to TTTTF and RRTTF races (";" to 2+), however, they were susceptible to TTKSK, TKTTF, TRTTF and JRCQC races. This could be due to the broadest virulence spectrum of the current races. Enkoy carrying the Sr*36* gene for stem rust resistance was considered to have durable resistance to stem rust, despite complete crop failure in 1993 (Temesgen *et al*., 1995) and in the current study, it was resistant at the seedling stage to TTTTF and RRTTF races (IT = ;1+).

Moreover, Hawi and Laketch varieties were susceptible to all races except TKTTF (;1). Hawi was postulated to have a seedling stem rust resistance gene(s), Sr30+/ Sr31 (Naod *et al*., 2005), both stem rust resistance genes were not effective to the virulent stem rust races in the major wheat growing regions of Ethiopia (Admassu and Fekadu, 2005; Admassu *et al*., 2009; Abebe *et al*., 2012). Dashen and Shorima were resistant to TTTTF, TTKSK, TKTTF, and RRTTF races but susceptible to TRTTF and JRCQC races. ET-13A2 variety was susceptible to all virulent races tested but resistant to TTTTF race. Hailu *et al*. (2015) also reported that this wheat variety was susceptible to TTKSK and TRTTF at the seedling stage during the 2014 main cropping season. Kingbird variety was susceptible to TTTTF, TKTTF, and JRCQC races but resistant to TTKSK, TRTTF and RRTTF races whereas Ogolcho variety was resistant to TKTTF, TRTTF, RRTTF and JRCQC dominant races but susceptible to TTTTF and TKTTF races (Table 16).

The seedling evaluation result indicated that most varieties were susceptible to race TTTTF. This might be due to the widest virulence spectrum of TTTTF race. Similarly, Lemma *et al*. (2014) reported TTTTF was more virulent race on durum wheat cultivars they tested. Pavon-76 was susceptible (IT=3-) to all virulent races tested but resistant to RRTTF race. The susceptibility of Pavon-76 in the seedling stage was previously reported by Singh *et al*. (2007)

and Hailu *et al*. (2015). Biqa variety showed resistant to TTTTF, TKTTF and RRTTF whereas susceptible to TTKSK, TRTTF and JRCQC races. Galil was resistant to TTTTF, TTKSK and RRTTF whereas susceptible to TKTTF, TRTTF and JRCQC races.

Finally, KBG-01 and K6295-4A varieties were susceptible to all dominant races but resistant to TRTTF race. KBG01 is postulated to have Sr9a and this Sr gene is very susceptible to Ug99 (TTKSK) and its variants (Naod *et al*., 2005). According to Lemma *et al*. (2014), KBG01 was susceptible at seedling stage but resistant at the adult stage during 2007/2008 main cropping season. This tells us there was adult stage resistance gene in this variety. Sr*2* is a well-known and effective adult plant resistance gene (minor gene) that confers resistance against stem rust. The stem rust check McNair was susceptible to all races at the seedling stage in the greenhouse. Admassu *et al*. (2009) also reported a similar finding that McNair 701 (Sr*McN*) was susceptible to all of the races identified.

Table 16: Reactions of bread wheat varieties against dominant stem rust races at seedling stage in the greenhouse

Varieties	Seedling Reaction (IT)					
	TTTTF	TTKSK	TKTTF	TRTTF	RRTTF	JRCQC
Danda'a	3-	3-	3-	3-	3-	3-
Enkoy	3-	3	3-	;1+	;1+	3-
Kakaba	3	3	3-	3-	3	3-
Mitikie	3-	3-	3-	;1+	2+	3-
Honqolo	;1+	;1	;1	;1	;1	;1
Huluka	;	;	;1	;1	;1	;1
ETBW-7638	3-	3-	3-	3-	3-	3-
ETBW-9042	2-	;1	;1	2	;1	;1
ETBW-7956	;1+	3-	;1	;1	;1	;1
Laketch	3-	3-	;1	3-	3-	3-
Dereselign	3	3	3-	;	;1	3-
ETBW-9017	;1+	2-	1+	;1	;1	;1
Alidoro	3	3-	3-	;1+	;1	3-
Dilfiker	;1+	;1	;1	;1	;	;1+
Biqa	2+	3-	;1	3-	2	3
Hawi	3-	3-	2+	3-	3-	3-
Lemu	;1	3-	;1	;1+	;1	;1
Dashen	;1	;1	3-	;1	;1	3-
Galema	3	3-	3-	;1	;1	3-
Ogolcho	3-	;1+	3-	2	2	2-
Kingbird	3-	2+	3+	2	;1	3-
Shorima	;1	;1+	3	;1	;1	3-
ET-13A2	2	3-	3-	3-	3-	3-
Hogana	;1	3-	;1	;1	;1	;1
Wabe	;1	;1+	;1	;1	;1	;1
Pavon-76	3-	3-	3-	3-	;1+	3-
Millennium	;1	;1+	;1	;1	;1	;1
Kubsa	3	3-	3-	3	3-	3-
KBG-01	3	3-	3-	;1+	3-	3-
K6295-4A	3-	3	3-	;1+	3-	3-
Digalu	3-	;	3	3-	3-	3-
Hidassie	3-	;1	3-	3-	3-	3-
Shehan	3-	3-	3	3-	3-	3
Madawelabu	3-	3-	3-	;1	;1	3-
Galil	2	;1	3-	3-	;	3-
Mekelle II	3-	3-	3-	3-	3-	3-
Mekelle III	3-	3-	3-	3-	3-	3-
Mekelle IV	3	3	3	3	3	3-
Mekelle I	3	3	3-	3	3-	3-
McNair	3	3	3	3	3	3+

*The scale described by Stakman *et al*. (1962) with ITs ; 1, 2 considered resistant and 3, 4 considered susceptible. Negative (-) = smaller uredinia than the normal size and + larger than the normal uredinia.

Of the total 39 bread wheat varieties evaluated; 23, 22, 15, 14, 14 and 11 were resistant to RRTTF, TRTTF, TTTTF, TTKSK, TKTTF and JRCQC races, respectively. Infection type coding resistance was ranging from (; - flecks) to (2+) were regarded as resistant whereby the frequent infection type mostly displayed was ";1" on the primary leaves of the seedling. On the other hand; 28, 25, 25, 24, 17 and 16 varieties were susceptible to JRCQC, TKTTF, TTKSK, TTTTF, TRTTF and RRTTF races, respectively.

Moreover, high frequency of 58.97% and 56.41% of bread wheat varieties were resistance to RRTTF and TRTTF races, respectively, with low infection types ranging from (flecks) to (2+). The high frequency of susceptibility was 71.79% for JRCQC followed by TTKSK and TKTTF which made 64.10% of the evaluated varieties susceptible (Figure 11).

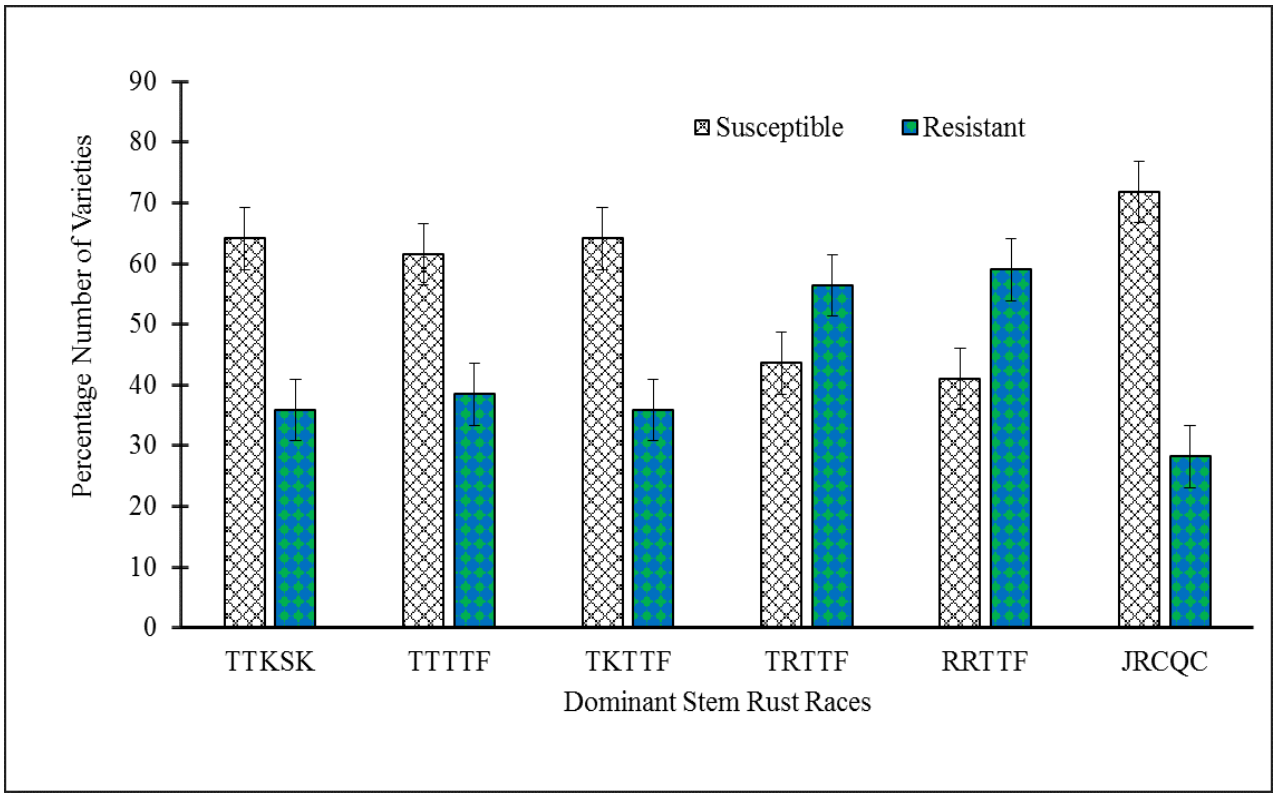

Figure 11: Percentage of 39 bread wheat (*Triticum aestivum*) varieties exhibiting a resistant and susceptible reaction to dominant stem rust races

In Ethiopia devising a breeding strategy to develop stem rust-resistant wheat varieties is valuable and will definitely contribute to the stability of wheat production in the country. Several efforts were made towards resistant cultivars development in the country and many bread wheat cultivars with various levels of rust resistance were released for production. However, most of the released bread wheat varieties succumb to stem rust soon after their release due to either introduction of exotic races or evolvement of new local races and changes in environmental factors (Singh *et al*., 2015; Alemu and Fininsa, 2016).

According to Ambika and Meenakshi (2018), Changing temperature and rainfall patterns have encouraged the emergence of new stem rust races that overcome the currently resistant and popularly grown wheat varieties remain at constant stake of losing their resistance to it. In this study, none of the tested varieties were immune to stem rust infection. Out of the tested bread wheat varieties, some varieties recorded low infection types (fleck (;) to 2+). These could be due to these varieties carry effective race-specific or seedling stem rust resistance genes to the virulent races. Seedling stage resistance can be responsible for a large amount of the resistance to a particular race of a pathogen in their action and effective through all plant growth stages, it functions against certain stem rust races or biotypes but not against others (Babiker *et al*., 2009; Sheikh *et al*., 2017).

Seedling resistance can be very powerful and can sometimes offer the plant near immunity against a specific race of the pathogen. This is the reason that seedling type of resistance has been used for years and is frequently very successful. However, in almost all cases the pathogen overcome effectiveness of the genes because of once a seedling resistance gene is discovered it is often deployed over a broad area, which exposes the gene to incredible amounts of inoculum (Hulbert & Pumphrey, 2014). Once a pathogen overcomes a seedling gene, use of the gene often becomes futile (Keane, 2012).

There was no obvious way to predict the durability of seedling resistance genes, only time will tell if they are long-lasting. Since this type of resistance is race-specific and in most cases only one seedling gene is used, the pathogen will eventually overcome it. This is because of the ability of the pathogen to change by sexual recombination and mutation (Ayliffe *et al*., 2008; Hulbert & Pumphrey, 2014). Sexual recombination can occur in any areas where the

alternate host (*Berberry holstii)* is present. It is evident that the alternate host (*Berberry holstii)* present in proximity to wheat production areas of Ethiopia and the pathogen is able to complete its life cycle in the country (Woldeab *et al.,* 2016).

Moreover, low infection types scored on some of the varieties evaluated against virulent races could be due to the presence of one or more major stem rust genes. This result is in agreement with Ogutu *et al*. (2017) who reported cultivars that exhibited low infection types at seedling stage could be either due to one or more of the stem rust genes or a combination that had similar infection type pattern towards the races. Major gene resistance/seedling resistance can offer complete protection and significant economic benefits to farmers. Therefore, these varieties can be used as sources of stem rust resistance when the aim of the breeding program is for the major gene (Cheruiyot *et al.*, 2015).

However, stem rust resistance at the seedling stage may not be indicative of the reaction at the adult plant stage because some genes are effective only at specific growth stages (Ogutu *et al*., 2017) and cultivars that exhibited high infection type may display minor gene resistance at adult plant stage. In the absence of all stage resistance gene means major gene resistance, varieties only with adult plant resistance will still be susceptible to stem rust at the seedling stage (Cheruiyot *et al.*, 2015).

Therefore, combining seedling resistance with adult plant resistance in the field will provide valuable indications to select resistant varieties. The recent experience in Ethiopia emphasizes the need for genes with broader resistance or for combinations of resistance genes that can confer a broader and more durable resistance (Zhang *et al*., 2017). Hence, this study investigated that, from the tested bread wheat varieties at seedling stage against six virulent stem rust, seven of them showed infection type fleckof (;) to 2 which was regarded as resistant. Those are Honqolo, Huluka, ETBW-9017, ETBW-9042, Dilfiker, Wabe, and Millennium (Table 16). Despite these efforts, those resistant varieties may have the potential and were used as a source of resistant in the seedling stage.

5. SUMMARY AND CONCLUSION

Wheat (*Triticum* spp.) is a stable food crop for billions of peoples worldwide. It is an excellent sources of nutrition and the most important cereal crop cultivated in Ethiopia. However, wheat stem rust pathogen have hindered and seriously threatening the production since the domestication of the crop. Its susceptibility to this pathogen poses a constant threat to sustainable production and hence food security in the study area.

During the assessment, wheat stem rust was prevalent in all assessed zones of the region at variable levels and its intensity varied from slight to complete infection in wheat fields. The mean prevalence of the pathogen was 85.55% in Southern, 62.22% in Eastern and 53.33% in Southeast zones. The overall mean prevalence of stem rust was 70.53% across all assessed zones of Tigray region. The highest rates of disease incidence 78.67%, 66.50% and 47.00% were recorded in Kilte Awulaelo, Raya Azebo and Ofla districts, respectively with the corresponding severities of 43.67%, 35%, and 20.67%.

The overall mean incidence and severity of stem rust was 41.88% and 21.36%, respectively. The results indicated that the present distribution of the disease is remarkably on increasing trend, possibly associated with the evolution of new pathogen races, extensive cultivation of the susceptible varieties and the current climate change (warmer temperature and humid conditions). Moreover, the study confirmed that stem rust is a shifty, changing and constantly evolving enemy which remains a major challenge to wheat production in the region due to the variability of virulence pattern in the pathogen population, the evolution of new races and an exponential reproduction capacity of the pathogen.

Forty-seven stem rust isolates were analyzed on 20 stem rust differentials and resulted in the identification of six races namely; TTTTF, TKTTF, TRTTF, TTRTF, RRTTF and TKPTF. TKTTF was a common race detected from all districts of the three surveyed zones and TTTTF was detected from all districts except Saesia Tsaedaemba district. TTRTF race was detected for the first time in Ethiopia and it has high virulence spectrum that makes 17 (85%) stem rust differential resistance gene non-effective. The study confirmed the presence of high virulence spectrum and high variable populations among the six identified stem rust races.

The highly virulent race TTTTF was detected with a higher frequency of 53.19% of the races identified and virulent to 90% of the stem rust resistance genes. TKTTF was the second most virulent race with 38.30% frequency and showed 85% virulence to stem rust resistance genes. The study showed that the majority of the resistance genes were ineffective against most of the races identified. The stem rust resistance gene Sr24 and Sr31 were the only effective gene that showed resistance to all identified races. Hence, the stem rust resistance gene Sr24 and Sr31 can be used as sources of resistance in the wheat breeding program. Therefore, it is mandatory to monitor the virulence composition and dynamics in the stem rust population and utilize currently effective stem rust resistance genes in the wheat improvement program.

Six dominant races; TTTTF, TTKSK, TKTTF, TRTTF, RRTTF and JRCQC were inoculated on 39 bread wheat varieties at seedling stage in the greenhouse to evaluate their reaction responses. The results showed no complete resistance (zero infection type) observed among the bread wheat varieties evaluated at seedling stage as all varieties allowed lesion formation by the pathogen. Of the 39 tested varieties, however, Honqolo, Huluka, ETBW-9017, ETBW-9042, Dilfiker, Wabe, and Millennium showed resistance reactions (IT = fleck (;) to 2) to all virulent races. These resistance reactions implying the presence of seedling resistance genes towards all virulent races while Danda'a, Kakaba, Kubsa, Shehan, Mekelle I, Mekelle II, Mekelle III, Mekelle IV and ETBW-7638 showed susceptible reactions (3- to 3+).

In conclusion, there is a need to develop a coordinated network on the assessment of wheat stem rust research program along with a regular evaluation of commercial wheat varieties. Based on the findings of the resistant varieties at seedling stage obtained from this study, it is recommended for further utilization in wheat improvement programs and effective genes Sr24 and Sr31 can be useful for developing resistant varieties for the currently available races in the country. Therefore, the best strategy for achieving long-lasting resistance involves the use of several different stem rust resistance genes with complementary race specificity (major gene resistance) in combination with non-specific (minor gene resistance). Thus, further research on adult stage resistance should be conducted to come up with a full package of recommendation on those varieties against the dominant races in the country.

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7. APPENDICES

Appendix 1: A questionnaire for assessment of wheat stem rust in major wheat growing zones of Tigray region

1. Region _____
2. Zone _____
3. District (Woreda) _____
4. Kebele (PA) _____
5. Name of the farmer _____
6. Weather condition of the area _____
7. Altitudinal zone a. Low ☐ b. Mid ☐ c. High ☐
8. Estimated area cultivation _____
9. Variety used for production a. Local variety ☐ b. Improved variety ☐
10. Source of seed

    a. Own seed local b. Improved varieties c. Other

11. Major disease of wheat in the area

    a. Stem Rust c. Yellow Rust e. Smut

    b. Leaf Rust d. Head blight f. Leaf blotch

12. Major insect pest _____
13. Type of cropping system _____
14. Disease management practices (measure taken by farmers to control the disease)

    1. Present ☐ 2. Absent ☐

15. Describe the disease management practice in the study area

_____

_____

16. Weed management: Good ☐ Fair ☐ Bad ☐

Appendix table 1: Nested ANOVA table for the intensity of wheat stem rust in major wheat growing zones of Tigray region

Source of variation	Degree of freedom	Mean square	
		Disease Incidence	Disease Severity
Model	94	7407.05**	2269.46**
Zone	2	8678.88**	1701.90**
District (Zone)	4	55346.88**	18050.89**
PA (Zone*District)	12	5694.09**	1948.88**
Error	380	28.82	29.66

Appendix table 2: ANOVA table for the intensity of wheat stem rust across altitude, growth stage and weed management in the zones of Tigray region

Source of variation	Degree of freedom	Mean square	
		Disease Incidence	Disease Severity
Variety	13	3020.82**	845.03*
Altitude	1	5555.11**	3235.17**
Growth Stage	3	2574.98**	518.84*
Weed management	2	26376.69**	5701.15**
Error	75	487.32	180.29

Appendix table 3: Intensity of wheat stem rust with weed infestation level

Weed infestation level	Number of farms	Incidence (%)		Severity (%)	
		Range	Mean	Range	Mean
Good (rare, scattered)	48	0-100	19.69[c]	0-90	10.52[b]
Fair (medium)	23	0-100	37.17[b]	0-50	18.26[b]
Bad (heavy)	24	0-100	90.63[a]	0-70	45.21[a]
LSD (0.05)			13.26		8.06
CV %			5.40		3.81

*Means with the same letter(s) within the column are not significantly different at $p<0.05$.

Appendix table 4: Average monthly temperature ranges over five months in 2017 main cropping season in the zones of Tigray region

		Temperature (°C)					Annual
Zones	Districts	July	Aug	Sept	Oct	Nov	
Southern	Ofla	12.38-23.14	12.12-23.00	11.23-22.80	10.25-22.72	9.69-22.1	10.95-23.31
	Raya Azebo	19.42-31.18	17.35-29.80	16.49-30.15	15.75-29.75	13.50-29.63	15.33-30.30
	Enda Mehoni	15.00-24.32	13.47-22.84	11.33-23.59	9.72-22.02	7.23-21.36	10.25-22.93
Eastern	Kilte Awulaelo	14.51-27.61	15.12-25.72	13.10-28.56	11.25-27.27	9.47-25.67	11.86-27.86
	Ganta Afeshum	11.74-23.43	10.74-22.26	7.60-24.19	6.24-23.97	4.90-23.00	8.37-24.21
	S/Tsaedaemba	12.83-23.67	13.15-22.28	12.30-24.49	11.32-24.12	9.15-23.65	11.11-24.42
Southeast	Enderta	14.03-24.72	13.68-23.12	11.84-25.39	11.04-24.82	10.22-23.60	11.78-24.86

Source: Mekelle Meteorological Agency Office, Gebrekristos *et al*. (2016)

Appendix table 5: Total monthly rainfall over five months in 2017 main cropping season

		Rainfall (mm)					
Zones	Districts	July	August	Sept	Oct	Nov	Annual
Southern	Ofla	155.80	336.90	0.00	51.20	0.90	750.60
	Raya Azebo	46.00	297.80	57.70	16.40	2.20	673.40
	Enda Mehoni	96.20	234.10	78.80	16.00	11.00	652.60
Eastern	Kilte Awulaelo	181.10	406.10	12.00	0.00	0.00	689.60
	Ganta Afeshum	60.90	217.50	12.30	16.60	3.90	493.00
	S/Tsaedaemba	127.00	207.30	30	0.60	3.60	540.00
Southeast	Enderta	113.00	208.60	16.40	0.00	0.00	440.50

Source: Mekelle Meteorological Agency Office, Gebrekristos *et al*. (2016)

Appendix table 6: Description of infection types used in classifying the reactions to stem rust on seedling stage

Class	IT	Description of symptom
Immune	0	No sign of infection to the naked eye
Very Resistant	0;	No uredia, but district flakes of varying sizes, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic and necrotic area
Moderately Resistant	2	Small to medium sized uredia, typically in a dark green island surrounded by a chlorotic area
Mesothentic/ heterogeneous	X	A range of infection type from resistant to susceptible scattered randomly on a single leaf caused by a single isolate not a minute
Moderately Susceptible	3	Medium sized uredia, usually surrounded by a light green chlorotic
Susceptible	4	Large uredia with limited amount of chlorotic; may be diamond shaped
	Modified Character	
Lower Uredinia	=	Uredia much smaller than typical and at the lower limit of the IT
Small Uredinia	_	Uredia smaller than normal
Larger Uredinia	+	Uredia larger than normal
Largest Uredinia	++	Uredia much larger than typical and at the upper limit for the IT

*IT = Infection Type

Sources: Stackman *et al*. (1962)

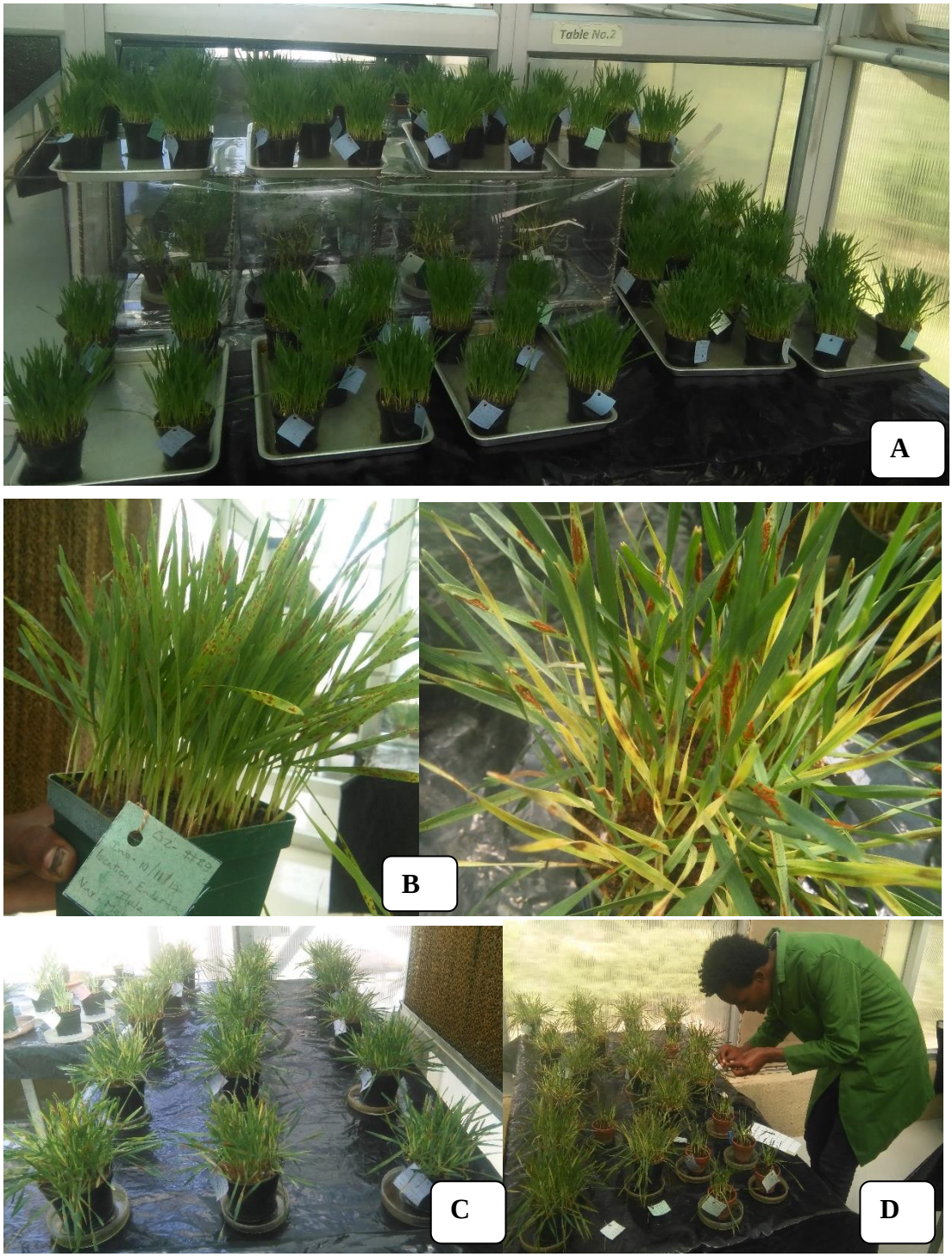
Appendix table 7: Wheat stem rust race analysis differentials scoring sheet

Region_____ Zone _____ District _____ Kebele _____

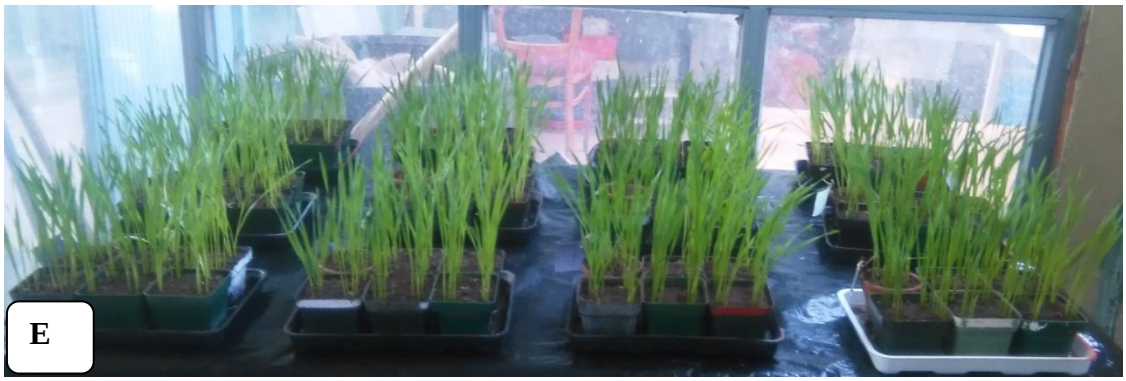
Altitude _____ Inoculation Date _____ Recording date _____

Set	Diff #	Line	Seed Source	Gene	Expected Low IT	Field # Iso-			Field # Iso-		
						Infection Type	H vs L	Name	Infection Type	H vs L	Name
I	1	ISe5-Ra	05 Aberdeen	**5**	**0,0:**						
	2	CnS_ T_mono	04 Aberdeen	**21**	**1,2-**						
	3	Vernstine	05 Aberdeen	**9e**	**:1+**						
	4	ISr7b-Ra	05 Aberdeen	**7b**	**2**						
II	5	ISr11-Ra	05 Aberdeen	**11**	**:2-,2+3-**						
	6	ISr6-Ra	05 Aberdeen	**6**	**0:**						
	7	ISr8a-Ra	05 Aberdeen	**8a**	**2**						
	8	CnSr9g	08 Aberdeen	**9g**	**2-**						
III	9	W2691SrTt-1	10 Aberdeen	**36**	**0,0:,X(LIF)**						
	10	W2691Sr9b	05 Aberdeen	**9b**	**22+**						
	11	BtSr30Wwst	05 Aberdeen	**30**	**2**						
	12	Combination V	05 Aberdeen	**17**	**0,:1**						
IV	13	ISr9a-Ra	05 Aberdeen	**9a**	**2-,23**						
	14	ISr9d-Ra	05 Aberdeen	**9d**	**:2-**						
	15	W2691Sr10	05 Aberdeen	**10**	**:1+**						
	16	CnsSrTmp	08 Aberdeen	**Tmp**	**2-**						
V	17	LeSr24Ag	08 Aberdeen	**24**	**2**						
	18	Sr31/6*LMPG	08 Aberdeen	**31**	**:1+**						
	19	VPM1	08 Aberdeen	**38**	**0:**						
	20	McNair 701	CDL Stock	**McN**	**:1**						
	21	Siouxland	Sr24,Sr31	**-**							
	22	DK42	Sr31								

Appendix figure 1: The overall pictorial descriptive for race analysis of wheat stem rust and bread wheat variety evaluation procedure

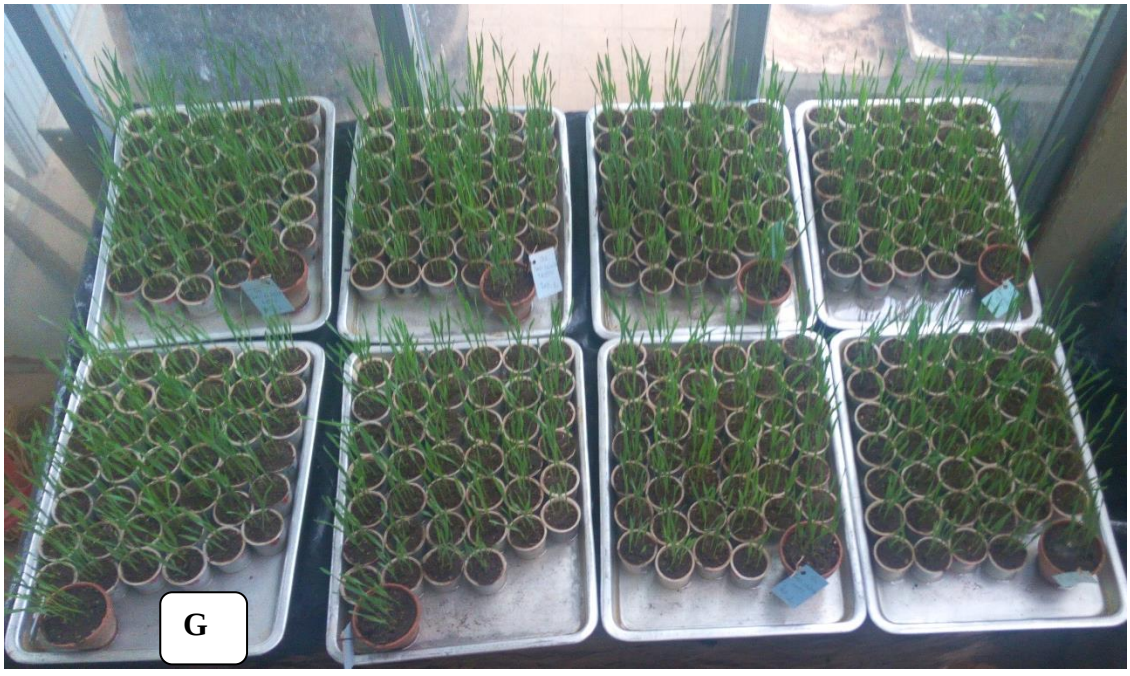


A. Isolates of stem rust inoculated on seedlings of McNair, B. Developed stem rust pustule after 14 days of inoculation, C. Development of a single pustule,
D. Collection of stem rust pustule for preservation from a single pustule developed

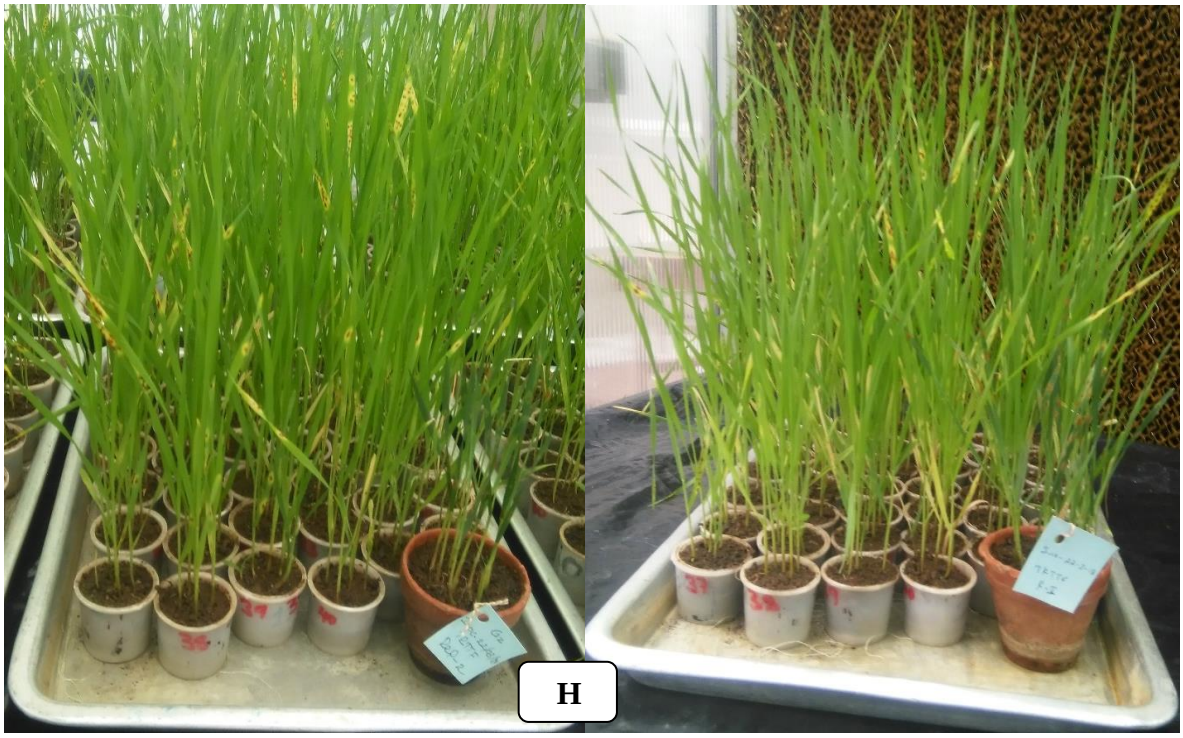


E. Stem rust differential sets inoculated with isolates of stem rust from different locations

F. Germinated bread wheat seeds transplanted on multi-pot trays filled with substrate



G. Inoculated bread wheat varieties aagainst dominant *Puccinia graminis* f.sp. *tritici* races



H**.** Development of dominant *Puccinia graminis* f.sp. *tritici* races on bread wheat varieties 14 days after inoculation