PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF THE WHEAT STEM RUST PATHOGEN *(Puccinia graminis* f.sp. *tritici)* AND VARIETIES' REACTION TO MAJOR STEM RUST RACES IN ETHIOPIA

MSc. THESIS

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A THESIS SUBMITTED TO SCHOOL OF PLANT AND HORTICULTURAL SCIENCE, COLLEGE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES,

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ADVISORS' APPROVAL SHEET

(Submission Sheet-1)

This is to certify that the thesis entitled "**PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF THE WHEAT STEM RUST PATHOGEN *(Puccinia graminis* f.sp. *tritici)* AND VARIETIES' REACTION TO MAJOR STEM RUST RACES IN ETHIOPIA**" submitted in partial fulfillment of the requirements for the degree of Master of Science with specialization in **Crop Protection** of the graduate program of the school of **Plant and Horticultural Sciences,** collage of Agriculture, is a record of original research carried out by **Tsega'ab Tesfaye Wanore** under our supervision, and no part of a thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, we recommend that it be accepted as fulfilling the thesis requirements.

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We, the undersigned, members of the Board of Examiners of the final open defense by **Tsega'ab Tesefaye** have read and evaluated his/her thesis entitled **"PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF THE WHEAT STEM RUST PATHOGEN *(Puccinia graminis* f.sp. *tritici)* AND VARIETIES' REACTION TO MAJOR STEM RUST RACES IN ETHIOPIA"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in plant Science with Specialization in **Crop Protection.**

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DEDICATION

I dedicate this thesis to my mother BEZABISH ATEBO and father, TESFAYE WANORE, who sacrificed everything to ensure my better future, wellbeing and welfare.

STATEMENT OF THE AUTHOR

I declare that this thesis is my independent work and all sources of materials used for this thesis have been duly acknowledged. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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Date of Submission: …….

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LIST OF ABBREVATIONS

ABL	Advanced Breeding Line
APPRC	Ambo Plant Protection Research center
APR	Adult Plant Resistance
BW	Bread Wheat
CDL	Cereal Disease Laboratory
CIMMYT	The International Maize and Wheat Improvement Center
CPC	Crop Protection Compendium
CRD	Completely Randomized Design
CSA	Central Statistical Agency
DRRW	Durable Rust Resistance in Wheat
DW	Durum Wheat
DZARC	Debre Zeit Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
EWRTN	Ethiopian Wheat Rust Trap Nursery
f.sp.	*formae speciales*
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographical Information System
KARC	Kulumsa Agricultural Research Center
Masl	Meter above sea level
McN	Mac Nair
MR	Moderately Resistant

MS	Moderately Susceptible
Pgt	*Puccinia graminis tritici*
RH	Relative Humidity
SNNPRS	Southern Nations, Nationalities and peoples Regional State
Sr	Stem rust
Ug99	Uganda 1999
USDA	The United States Department of Agriculture

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ABSTRACT

Stem rust caused by Puccinia graminis f.sp. *tritici (Pgt) is one of the most important diseases of wheat (Triticum aestivum) across the globe. Because of the sudden changes in stem rust race patterns, commercial varieties are becoming vulnerable globally at large and particularly in Ethiopia. The objectives of this study were to i) assess the distribution of wheat stem rust across selected major wheat producing regions of Ethiopia, ii) study population diversity of pgt races and iii) identify physiological race and the reaction of wheat genotypes to major races of the pathogen. For this purpose, a total of 464 wheat fields were surveyed in eight zones and 37 wheat producing districts of Amhara and Oromia regional states of Ethiopia. Besides, 60 disease samples were collected for race analyses using 20 differentials in a greenhouse trial and molecular diversity of the pathogen was studied using custom PgtSNP 3.0K chip. The reaction of 75 wheat genotypes and 1 universal susceptible check to major races of the pathogen was also evaluated in the greenhouse. Stem rust prevalence in the surveyed areas ranged from 0 to 100%, while disease incidences and severity varied between 0 and 25% and 0 and 11.5%, respectively. Greenhouse trials involving 20 differential cultivars resulted in the identification of three Pgt races (TKTTF, TTKSK and TTRTF) among the collected stem rust samples. Of these, TTRTF has not been previously reported in Ethiopia and hence, this is the first report of TTRTF in Ethiopia. Race TKTTF, also known as "Digalu race", was the most frequent (75% frequency) of all the races of Pgt. The remaining two races TTKSK (Ug-99) and TTRTF were detected with frequencies of 4.16 and 22.91%, respectively. Eighty five percent of the stem rust resistance genes (Sr5, Sr21, Sr9e, Sr7b, Sr6 Sr8a, Sr9g, Sr9b, Sr17, Sr9a, Sr9d, Sr10, Sr38 and McN) were ineffective to all the races detected and 30% of the resistance genes were found to be effective to one or more of the races identified. However, in the present study only the differential host carrying Sr24 was effective to all the races identified in the study areas. The presence of the three Pgt races was also confirmed by molecular study, which categorized the isolates into three clades as I, IVB and IIIB for TTKSK, TKTTF and TTRTF, respectively. The resistance screening trial revelaed that five durum wheat genotypes namely Bichena, Tob-6, Assasa, Kokate and DW/NVT-LMA and six bread wheat genotypes namely, Sulla, Hidase, Wane, 36ESWY, ETBW 8459 and 36ESWYT were resistant against all the races at the seedling stage. These resistant genotypes can be used in wheat improvement programs after being checked under field condition for their adult stage resistance.*

Keywords: Differentials, Genotype, Phenotype, Race analysis, Wheat rust,

1. INTRODUCTION

1.1. Background and Justification

Wheat (*Triticum* spp.) is the most widely grown crop worldwide and is the crucial component for the global food security. Wheat provides one-fifth of the total caloric intake for the world population and is produced on an area of over 200 million hectares worldwide (Wang *et al*., 2015). It is cultivated on 15.4% of the arable land, accounting for around 30% of global grain production and 44% of cereals used as food (FAO, 2017a).

Although the crop is most successful between the latitudes of 30° and 60°N and 27° and 40°S, wheat can be grown beyond these limits, from within the Arctic circle to higher elevations near the equator. Negash and Heinrich (2013) have suggested that wheat can be cultivated at elevations ranging from sea level to 3,000 meters above sea level (m.a.s.l.). Although wheat grows on a wide range of soils, neutral to slightly acidic soils are most suitable for its production. Wheat has become successful because of its adaptability and high yield potential and also its gluten protein fraction, which confers the viscoelastic properties that allow dough to be processed into bread, pasta, noodles and other food products. Minerals, vitamins, essential amino acids, beneficial phytochemicals and dietary fiber components can also be obtained from wheat (Shewry, 2009).

Bread wheat (*Triticum aestivum L.*) and durum wheat (*Triticum turgidum* var. *durum* Desf.) are the two principal commercial types of wheat, of which bread wheat covers majority of the world's wheat area, where as durum wheat is not widely distributed and represents only 5% of the total wheat crop (Roberto, 2015). A rapid increase in global wheat production has taken place during the last five decades, mainly due to increased productivity (FAO, 2017b). Bread wheat is one of the major crops cultivated in the highlands of Ethiopia.

The country is the largest wheat producer in Sub-Saharan Africa (FAO, 2017b). During the last 14 years, the area covered by wheat has increased from 0.77 million ha in 1997 to 1.7 million ha in 2016, and the crop ranked fourth in land coverage and total production after tef, maize and sorghum (CSA, 2016/17). Both bread and durum wheat are grown in Ethiopia and about 87% is grown during the main growing season (meher). The major wheat producing areas of Ethiopia include Oromia, Amhara, SNNPRs and Tigray regions, and 99% of wheat comes from these regions (Netsanet, 2014). The highlands of Ethiopia are known for wheat production and 75% the total production comes from Arsi, Bale and Shewa regions. Small amount of wheat is produced in the rest of the northern and southern regions (CVRI, 2013).

Ethiopia is a country, where small scale wheat production is prominent, allowing it to meet more than 70% of the demand from domestic production (Shiferaw *et al*., 2011). According to CSA (2016/17), in Ethiopia wheat is produced on 1.7 million hectares of land with annual average productivity of about 2.6 metric tons/ha, which is by far below from the world's average yield of about 3.3 tones/ha. These statistics indicate the critical importance of improving the productivity and production of wheat through generation and development of improved technologies in order to promote broad-based economic growth and poverty reduction in Ethiopia. Different constraints play a role in reducing the productivity of wheat in Ethiopia. Among these constraints, low soil fertility, weeds, types of varieties grown, pests and diseases are the major ones. Socio-economic constraints such as, unavailability of improved inputs, seasonal labor shortages, land shortages and low price were the major ones (Netsanet *et al*., 2017). Recently, agro climatological constraints such as warm temperature is becoming a problem for wheat production in Ethiopia. This emphasizes that attempts to identify areas with high production should focus on strategies to overcome heat stress and on searching for resistance to wheat diseases related with warmer environments (DRRW, 2010).

Abiyot *et al*., (2014) reported that serious wheat diseases like rusts are the key factors for wheat yield loss in Ethiopia. Stem rust, which is also known as black rust, is a disease of wheat caused by the fungus *Puccinia graminis* f. sp. *tritici* (Pgt). It is one of the most devastating diseases in Ethiopia causing up to complete destruction of wheat crops over wide areas during epidemic years (Teklay *et al.,* 2012). Humid condition and relatively warm temperature are favorable for the disease. Stem rust is mostly severe at a later stage of a crop development (Fassil, 2008). Different types of wheat stem rust pathogen races can exist in different pathogenicity and aggressiveness level. Stalkman and Piemeisal (1917) have described such physiological specialization of a pathogen. According to Singh and Rajaram (2006), mutation or recombination between different wheat stem rust races are believed to serve as sources of new races. Ethiopian highlands are sources of many new virulent races of wheat stem rust pathogen, which are also the most aggressive ones in the world (Endale *et al.*, 2016). After the identification of Ug99 race group of *Pgt*, which had been the most devastating and serious race in east Africa, Digalu race that has caused epidemics in Ethiopia emerged as a threat and forced the wheat variety "Digalu" out of production in 2013/14 (Olivera *et al*., 2015). The continuous mono cropping of popular, major gene protected varieties by Ethiopian wheat growing farmers favored epidemics to occur in the last decades. For instance, varieties like Enkoy in 1993/94 in Oromia region (Arsi and Bale) and in 2013/14 and 2014/15 in SNNPR became vulnerable and contributed to stem rust disease epidemics (Olivera *et al*., 2015).

1.2. The Research Gap

Wheat stem rust is among the major threats of wheat production causing up to 100% yield losses during epidemic years (Hodson, 2015; Abiyot *et al*., 2014). East Africa is considered to be a hot–spot for stem rust and for the emergence of new and virulent races (Singh *et al*., 2006).

Abiyot *et al.,* (2014) reported that most of the previously identified races were virulent on most of the varieties grown in the country. Wheat stem rust can be effectively controlled by growing resistant varieties as genetic resistance is the safest and best control strategy especially for resource-poor farmers (DRRW, 2010). However, the development of wheat rust resistant varieties requires knowledge of the virulence diversity, race distribution and identification of resistance genes that are effective against the virulent races. Virulence surveys and pathotype analysis are important for studying the evolution of new races and forecasting the virulence shifts in a population. Pathotype analysis is used to monitor the origin, occurrence and spread of new pathotypes; to understand how new pathotypes develop; to determine the degree and range of pathogenic variation in particular regions; to monitor the resistances used in commercial cultivars and advanced breeding lines; to confirm suspected pathogenicity changes from field observations and to obtain new or relevant pathotypes for use in breeding programs. Even though the virulence surveys and pathotype analysis are mandatory, frequent and successive pathogenicity survey were not conducted in the study areas and previous phenotypic and genotypic studies are insufficient to have a comprehensive and up to date understanding of the pathogen diversity, which is key for resistance breeding. Therefore, this research was proposed with the following objectives:

1.3. Objectives

- To assess the distribution of wheat stem rust disease across selected major wheat producing regions of Ethiopia
- To identify the physiological race(s) of stem rust *Puccinia graminis* f.sp. *tritici* and elucidate the genotypic diversity of races in the study areas.
- To evaluate the seedling resistance of advanced breeding lines and recently released wheat genotypes against the major wheat stem rust races

2. LITERATURE REVIEW

2.1. Wheat Stem Rust (*Puccinia graminis* f. sp. *tritici) (Pgt*)

Rusts are the major diseases of wheat since no other wheat disease could result in greater loss over large area in a given year (Endale *et al*., 2016). Rusts can cause up to 60-100 % loss (Park *et al.*, 2007). Stem rust is the most feared disease of wheat and it is mainly found on the stems but also on leaves, sheaths, glumes and seeds. The early stages of the disease are characterized by raised, long and narrow, orange-red pustules on stems and leaves of susceptible cultivars (Marsalis and Goldberg, 2006). The pustules are about 6mm in length and they burst early, exposing a brown powder called urediniospore and are surrounded by epidermal fringes (Singh, 1998). Spores of stem rust arriving as late as one month before harvest can turn a previously healthy crop into a tangled mass of stems, which produces little to no grain (Singh *et al.*, 2008). The specific characteristics of stem rust fungi that could help it to survive or persist include a capacity to produce a large number of spores, which can be wind-disseminated over long distances and infect wheat under favorable environmental conditions and the ability to change genetically, thereby producing new races with increased aggressiveness on resistant wheat cultivars (Singh *et al*., 2008).

A narrow genetic base for stem rust resistance in most of the wheat producing areas of the world played a great role for the new virulent races like Ug99 (TTKSK) to be emerged. Race TTKSK commonly referred to as "Ug99" was 1st reported in Uganda in 1999 and has since then spread to Kenya, Ethiopia, Sudan, Yemen, Tanzania, Syria and Iran (Jin *et al*., 2008; Singh *et al*., 2008; Patpour *et al*., 2016). Several races related to Ug99 have also been reported in South Africa and Zimbabwe (Singh *et al*., 2015; Newcomb *et al*., 2016). The broad and unique combination of virulence found in the Ug99 race group, including Sr31 virulence, has led to estimates that 90% of the wheat cultivars grown globally are susceptible to the new races and

this may cause severe yield loss if the new races spread further to major production regions, such as South Asia, where it has not yet been reported (Singh *et al*., 2011). Resistant varieties have been used to combat stem rust pathogen (Jin *et al*., 2007). The re-emergence of a new virulent race TTKSK (Ug99) in Eastern Africa region (Pretorius *et al*., 2000), and the subsequent detection of its many variants in Kenya has rendered important commercial varieties susceptible (Jin *et al*., 2008). Currently, seven genetically related races have been detected in the Ug99 race groups which have spread across 13 countries (Singh *et al*., 2015). However, the Ug99 race group is not the only current stem rust threat. Recent epidemics in Ethiopia have been caused by non Ug99 race such as TKTTF (Olivera *et al*., 2015).

2.2. Distribution of Wheat Stem Rust in Ethiopia

Stem rust is one of the most important diseases of wheat in Ethiopia. The damage caused by wheat stem rust can be more serious than any other cereal disease. It can destroy thousands of hectares of a healthy crop with a high yield potential in less than a month (Endale *et al*., 2016). Hexaploid cogmmon bread wheat (*Triticum aestivum*), Tetraploid durum wheat (*Triticum turgidum var. durum*), Barley (*Hordeum vulgare*), Triticale *(×Triticosecale Wittm*) and wheat progenitors are primary hosts of the stem rust fungus (Roelfs *et al*., 1992). The source of primary inoculum of stem rust that infects the new wheat crop in the season differs depending on the region in which the crop is grown. The two basic sources of inoculum for cereal hosts are the Urediospores and Aeciospores, the uredospore originates from infected volunteer plants grown in the fields or roadside and or from long distance transport within or outsides the epidemiological zones where as aeciospore originates from the Barberry plants. (Roelfs, 1985). In Ethiopia, the stem rust disease was known to be an important and widely distributed disease as early as 1930 (Tanner *et al*., 1996). Severe epidemics occurred throughout the country in 1975 and 1976.

In 1977, moderate epidemics had occurred in Arsi, Shewa, Gondar and Gojjam regions (Temesgen *et al*.,1995). Loban (1988) described that National surveys conducted in Arsi and Shewa in 1986/87 and 1987/88 revealed the occurrence of stem and leaf rust on majority (>60%) of fields and the disease was highly prevalent in Debrezeit, Arsi and West Shewa zones in 1988. According to Shank (1994), the continuous mono cropping of varieties like Enkoy and Lakech in 1993/94 in Oromia region (Arsi and Bale) resulted in yield losses of 65-100% and a 1998 epidemic attacked the high yielding variety Kubsa and Shina, released in 1999 for north western Ethiopia. These varieties were forced out of production because of stem rust epidemics in 2001. Since then there have been no severe stem rust epidemics. But during the 2013/14 cropping season, the disease caused 100% yield losses in cultivar Digalu (a reportedly resistant variety to stem rust) in SNNPR and on the highlands of Oromia region (Olivera *et al*., 2015). The outbreak was recorded in high altitude areas (>2200 m.a.s.l) although stem rust mainly occurred in the low altitude areas of 1800 m.a.s.l, extending rust incidence to low, medium and high altitudes production areas. Therefore, the highlands of Oromia, SNNP, Tigray, and Amhara regions are potential areas where wheat is predominantly cultivated and areas with frequent epidemic occurrence in the country (Endale *et al*., 2016).

2.3. The Biology of wheat Stem rust pathogen *Puccinia graminis* f. sp. *tritici*

The fungus *Pgt* belongs to the division Eukaryota, kingdom Fungi, phylum Basidiomycota, Class *Urediomyctes*, Order *Uridinales*, Family *Pucciniacceae* and Genus *Puccinia* (CPC, 2003). It is heteroecious and heterothallic in its life cycle. The life cycle involves sexual and asexual stages. The sexual cycle needs the alternate hosts *Berberis* species or less commonly *Mahonia* species. The fungus has five distinct spore stages. These are uredinial, telial, basidial, pycnidial, and aecial stages (Fig. 1).

The pycndial and aecidial stages occur on alternate hosts for the completion of life cycle of the pathogen. Basidiospores and Pycnidiospores are haploid while the others are diakaryotic although teliospores over season in diploid form (Singh, 2009). During sexual cycle, the pathogen over-winters as a teliospore. The sexual cycle can produce a large amount of inoculum in the form of aeciospores, which initiates local epidemics, and then the resulting production of urediospores can cause regional epidemics (Roelfs, 1982). Teliospores remain dormant until the next season and up on germination they immediately enter into the uni- nucleated and haploid basidiospore stage (Alexopoulos *et al.,* 1996). Basidiospores can cause infection on the alternate host but not on wheat. On the young leaves of Barberry, the resulting infections produce specialized structure known as pycnia, which plays an essential role in sexual stage of the pathogen (Kurt, 2001). The fertilization of pycniospores of different mating types result in the formation of dikaryotic aeciospores successively in chains which infect wheat but not barberry (Singh, 2009). The stem rust pathogen reproduces asexually by Urediospores and survives the winter on volunteer cereal plants or on other gramineous hosts. The Urediospores again are converted into teliospores at the end of the season and repeating the cycle (Singh, 2009).

2.4. Epidemiology of Wheat Stem Rust

Eastern Africa has always been considered as a hot spot and is playing a major role for the emergence of new virulent races of wheat stem rust pathogen there by contributing a vital part in relation to the pathogen epidemiology. This is because of geographic location and climatic conditions of the region, and the continuous cultivation of wheat throughout the year providing green-bridge for inoculum survival (Singh, 2006). Rusts are able to spread over long distances. Studies revealed that the long distance spread of *Pgt* urediospores from Australia to New Zealand across 2000 to 5000 Km distance of ocean (Mc Ewan, 1969).

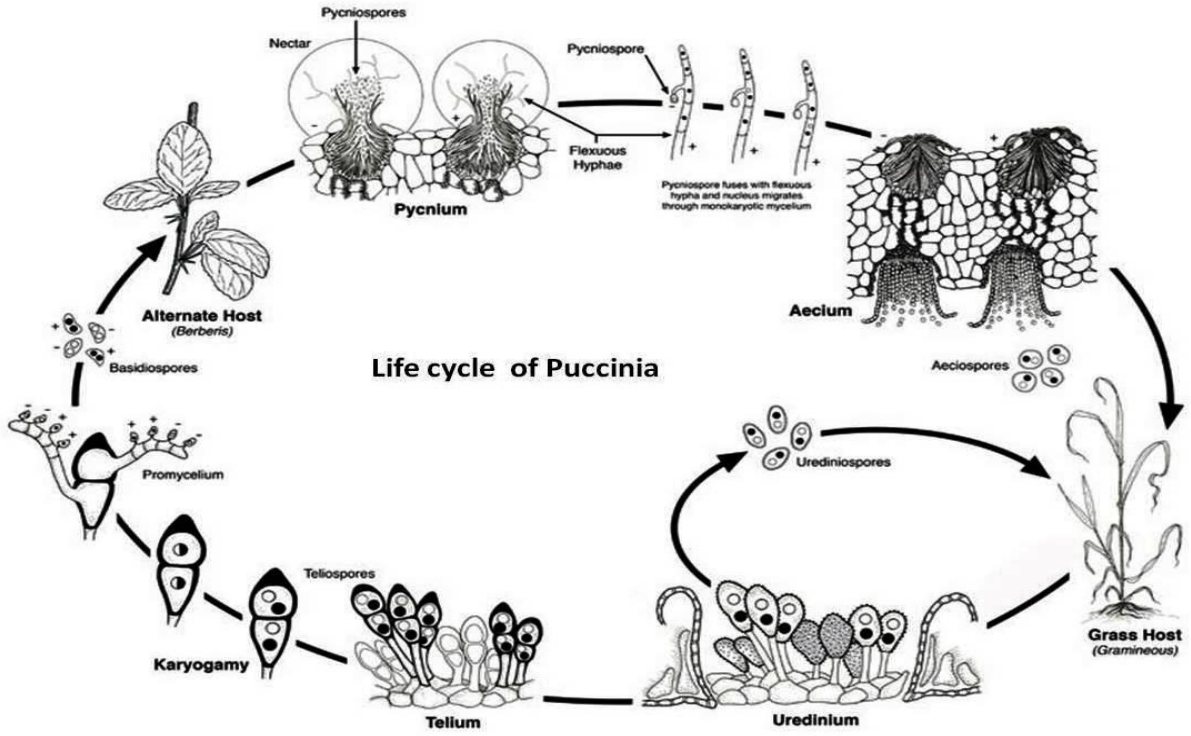


Figure 1. The life cycle of *Puccinia graminis* f. sp *tritici.*

Stalkman *et al*., (1917) demonstrated that in North America there was stem rust pathogen movement from its over-wintering sources in Southern Texas to fields in Dakota, Minnesota and across the Canadian border. Studies in the USA showed spore movement from the south west to north east (Roelfs,1982).

The minimum, optimum and maximum temperatures for spore germination are 2°, 15-24° and, 30°C, respectively, and for sporulation, 5°, 30° and 40°C, respectively, (Jin *et al*., 2008). Stem rust differs from leaf rust in requiring a longer dew period (six to eight hours). In addition, many penetration pegs fail to develop from the appressorium unless stimulated by at least 10 000 lux of light for a four-hour period while the plant slowly dries after the dew period. Maximum infection is obtained with 8 to 12 hours of dew at 18°C followed by 10 000+ lux of light while the dew slowly dries and the temperature rises to 30°C (Pretorius *et al*., 2000). Stem rust uredinia occur on both leaf and stem surfaces as well as on the leaf sheaths, spikes, glumes,

awns and even grains and uredinium can produce 10,000 urediospores per day (Nuttonson *et al*., 1955). Urediospores are long lived and are resistant to atmospheric conditions if their moisture content is moderate (20-30%) and can be carried long distance by the wind (Singh *et al*., 2003).

2.4.1. Primary Hosts

Stem rust is generally limited to *Triticum* species although naturally infected plants of *Secale* cereale, *Hordeum vulgaris*, *H. jubatum*, *H. pusillum*, *Elymus junceus* occur. Other *formae specialis* of *Puccinia graminis* attack many cereals and related grasses and many species are susceptible to more than one formae specialis. Wheat, barley, triticale and a few related species are the primary hosts for *Pgt.* However, the closely related pathogen, *Puccinia graminis* f. sp. *secalis*, is virulent on most barley and some wheat (Jin, 2011). In Ethiopia, Barberry plants were confirmed to be found in 19 localities, of which, 12 are in north Shewa zone, three in south Wello zone and four in south Tigray zone. The shrub was locally called by its by local name known as "Zinkila" in North Shewa and "Yeset aff" in Wello zones. The shrubs grow at altitudes ranging between 2488 to 2979 m.a.s.l. The lowest altitude was in south Wello, Desse zuria zone and the peak was in north Shewa zone. The majority of the locations had altitudes above 2800 m, while a few had below 2600 m and these locations are in south Tigray zone. The locations where Barberry shrubs grow have light dark/black soil color with black rocky stones. The plants grow in patches on hilly areas. Either wheat or barley or both crops as well as grass weeds grow around this alternate host (Getaneh *et al*., 2016).

2.4.2. Alternate hosts

The alternate host for *Puccinia graminis* f.sp. *tritici* is *Berberis vulgaris*. Because of its upright, bushy growth with many sharp thorns, it made an excellent hedge along field borders. Many species of *Barberry*, *Mahonia* and *Mahoberberis* are susceptible to *Pgt.*

The *Barberry* spp. are considered as major sources of new combinations of genes for virulence and aggressiveness in the pathogen. Barberry was a major source of stem rust inoculum in Denmark and North America (Roelfs, 1982). The success of reducing stem rust epidemics in northern Europe and North America following the removal of barberry near wheat fields has probably led to an over emphasis of the role of this alternate host in generating annual epidemics elsewhere. However, in Ethiopia, no Barberry eradication program has been implemented so far.

2.5. Disease Management

2.5.1. Resistant varieties

Disease resistance is the ability of the host plant to hinder a pathogen or a disease-causing agent. Generally, there are two types of resistance for stem rust namely, race–specific (Vertical) and race-nonspecific resistance (Horizontal or General). Race-specific resistance is controlled by a few genes having major effects and race-nonspecific resistance is governed by several genes each with minor effects (Van der Plank, 1984). Developing resistance within cultivars is the main and desirable method of disease control. It is the most effective, least expensive and environmentally safe (Pretorious *et al*., 2000). Genetics of resistance and pathogenicity in host–pathogen relationship is the key element in the development of resistant cultivar. For the last 50 years, researchers have been working on identifying sources of resistance and developing resistant varieties (Singh *et al*., 2008). Kolmer (2009) described that stem rust resistant genes were transferred from tetraploid sources, durum and emmer, to hexaploid wheat; this mechanism had served effectively for many years. The varieties named “Thatcher” and “Hope” were among a few varieties developed through this process, they possess adult plant resistance gene known as Sr2 (Singh *et al.*, 2008). In most cases, resistance to stem rust is mainly race specific. Varieties with race specific resistance became susceptible with in short period of time

after being released. This is because virulent races of pathogen found in a fungus population break the resistant gene (Ayliffe *et al*., 2008). Therefore, for the long term, breeders should think of accumulating many additive genes that can help to express strong quantitative resistance to stem rust (Herrera-Fossel *et al*., 2007).

2.5.1.1. All-stage resistance

All-stage resistance, sometimes called seedling resistance, is controlled by one or a few genes that confer highly effective resistance during the entire life of the wheat plant (Hare and MacIntosh, 1979). This type of resistance is relatively easy to transfer by conventional breeding techniques, and resistant lines are relatively easy to identify using artificial inoculation tests at the seedling stage. There are two main disadvantages with all-stage resistance. First, all-stage resistance genes that originate from alien species (wild wheat) are often associated with genes that have negative effects (linkage drag) on yield, quality, susceptibility to other diseases, and other undesirable agronomic characteristics. Second, all-stage resistance genes usually are race specific, i.e. they are effective against some races of the pathogen but ineffective against others. Widespread use of these genes can put selection pressure on the pathogen population to change in favor of mutants that render the specific resistance genes ineffective. Currently, it is not possible to accurately predict the durability of these resistance genes. Some all-stage genes are overcome by new races before being deployed in commercial cultivars. However, other genes like Sr31, the all-stage resistance gene overcome by Ug99, have been durable and provided useful resistance globally for several decades (Belayneh and Emebet, 2005).

2.5.1.2. Adult plant resistance (APR)

Cultivars with APR gene generally have characteristics of slow rusting and have intermediate levels of stem rust in the field. Compared to a very susceptible cultivar with no APR, the level

of APR can be low (susceptible), moderate (moderately susceptible) or high (moderately to fully resistant). Moderate and high levels of APR would be useful wherever wheat is prone to stem rust epidemics, and even low levels of APR may be sufficient to prevent losses in areas that are marginal for stem rust development. Identification of APR is more time-consuming than all-stage resistance because wheat plants need to be grown to maturity, often in multiple environments, and determining the level of resistance is more difficult. Molecular markers can be used to "tag" APR genes, which would allow plants containing APR genes to be identified as seedlings. However, finding molecular markers that are closely linked to APR genes is more difficult than for all-stage resistance genes because APR is more difficult to identify (Roelfs *et al*., 1992).

2.5.1.3. Gene combinations or gene pyramids

Combining resistance genes, or gene pyramiding, should provide more durable resistance than single genes based on the hypothesis that mutations to virulence in the pathogen are rare and independent events (Schaffer and Roelfs, 1986). If the probability of mutation to virulence for one gene is one in a million, then the probability of mutation for two genes is one in a million times one in a million, or one in a trillion. Thus, as more genes are added to the pyramid, the probability of simultaneous mutations to virulence becomes extremely unlikely. Gene pyramiding depends on having many genes available for use by breeders that are effective and which have not already been overcome by the pathogen. Increasing the availability of molecular markers for stem rust resistance genes will facilitate gene pyramiding efforts (Liu *et al*., 2010; Olson *et al*., 2010).

2.5.2. Cultivar mixtures and multiline cultivars

Growing a mixture of wheat cultivars that have different seedling resistance genes can both hinder an epidemic within a field and delay the time it takes for the pathogen to overcome a new resistance gene. Norman Borlaug developed this methodology for the control of wheat stem rust in the 1950s and demonstrated that it can be very effective (CIMMYT, 1988).

2.5.3. Gene deployment

Regional deployment of resistance genes, or gene deployment, is the geographic distribution of resistance genes among different wheat-producing regions so that each will have effective resistance genes that are not used in other regions. The idea behind this strategy is that inoculums (spores) produced in one region will not be adapted to or "match" the resistance genes used in another region and therefore, be unable to infect the cultivars present, thus minimizing spread of the disease (Frey *et al*., 1977).

2.5.4. Cultural control

Cultural methods for stem rust disease control include mainly, early planting and growing early maturing cultivars and known as escape mechanism. Although not a form of disease resistance, escape occurs when a susceptible plant is not infected as the result of factors such as random chance and maturity date. In most areas, stem rust develops late in the growing season when conditions become conducive for disease development; altering planting date is another mechanism by which disease escape may occur. Therefore, cultivars that mature early may escape severe stem rust. These helps in reducing the time of the cultivars to be exposed to the pathogen and hence, reduce the yield loss (Netsanet, 2014). Using early maturing wheat varieties reduce the risk of stem rust epidemics and reduce the number of urediniospores that can contribute to the spread of the disease to other areas. The disease development is directly

related with the date of disease onset (Hamilton and Stalkman, 1967). Clearing green bridges and eradication of alternate hosts that can carry inoculum from one field to the next is the other important cultural practices. Alternate host eradication plays a great role in altering the sexual cycle of the fungus. Removing green bridges, including volunteer cereals or wild accessory hosts, is essential to reduce the carryover of *Pgt* to the next season wheat crop (Schumann and Leonard, 2000).

2.5.5. Chemical control

Currently, the large majority of bread wheat varieties are susceptible to either stem rust and/or yellow rust. The development of cultivars with effective resistance to emerging races may take 10 years or more. Consequently, chemical control will likely be an important tool if the new races of stem rust arrive prior to widespread deployment of new resistant cultivars. 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 (Wanyera *et al.*, 2009). A number of fungicides are highly effective against stem rust and have been used to successfully manage the disease. Products containing a triazole fungicide for instance triadimefon (Baylaton and Noble) 25% WP at 0.5 l/ha, and propiconazole (Tilt and Bumper) 250 EC at 0.5 l/ha were generally the most effective in limiting disease development and/or reducing disease severity and differences in efficacy were noted among individual triazole fungicides, indicating that not all provide the same degree of control (Wanyera *et al.*, 2009; Bekele, 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 wheat stem rust but early detection and rapid action are crucial, so integrated management strategies in the long run (FAO, 2017a).

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

The wheat stem rust survey was carried out in major wheat growing areas of Amhara and Oromia regional states of Ethiopia (Fig. 2). The study areas in Amhara region are located within 10.06172 to 11.96160 N latitude and 36.51448 to 39.64268 E longitude, and had altitude ranges of 1804 to 3450 m.a.s.l. In Oromia regional state the disease survey was carried out at altitude range from 1704 to 3334 m.a.s.l with latitude of 6.55038 to 8.93800 N and longitude of 37.16835 to 39.28679 E (Table 6). The stem rust race analysis and evaluation of advanced breeding lines were done in Ambo Plant Protection Research Center (APPRC) located at 8.261833 N latitude and 35.32694 E longitudes at an altitude of 2147 m.a.s.l.

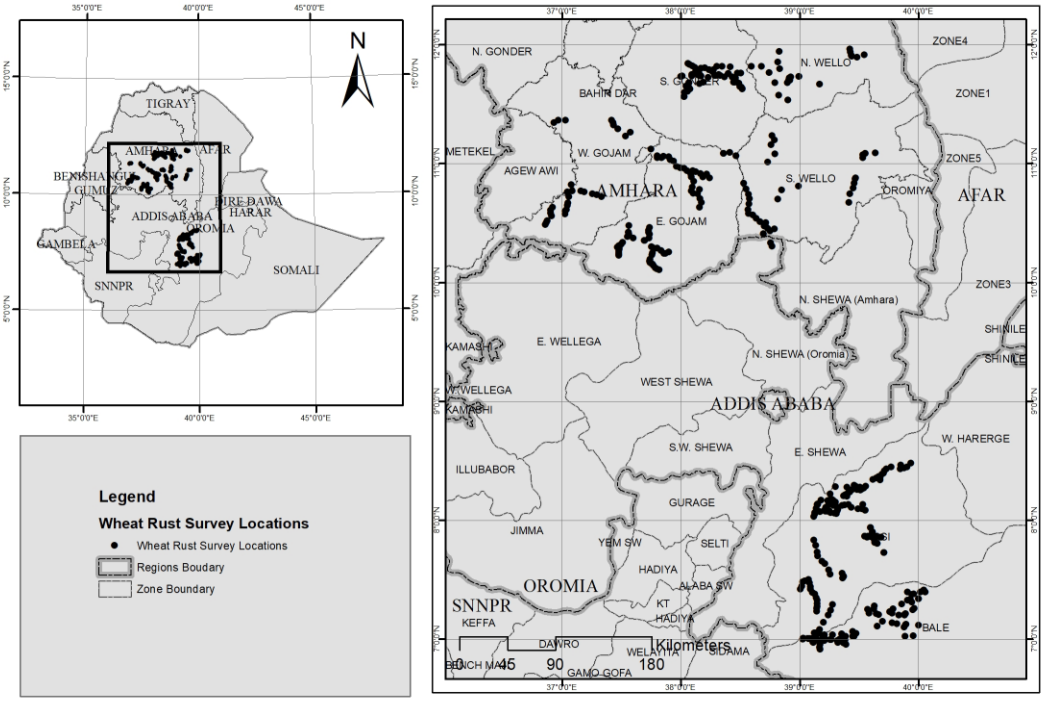


Figure 2. Map showing wheat stem rust survey area.

3.2. Field Survey

The surveys of wheat stem rust were carried out in selected main wheat growing districts of Oromia and Amhara regional states from 3 October to 30 December, 2017. A total of 464 wheat fields in eight zones and 37 districts were surveyed in both Oromia and Amhara regional states. Three zones namely, Bale, East Arsi and West Arsi from Oromia and five zones namely, East Gojjam, West Gojjam, North Wello, South Wello and South Gondar from Amhara were assessed for wheat stem rust intensity. The surveyed areas were selected based on wheat area coverage and the importance of the disease in the area. Farmers' fields, state farms and experimental stations growing wheat were surveyed at 5-10 km interval. Out of 464 wheat fields surveyed, 224 fields were from Oromia and 240 were from Amhara regional states. In each field, the survey was carried out during flowering to grain filling stages (Table 1). Stem rust assessment was carried out along the two diagonals of the field in an "X" pattern at five points of the field and samples were taken using 0.5m x 0.5m (0.25 m^2) quadrant.

For each field, wheat plants within a quadrate were counted and recorded as diseased/infected and healthy /non- infected, and assessed for stem rust severity using the modified Cobb's scale (Peterson *et al.* (1948). The stem rust intensity was calculated as follows.

$$\textbf{Prevalence (\%)} = \frac{\text{No of fields affected}}{\text{Total fields assessed}} \times 100$$

$$\textbf{Disease Incidence: DI (\%)} = \frac{\text{Number of diseased plants in a quadrant}}{\text{Total No. of plants in a quadrant}} \times 100$$

$$\textbf{Disease severity: DS (\%)} = \frac{\text{Area of a plant tissue affected}}{\text{Total area of a plant part assessed}} \times 100$$

The modified Cobb's scale (Fig. 3) was used for disease severity assessment, which for instance states that for 100% disease severity, the actual leaf/stem area covered by rust pustule is 37% (Jin, 2007).

Ten plants from a single quadrate and five quadrants per were used for the estimation of disease severity from a single wheat field and average severity was used for analysis.

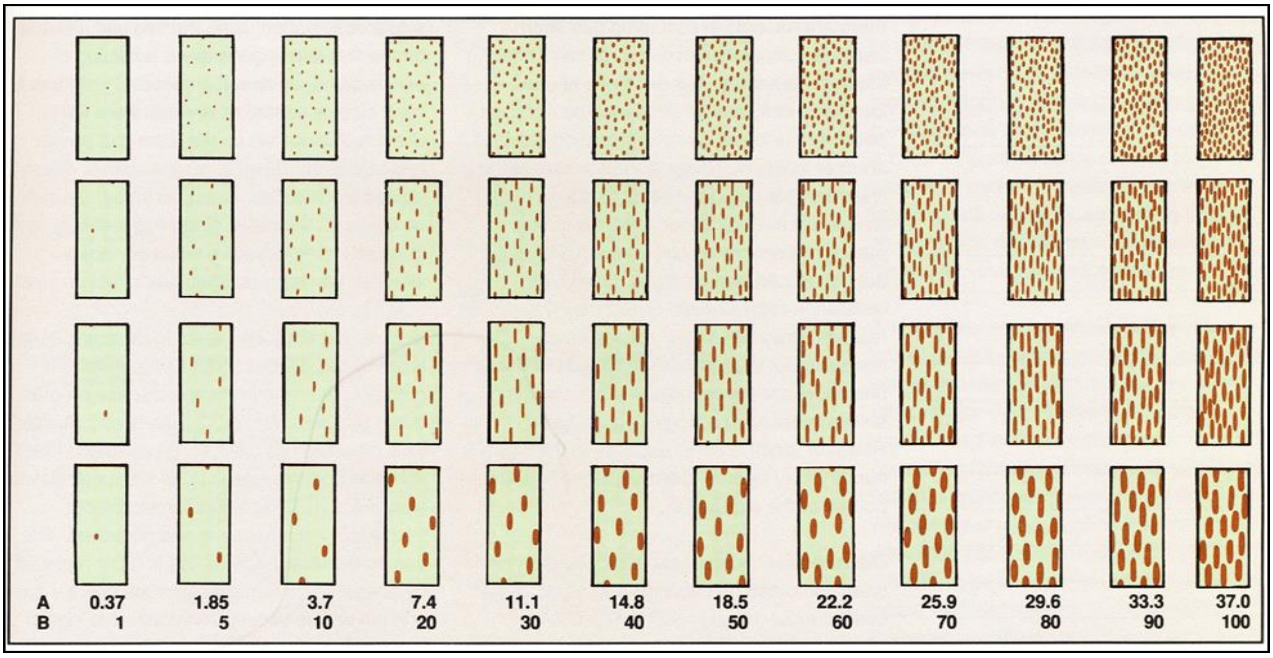


Figure 3. Modified cob scale Peterson *et al.*, 1948 (The actual percentage occupied by rust Uredinia (A) through considering combination of pustules size and rust severities (B).

Table 1. Wheat Varieties assessed during the Survey.

No	Varieties Name	Released year	Type of wheat	Remark
1	Akbir	1983	Bread Wheat	
2	Alem tena		Durum Wheat	
3	Batu	1984	Bread Wheat	
4	Danda'a	2010	Bread Wheat	
5	Dashen	1984	Bread Wheat	
6	Digalu	2005	Bread Wheat	
7	Enkoy	1974	Bread Wheat	
8	ETBW 7690		Bread Wheat	
9	Galama	1995	Bread Wheat	
10	Gundile		Bread Wheat	The farmers call it "Nebar zer"
11	Hidase	2012	Bread Wheat	
12	Hoggana	2011	Bread Wheat	
13	Hulluka	2010	Bread Wheat	
14	Improved Unknown		Bread Wheat	The farmers call it "Mirt Zer"
15	K6295-4A	1980	Bread Wheat	
16	Kakaba	2010	Bread Wheat	
17	Kubsa	1995	Bread Wheat	
18	Kursht		Bread Wheat	The farmers call it " Nebar zer"
19	Lemu	2016	Bread Wheat	
20	Local Unknown		Bread Wheat	
21	Maddaa Walabu	2000	Bread Wheat	
22	Mixed		Breads Wheat	Farmers mix more than two varieties
23	Ogolcho	2012	Bread Wheat	
24	Pavon 76	1982	Bread Wheat	
25	PBW 343	1996	Bread Wheat	
26	Shorima	2011	Bread Wheat	
27	Sorra		Bread Wheat	
28	Tesfaye		Durum Wheat	
29	TT14 (Logaw Shibo)		Triticale	
30	Ude	2002	Bread Wheat	
31	Wane	2016	Bread Wheat	

Source: Crop Variety Register Issue No.16

3.3. Physiological Race Analysis

3.3.1. Collection of Wheat Stem Rust Samples

Four to six stems of samples infected with Pgt were collected from a farmer's field and experimental stations following the international stem rust live sampling protocol (Park *et al*., 2013). A total of 60 samples were collected from the study areas.

The core tissue and sheaths of the stem samples were removed and cut into a small piece of 5-10 cm. The core sheaths infected with stem rust were then put in a glycine paper bag. This method helped the samples to be easily dried and avoids spore germination before the actual race analysis work in the green house. The samples collected in the glycine paper bags were labeled with sample number, sample code, date of collection, name of collector, location, GPS data, and the name of cultivar and other relevant information such as incidence and severity of the disease.

3.3.1.1. Isolation and Multiplication of Single-Pustules

The spores from the collected infected stem samples were harvested using motorized collector known as vacuubrand diaphragm pump atomizer, which is manufactured in Germany by vacuubrand technology manufacturer located at Berlin, Germay and multiplied on universally rust susceptible variety" Mc Nair701" that does not carry stem rust resistance genes (Roelfs *et al*., 1992). Five seedlings of this variety were raised in 8 cm diameter pots that were filled with steam sterilized soil, sand and manure mixture at 2:1:1 proportion. Green house inoculation was carried out using the methods and procedures developed by Stalkman (1962). Seven-day old seedlings were inoculated with 3-5 mg *Pgt* spores in 1ml of solTrol-130 vacuubrand diaphragm pump atomizer. Distilled water was used to moisten plants and the moistened plants were then placed in an incubation chamber for 14 hrs in the dark at temperature of 18-24^{0}C. After incubation, seedlings were exposed to florescent light for 4 hrs to provide favorable condition for infection and seedlings were allowed to dry / remove their dew for about 1-2 hrs. After this, the seedlings were transferred from dew chamber to glass compartments of the green house where conditions were regulated as 12hrs photoperiod, temperature of 18- 25^{0}C and RH of 60-70%. The remaining rust spores which were not used for inoculation were stored at -80^{0}C and

used to replace samples which fail to produce infection on the universally susceptible line Mc Nair701.

Table 2. List of stem rust differential lines used in the study, their corresponding Sr genes and Origin/pedigree.

No.	Differential host	*Sr* genes	Origin/pedigree
1	Lc*Sr*24Ag	24	Little Club/Agent (Cl 13523)
2	W2691*Sr*Tt-1	36	Cl12632 T.timopheevii
3	I*Sr*7b-Ra	7b	Hope/Chinesen Spring
4	I*Sr*8a-Ra	8a	Rieti/Wilhelmina//Akagomughi
5	CnSSrTmp	Tmp	Triumph 64(C/13679)/Chinese Spring
6	*Sr*31 (Benno)/6*LMPG	31	Kavkaz
7	CnS-T-mono-deriv	21	Einkorn Cl 2433
8	Trident	38	Spear *4/VPM(p1519303)
9	I*Sr*9a-Ra	9a	Red Egyptian/Chinese spring
10	I*Sr*9d-Ra	9d	Hope/Chinese spring
11	Combination VII	17	Esp 518/9
12	I*Sr*5-Ra	5	Thatcher/Chinese Spring
13	I*Sr*6-Ra	6	Red Egyptian/Chinese spring
14	W2691*Sr*9b	9b	Kenya 117A
15	Vernsteine	9e	Little club//3*Gabo/2*
16	W2691*Sr*10	10	Marquis*4/Egypt NA95 /2/2*W2691
17	Bt*Sr*30Wst	30	Festival/Uruguay C10837
18	Cns*Sr*9g	9g	Selection from Kubanka (C11516)
19	I*Sr*11-Ra	11	Kenya C6402/pusa4/Dundee
20	McNair701	McN	C115288

Seed source: Ambo Plant Protection Research Center

After seven to ten days of inoculation (when the flecks/symptoms were clearly visible), leaves containing a single fleck that has produced single pustule were selected from the base of the plant and the remaining seedlings within the pot were removed using scissors. Only 2-3 leaves per pot which contain single pustule were left. Each pot with single pustule was covered with cellophane bags (145 x 235mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004). After two weeks (14-15 days) following inoculation (when the mono pustule was developed), each mono pustule was collected using spore collector known as vacuubrand diaphragm pump atomizer, and stored in a separate gelatin

capsule. Seven-day old seedlings of a universally susceptible variety "Mc Nair " were inoculated with one gelatin capsule of *Pgt* spore suspension prepared by mixing 3-5 mg urediospores with 1ml of mineral oil (solTrol-130). After inoculation, the seedlings were placed in a humid chamber in dark condition at 18-22^{0}C for 14 hrs and then in the light for 4 hrs, after which they were transferred to a greenhouse with temperature of 18 - 25^{0}C and RH of 60 - 70% 14-15 days After inoculation, the multiplied spores of each mono pustule were collected in separate test tubes and stored at -80^{0}C till they were inoculated on the standard differential sets. This procedure was repeated till sufficient number of spores were produced in order to inoculate the set of stem rust differential hosts (Table 3).

3.3.1.2. Inoculation of Wheat Stem Rust on the Differential Hosts

Five seeds, from each of 20 stem rust differential hosts with known resistance genes (Table 2) and one susceptible variety “Mc Nair” were grown in 3cm diameter pots separately in Greenhouse. The susceptible variety “Mc Nair” (with no *Sr gene*) was used to determine the viability of spores inoculated on the differential hosts. Twenty pots were used for each of the 20 differential lines, for one differential line one pot was used. A total of 960 pots were planted with 20 differential lines for analyzing 48 samples collected from the study area. The experiment was arranged in CRD with three replications and a single pustule isolate spores (3mg of spores per 1ml light weight mineral oil (SolTrol-130) sprayed/ inoculated onto seven-day-old seedlings using vacuum pump inoculator known as vacuubrand diaphragm pump atomizer. After inoculation, plants were moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 14 hrs in the dark at 18-24^{O}C and were supplemented 4 hrs of additional cool white fluorescent tubes. After removing from the dew chamber, plants were placed in separate glass compartments in a glass house where air condition was maintained by an instrument known as control panel, which works by

sensing the glass room's temperature, moisture, light and humidity. It is this control panel which control the environment under the green house (facilitate infection to occur) in the compartments after inoculation. The temperature inside in each compartment were adjusted to 18-24^{o}C (Minimum -Maximum) and the humidity is maintained through water pad (fixed with external water line) and installed inside the compartment, light sensors functions during prolonged cloudy days (Especially in June, July and August) numerous fluorescent tubes were used and arranged directly above plants to supplement the compartment with natural day. Detailed procedure of greenhouse procedures (incubation, inoculation and greenhouse conditions) were similar as described in the previous section 3.3.1.1. The data on stem rust infection types were scored 14 days after inoculation using the 0-4 scale of Stalkman *et al* (1962) (Table 5). Infection types were grouped into two, where, Low (resistance) or incompatibility (infection phenotype 0, 0; (fleck), 1, 2, and 2+) and High (susceptible) or compatibility (infection phenotype, 3, 3+ and 4) (Fig. 4).

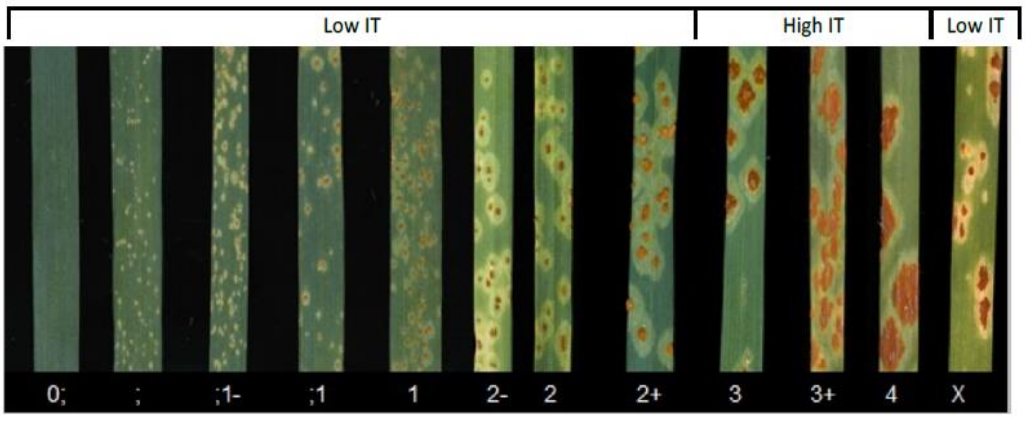


Figure 4. Pictorial description of infection types used in classifying the reactions of stem rust on leaves of wheat seedlings (Stalkman *et al*., 1962).

3.3.1.3 Designation of Races

The Northern American nomenclature system was used and the designation of the races were done by grouping differential lines each with a different single resistance gene, which can distinguish races by their qualitative differences in reactions to different pathogen isolates. The

differentials were put in the following order (Table 3). **Set I**: *Sr5, Sr21, Sr9e, Sr7b,* **Set II:** *Sr11, Sr6, Sr8a, Sr9g,* **Set III:** *Sr36, Sr9b, Sr30, Sr17,* Set **IV:** *Sr9a, Sr9d, Sr10, SrTmp,* **Set V:** *Sr24 Sr31, Sr38, Sr McN*. The five-letter race code was given to each isolate based on the reaction on the differential lines (Fetch & Dunsmore, 2004). For example, low infection types on the four lines in a set is assigned with the letter 'B' while high infection types on the four lines is assigned with letter 'T'. Hence, when an isolate produced low infection type (resistant reaction) on the 20 differential lines, the race was designated with a five-letter race code 'BBBBB'. Similarly, an isolate which produced a high infection type (susceptible reaction) on the 20 wheat differential lines was given a race code 'TTTTT'. When an isolate produced a low infection type on Sr11, Sr24, and Sr31, but a high infection type on the remaining 17 differential lines, the race was designated as TKTTF (Table 3). The experiment was repeated three times and only differential lines that produced similar infection types were considered for the data analysis. When there was infection type 0 (immune reaction), the test was done again to exclude the possibility of disease escape.

Table 3. Code for the 20 differential lines for *Puccinia graminis* f.sp. *tritici.*

Infection phenotype of pathogen and wheat *Pgt* gene					
Pgt-code	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
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

Source: Fetch and Dunsmore, 2004 Low/Resistant infection type (0 to 2+), High/ Susceptible infection type (3- to 4).

3.4. Genetic Characterization of *P. graminis* f.sp. *tritici* isolates

A total of 48 *Puccinia graminis* f.sp. *tritici* infected samples collected from farmers' fields and experimental stations of major wheat growing areas of Oromia and Amhara regional states in 2017 (from September to November) were used for genetic analysis at the United States Department of Agriculture (USDA), Cereal Disease Laboratory (CDL), Minnesota, USA. The 45 isolates were sequenced and genotyped using custom PgtSNP 3.0K chip (Newcomb *et al*., 2016, Olivera *et al*., 2015). The remaining 3 isolates did not yield quality DNA, hence not processed for the confirmation. A Total of 12 reference isolates were used for the isolates genotyped to compare whether they are similar or not (Table 4).

Table 4. List of clades number and reference isolates used for comparison.

No	Clade -Number	Race to be referred	Reference Isolate	Remark
1	Clade – I	TTKSK	04 Ken 156104	Ug-99
2	Clade – I	TTKST	06 Ken 19V-3	Variant race for TTKSK
3	Clade – I	TTTSK	07 Ken 24-4	Variant race for TTKSK
4	Clade – II	JRCQC	13 Eth 28-2	Durum wheat race
5	Clade – II	JRCQC	14 Yem 123-1	Durum wheat race
6	Clade III B	TTRTF	14 Geo 189-1	New race for Ethiopia
7	Clade III B	TTRTF	15 Geo 271-1	New race for Ethiopia
8	Clade IV A-I	TKTTF	13 Eth 18-1	Digalu race
9	Clade IV A-I	TKTTF	13 Ger 02-1	Digalu race
10	Clade IV A-I	TKTTF	14 Eth 128-1	Digalu race
11	Clade IV-B	TKTTF	14 Eth 126-1	Digalu race
12	Clade IV-B	TKTTF	14 Eth 132-2	Digalu race

3.4.1 Stem Rust Sampling for DNA Analysis

A distinct single large pustule was identified and stem immediately above and below pustule was cut using sharp disinfected scissors (Appendix 5). The scissors were disinfected by washing and wiping the scissor with lab soft tissue and discarding wipes, the process was repeated for each sampling for the entire samples. Sheath tissue was cut at a side of pustule and then the core tissue containing a single pustule was removed and placed in a tube filled with 75-80% volume of 80% ethanol. The tube was labeled following the standard naming convention. In the label, two numbers for year, three letters for country code and three numbers for sample code were used based on the standard DNA sample protocol for wheat stem rust sampling (eg.17 ETH 001, 17 ETH 002 etc.). Accordingly, the number “17” stands for the year (2017) samples were collected, the “ETH” stands for the country (Ethiopia) samples were collected and the numbers 001and 002 stands for the sample codes given for sample number one and sample number two, respectively. Scissors were cleaned and disinfected by washing and wiping the scissor with lab soft tissue and discarding wipes after processing between each sampling.

The single pustule in the cryogenic vials with (12mm X 32mm with 9mm screw thread) was then kept inside 70% ethanol for about 7days. The ethanol was then poured off and the tube with a single pustule was air dried for 2 days. Finally, the tubes were sealed and sent to University of Minnesota, U.S Department of Agriculture, Cereal Disease Laboratory for DNA analysis and genotyping (Olivera *et al*., 2015).

3.4.2. DNA extraction and genotyping

DNA was extracted from 25-50 mg purified *Pgt* Uredospore and the DNA isolation was carried out using Omniprep DNA extraction kit (G-Bioscience, St Louis, USA) following the procedures described by the manufacturer (Olivera *et al*., 2015). Samples were genotyped using AB Infinium custom single -nucleotide polymorphism (SNP) chip (PgtSNP 3.0k chip). This chip is an expansion of the original PgtSNP 1.5k chip and increases the coverage from 50 to 98% of the *P. graminis* f.sp. *tritici* assembled genome (Newcomb *et al.,* 2016). The SNP chip assay was performed as described by the manufacturer using 500ng of DNA per sample and each sample was run in duplicate. The quantity and purity of DNA was determined using a Nano drop (model ND-1000) and a custom 1,536 Single Nucleotide Protein (SNP) Golden Gate chip (*Pgt* SNP) was used for genotyping. The extracted genomic DNA was sequenced by Roche GS FLX 454 technology (Upadhyaya *et al*., 2015).

A total of 17 molecular markers have been used and the phylogenetic analysis of the data (races) was performed using DARwin software version 6.0 (Dissimilarity Analysis and Representation for windows) (Perrier, 2010). The sequenced nucleotides were converted into numerical matrices into alternate allele and reference allele for the clustering of the isolates into their respective race group to which they belong to.

3.5. Seedling evaluation of bread and durum wheat genotypes for stem rust resistance using 7 races

A total of 75 wheat genotypes (Appendix 1) and one stem rust universal susceptible check McNair 701 were tested for their major gene response against virulent races in the country. Of these, 40 were durum wheat genotypes (17 advanced breeding lines and 23 released varieties) and the remaining 35 were bread wheat genotypes (9 commercial varieties and 26 advanced breeding lines). Seven virulent wheat stem rust races namely TTKSK (Ug-99), TKTTF (Digalu race), TRTTF, JRCQC, RRTTF, TTTTF and TTRTF were used for screening of the genotypes. These races were identified and maintained at Ambo Plant Protection Research Center from samples collected during previous years and 2017 surveys. Among these, TTRTF was newly detected race in Ethiopia and was not reported in the country in previous years. From the races mentioned above, TTTTF and TKTTF were virulent on more than 80% of the Sr genes in the differential lines and were predominantly distributed in Ethiopia since 2013 (Endale *et al.*, 2016). The spores of prevalent and virulent stem rust race(s) identified from the study areas were multiplied on the universally susceptible variety Mc Nair with no Sr gene following the procedures mentioned in 3.3.1.1 and collected in separate test tubes to inoculate a total of 76 bread and durum wheat cultivars and advanced breeding lines. Five plants per pot were used for the experiment. Pots were arranged in a Completely Randomized Design (CRD) and each genotype within a pot was replicated three times during the greenhouse experiment.

Seven-day-old seedlings (the first leaf is fully expanded and the second leaf is just emerged and beginning to grow), were inoculated with spores (3-5mg) of per 1ml of Soltrol-130 mineral oil suspension) of virulent races following the procedures mentioned earlier in section 3.3.1.1. Data on infection types were recorded 14 days after inoculation according to the host response (Table 5) and pictorial description of infection types used in classifying the reaction of wheat seedlings

to stem rust isolates (Fig 4). The experimental procedures in inoculation and disease assessment described by Jin *et al.* (2007) were followed. Plants were evaluated for their infection types (ITs) 14 days after inoculation using the 0-4 scale according to Stakman *et al*., (1962), where ITs of 0; 1, 2, or X were considered as low ITs and ITs of 3 or 4 considered as high ITs. Lines giving variable reactions between experiments were tested again to confirm the most likely reactions. The lines and varieties were categorized into two, those with susceptible (compatible reaction) and those with seedling resistance. The lines and varieties that were scored with 0, 1, 2, 2^+ were under the category of seedling resistance, while those lines and varieties with scoring values of 3, 3^+, and 4 were under the category of susceptible reaction type.

Table 5. Description of infection types used in classifying the reactions of stem rust on leaves of wheat seedlings.

Class	ITs	Description of symptoms
Immune	0	No sign of infection on the naked eye
Very Resistant		No uredia, but distinct flackes of varying size, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic area
Moderately Resistant	2	Small to medium sized uredia, typically in a dark green island surrounded by a chlorotic area
Mesothetic/Heterogeneous	X	Mixed type of infection on a single leaf
Moderately Susceptible	3	Medium sized Uredia. Usually surrounded by a light green Chlorotic
Susceptible	4	Large uredia with a limited amount of chlorosis: may be

Source: (Stalkman *et al.,* 1962.)

3.6. Data Analysis

Race nomenclature was done using the International system of nomenclature for *Puccinia graminis* f. sp. *tritici* (Roelfs and Martens, 1988) and survey data (prevalence, incidence, severity, means over districts, varieties, altitude range and crop growth stages), data on physiological race analysis and screening of wheat cultivars were analyzed by using the descriptive statistics (Gomez and Gomez 1984). SPSS IBM20 and Arc GIS version 10.5.1 computer software programs were used to compute the mean, statistical analysis and to generate maps.

4. RESULTS AND DISCUSSION

4.1. Distribution of wheat Stem Rust in Selected Major Wheat Growing Districts Ethiopia

Typical stem rust symptoms i.e. (Fig. 5) were evident on wheat crop during the current survey. From 464 fields surveyed, 20 (0.43%), 51 (10.99%), 116 (25%), 244 (52.58%), and 51 (10.99%) of wheat fields were at booting, flowering, milky, dough and maturity stages, respectively. Stem rust was observed in 10 (19.60%), 15 (12.93%), 44 (18.03%) and 3 (5.88%) of fields in flowering, Milky, dough and maturity stages, respectively, however, the stem rust was not recorded at booting growth stage.

Table 6. Varieties, altitude ranges and growth stages of wheat by region, zones and districts.

Zone	Districts	NF	Varieties	Altitude (m.a.s.l.)	Growth stage
Bale	Agarfa	10	Ogolcho, Tesfaye, Danda'a, Improved Unknown, Alemtena	2388 - 3046	FS-DS
	Goba	9	Ogolcho,Hidase, Ude	2356-2745	FS-DS
	Sinana	20	Ogolcho, Mada wallabu, Hulluka, Improved Unknown, Kakaba, Hidase, Danda'a	2370-3062	FS-DS
East Arsi	Arsi robe	32	Improved Unknown, Ogolcho, Danda'a, Hulluka, Hogena, Madda walabu, Shorima.	2372-3105	BS-MS
	Digalu tijo	15	Digalu, Kakaba, Hidase, Improved Unknown, Danda'a	2337-2619	FS-DS
	Hetosa	31	Hidase, Improved Unknown, K 6295-4A, Kubsa, Lemu, Madda walabu, Mixed, Ogolcho.	1714-3334	FS-DS
	Jeju	16	Danda'a, Enkoy, Kubsa, Pavon76,	1913-2554	MiS-DS
	Lemu bilbil	10	Hidase, Improved Unknown, Lemu,	2488-3003	Mi--DS
	Merti	16	Galama, Improved Unknown, Ogolcho, Pavon76, Wane,	1775-3142	FS-MS
	Sire	17	Batu, Hidase, Improved Unknown, K6295-4A, kakaba,Ogolcho	1704-3069	FS-MS

Table 6. Continued…..

West Arsi	Adaba	12	Danda'a, Digalu, Hidase, Hulluka, Improved Unknown, Ogolcho	2348-2568	MiS DS
	Asasa	10	Danda'a, Hidase, Hulluka, Improved Unknown, Kubsa, Ogolcho	2379-2662	MiS-MS
	Dodola	26	Digalu, Hidase, Kubsa, Ogolcho	2365-2693	MiS-DS
East Gojjam	Aneded	8	Danda'a, Improved Unknown	2304-2519	FS-DS
	Basoliben	10	Improved Unknown	2289-2392	FS-MS
	Debre elias	10	Improved Unknown	2186-2246	FS-DS
	Enarj Enawga	7	Improved Unknown	2497-2584	MiS -MS
	Goncha siso	11	Improved Unknown	2506-2739	FS – DS
	Gozamen	9	Danda'a, Improved Unknown	2224-3046	FS – MS
	Hulet eju	10	Improved Unknown, Local Unknown	1804-2495	DS –MS
	Mechakel	5	Danda'a, Digalu, Improved Unknown, Local Unknown	2219-2425	FS – MS
North Wello	Gubalafto	6	Pavon76, PBW343, Kakaba	1860-2974	MiS DS
	Meket	13	Danda'a, Digalu, Improved Unknown, Local Unknown	1968-3037	MiS DS
	Wadla	20	Danda'a, Improved Unknown, Local Unknown, Hidase, Sorra, Kakaba,	1957-3078	FS – DS
South Gondar	Farta	26	Improved Unknown, Triticale	2178 - 3450	FS –MS
	Lay gayint	19	Akbir, Damda'a, Improved Unknown, Triticale	2130 - 3552	FS –MS
	Tach gayint	7	Improved Unknown, Local Unknown	2799 - 3262	FS –MS
South Wello	Borena	6	Danda'a, Local Unknown, Gundile (local)	2424 - 2589	MiS DS
	Dessie Zuria	8	Danda'a, Pavon76	2231 -2938	FS – DS
	Jamma	13	Danda'a, ETBW-7690, Hidase, Kursht (local)	2570 - 2645	MiS DS
	Legambo	7	Danda'a, Kursht (local)	2632 - 2917	MiS DS
	Tenta	8	Danda'a, Local Unknown	2911 - 3282	MiS DS
	WereIlu	8	Danda'a, Improved Unknown, kakaba, Kursht(local), Pavon76	2612 - 2968	FS – DS
West Gojjam	Achefer	3	Improved Unknown, Pavon 76	2685 - 3041	FS –MS
	Burie	7	Improved Unknown	2014 - 2418	FS –MS
	Womberima	7	Improved Unknown	2023 - 2096	DS –MS
	Yilman densa	6	Improved Unknown	2239 - 2383	FS – DS

BS: Booting stage, FS: Flowering stage, MiS: Milking stage, DS: Dough stage, MS: Maturity stage, NF: Number of fields

In addition to the disease parameters (Prevalence, Incidence and Severity), GPS data, environmental conditions, wheat type and variety grown, agronomic practices (sowing dates, fertilizer application and weed status), date of planting and plant growth stage were recorded.

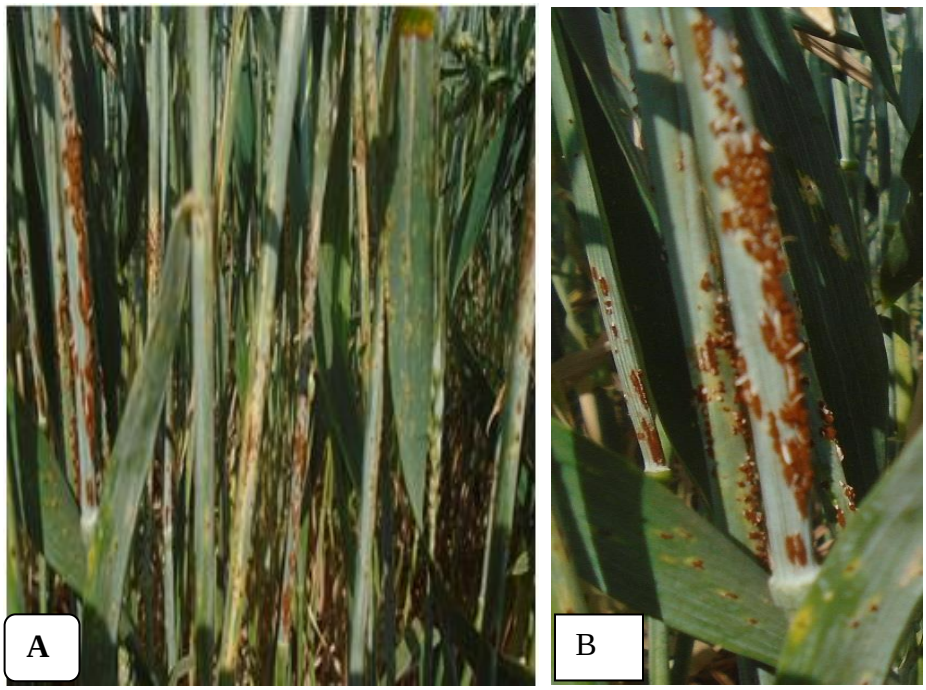


Figure 5. Typical symptoms of stem rust (A and B) observed during survey in the study area.

4.1.1. Intensity of Stem Rust Across Locations

Of the 464 wheat fields surveyed in Oromia and Amhara regions, 15.51% were affected by stem rust disease. The overall mean prevalence of stem rust in the study areas of Oromia and Amhara regions were 18.30 % and 12.91 %, respectively, (Fig. 6), while stem rust incidence averaged on 2.84% and 2.49% in Oromia and Amhara, respectively. The overall mean severity was 0.92% and 1.54 % in Oromia and Amhara regions, respectively.

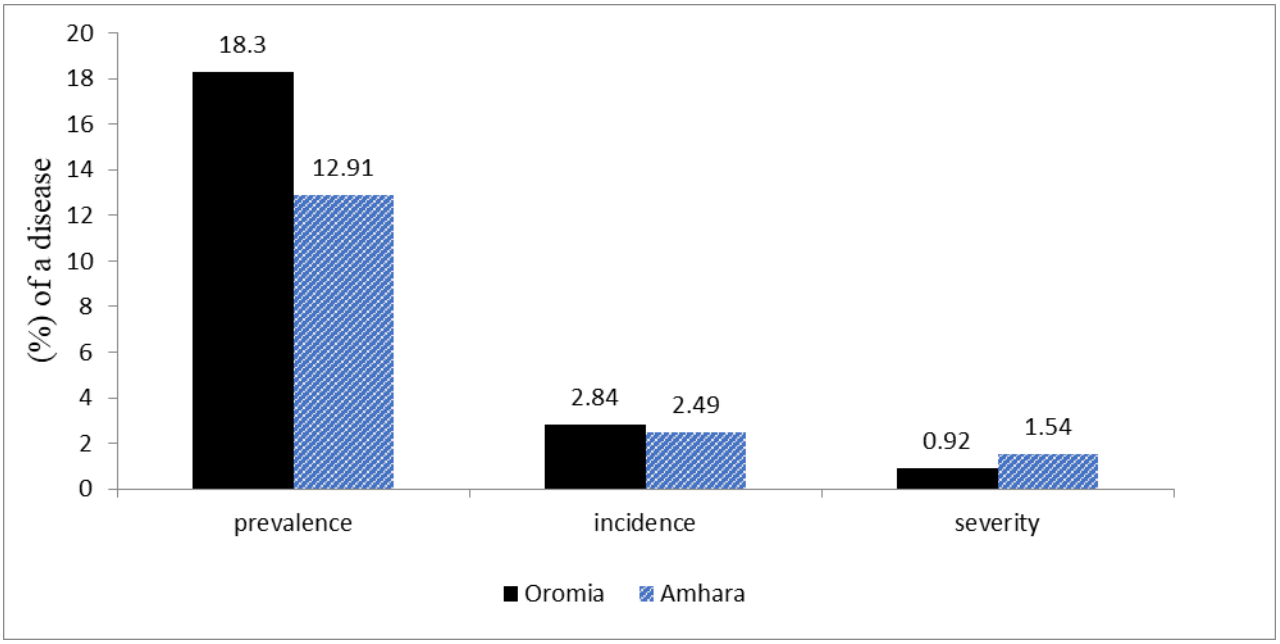


Figure 6. The overall mean prevalence, incidence and severity of Stem rust in the study.

The stem rust prevalence varied from 0 to 100% in surveyed fields and districts of the study areas. The trace (TR), Moderately resistance (MR), Moderately susceptible (Ms) and Susceptible (S) types of reactions were observed in the assessed fields during the study period. Among the surveyed areas in Oromia, the overall mean prevalence of stem rust was the highest in Bale zone (81.7%) followed by West Arsi (9.18 %) and East Arsi (2.27 %) (Table 7). The mean incidence of stem rust in the region varied from 0.26% in East Arsi zone to 14.6% in Bale zone, while the mean severity varied from 0.09% in East Arsi to 4.23% in Bale. Among the surveyed zones of Oromia, the highest disease incidence (14.64%) was recorded at Bale zone. Goba and Agarfa districts had the highest severity of about 7%. On the other hand, stem rust severity was nil in the districts of Digalu tijo, Jeju, Lemubilbilo, Merti and Sire. In Amhara region, the stem rust prevalence varied from 0 to 100%. The highest stem rust prevalence of 100% was recorded in West Gojjam zone at Achefer district, followed by Enarj Enawga districts of East Gojjam which had disease prevalence of 42.85%. Whereas, the lowest stem rust prevalence (0%) was recorded in 10 of the 24 districts across the four different administrative zones (Table 6). The mean incidence of stem rust in the region varied from 0.66% in East

Gojjam zone to 6.52% in south Wello zone. At the district level, the highest stem rust incidence of 25% was recorded from Dessie Zuria district of South Wello zone followed by Jamma district of same zone with 7.69%. The maximum disease severity was scored in South wello zone with the mean value of 2.7%, the Dissie zuria districts of South Wello zone accounted the highest mean severity value of 11.25%. Following South Wello zone, the West Gojjam accounted about 2.48% of mean severity.

In Ethiopia, wheat stem rust surveys have been conducted annually over the past two decades. Although the disease incidence and severity varied from season to season, and from location to location, the stem rust disease was among the major ones that had occurred in the country (Abraham, 2006). The wheat pathology progress report (HARC) from 1998/99 to 2000 and 2003/04 to 2005 clearly showed that the three rusts i.e. yellow, leaf and stem rust were the predominant diseases in the study areas. Recent wheat disease survey in East Arsi, West Arsi and Bale indicated that yellow rust followed by stem rust were found to be the most important diseases (KARC Progress report, 2005). According to KARC progress reports (1989-2005), surveys made in the last two decades indicated that stripe rust (Yellow rust) has been widely distributed in the central highlands of Arsi and Bale. However, the recent disease wheat disease progress reports from Ambo Plant protection Research Center (APPRC, 2016/17) showed that the yellow rust disease has been replaced by stem rust disease in terms of area coverage. Several rust outbreaks were seen in the country especially on major wheat growing districts and zones of Oromia. In very recent years, 2014/15, for instance, continuous outbreaks of stem rust and yellow rust were recorded in Arsi Robe area and Bale zone with devastating losses in majority of the fields (Hodson, 2015).

Table 7. Intensity of wheat stem rust in the study areas in 2017.

Zone	Districts	NFI	Prevalence (%)	Incidence (%)		Severity (%)		Host response
				Range	Mean	Range	Mean	
Bale	Agarfa	10	80	0-100	25.50	0-40	7	Tr-S
	Goba	9	100	1-100	19.56	0-30	7.22	Tr-S
	Sinana	20	65	0-50	7	0-10	1.5	Tr-S
	Subtotal	**39**	**81.66**	**0-100**	**14.64**	**0-40**	**4.23**	**Tr-S**
East Arsi	Arsi Robe	32	6.25	0-5	0.31	0-4	0.19	Mr-Ms
	Digalu Tijo	15	0	0	0	0	0	0
	Hetosa	31	9.67	0-15	0.81	0-3	0.23	Mr-Ms
	Jeju	16	0	0	0	0	0	0
	Lemibilbilo	10	0	0	0	0	0	0
	Merti	16	0	0	0	0	0	0
	Sire	17	0	0	0	0	0	0
	Subtotal	**137**	**2.27**	**0-15**	**0.26**	**0-4**	**0.09**	**Mr-Ms**
West Arsi	Adaba	12	8.33	0-5	0.42	0-4	0.33	Ms
	Asasa	10	0	0	0	0	0	0
	Dodola	26	19.23	0-5	0.96	0-5	0.81	Ms
	Subtotal	**48**	**9.18**	**0-5**	**0.62**	**0-5**	**0.52**	**Ms**
East Gojjam	Aneded	8	12.5	5	0.63	50	6.25	S
	Basoliben	10	0	0	0	0	0	0
	Debre Eliyas	10	0	0	0	0	0	0
	Enrj enawuga	7	42.85	10	3.57	16	5.14	Ms-S
	Goncha siso	11	0	0	0	0	0	0
	Gozamen	9	22.22	5	1.11	5	0.78	Mr-S
	Hulet eju	10	10	1	0.1	1	0.1	Ms
	Mechakel	5	16.66	5	1	4	0.8	Ms
	Subtotal/mean	**70**	**13.02**	**0-10**	**0.8**	**0-16**	**1.63**	**0 -S**
North Wello	Meket	13	15.38	20	2.31	2	0.31	Mr
	Gubalafto	6	0	0	0	0	0	0
	Wadla	20	0	0	0	0	0	0
	Sub Total	**39**	**5.12**	**0-20**	**0.77**	**0-2**	**0.10**	**Mr**
South Gondar	Farta	26	11.53	60	3.27	50	2.15	MR-,S
	Lay Gayint	19	5.26	5	0.26	5	0.26	S
	Tach Gayint	7	0	0	0	0	0	0
	Sub Total	**52**	**5.59**	**0-60**	**1.73**	**0-50**	**1.17**	**Mr-S**

Table 7. Continued…..

South Wello	Borena	6	33.33	20	5	20	3.67	Mr-S
	Dessie Zuria	8	38.88	80	25	30	11.5	TrMs,S
	Jamma	13	7.69	100	7.69	32	2.46	Ms
	Legambo	7	0	0	0	0	0	0
	Tenta	7	0	0	0	0	0	0
	WereIlu	14	14.28	10	1.43	4	0.36	Tr-Ms
	Sub total	**55**	**15.69**	**0-100**	**6.52**	**0-32**	**2.70**	**Tr-S**
West Gojjam	Achefer	3	100	40	25	13	11	Ms-S
	Burie	7	0	0	0	0	0	0
	Wemberima	7	0	0	0	0	0	0
	Yilmandesna	6	33.33	5	1.17	20	4	Ms
	Sub total	**23**	**33.33**	**0-40**	**3.57**	**0-20**	**2.48**	**Ms-S**

Tr: Trace, Ms: Moderately susceptible, Mr: Moderately resistant, S: Susceptible, NFI: Number of Fields Inspected

The Ethiopian Wheat Stem Rust Nursery (EWRTN) 2011-2016 planted at different experimental substations in the Aris and Bale showed that stem rust was one of the dominant wheat diseases that had broken majority of resistance genes and caused the varieties to be out of production (EWRTN, 2011-2016-unpublished data). The findings of the current study did not agree with the national wheat disease progress report of 2015/16 that depict the distribution of stem rust was high in different districts of Arsi and Bale. The present study however revealed that the intensity of stem rust disease was low in Arsi and Bale in 2017 main season when compared with the previous years. The rate of *Puccinia graminis* f. sp *tritici* infection is heavily influenced by cultivar susceptibility, the virulence of the pathogen race as well as favorable 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). The possible reason for decrement of the disease pressure in these areas would be due to the prior intensive application of fungicides (KARC Progress report, 2005). The farmers in Arsi and Bale are familiar with the stem rust pathogen and they know what would happen if they miss to spray the fungicide (Personal communication). The advisory services from the government agricultural offices and research centers had been given to the farmers in the areas following previous years' experience. Therefore, they had sprayed before the sporulation leading to lower disease levels during the current survey period. The other reason would be the weather condition: the stem rust pathogen needs warm temperature and optimum moisture to geminate and sporulate However, during the current survey period, the temperature was very low and moisture was abundant (Personal communication with farmers and DA's). This weather condition, particularly the low temperature and high moisture might have disfavored the development of stem rust. Similar observations were made by (Hollaway, 2011), who reported that the pathogen is favored by humid conditions and temperatures of 15 to 30°C. Netsanet (2014) describes the environmental conditions such as temperature and moisture considerably affect disease expressions and consequently of yield.

Temperature is one of the important environmental variables most often associated with biological responses and it is measured almost universally in studies on plant disease epidemics. Due to changes in temperature and moisture, climate change may alter the host growth stage, development rate and pathogenicity of infectious agents, and also 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).

4.1.2. Intensity of Stem rust across altitude gradient

Out of the total fields surveyed in Oromia region, 20 (8.92%) fields were found at low-altitude (1500-2000 m.a.s.l), 139 (62.05%) mid altitude (2001-2500 m.a.s.l) and 65 (29.01%) were high altitude (2501-3560 m.a.s.l) areas according to Hurni, (1998). No stem rust was observed in all of the 20 fields assessed in the lowlands. However, from 139 wheat fields inspected in the mid-altitude range, stem rust was observed in 28 (20.14%) wheat fields with mean disease incidence and severity of 2.81% and 0.87%, respectively. Of the 65 wheat fields surveyed at high altitude range, the disease occurred in 13 wheat fields (20%) with 3.76 % mean incidence and 1.33 mean severity. In general, the survey results for the region indicated that the prevalence of stem rust increased from zero at lower altitude to 20% at mid and high-altitude ranges. In a similar way, mean incidence and severity of the disease increased with elevation. A total of 240 wheat fields were surveyed for stem rust disease in Amhara region. Among these, 7 (2.91%), 82 (34.16%), and 151 (62.91%) fields were found in low, mid and high-altitude ranges, respectively. Of the seven fields inspected in low altitude range, stem rust was observed in only one field with 14.28 % prevalence, 0.14% incidence and 0.11% severity, whereas, from 82 wheat fields located in mid altitude range, stem rust was observed in 11 fields with 13.41% prevalence, 1.85% mean incidence and 1.54% mean severity. From 151 wheat fields surveyed in high altitude range, stem rust was observed in 19 fields with 12.58% prevalence, 3.56% mean incidence and 1.78 mean severity. Very low mean incidence and severity were scored in lower altitude ranges, however, the maximum mean incidence (2.94%) and mean severity (1.6%) were registered in higher altitude ranges, followed by mid altitude with 1.84 % and 1.54% mean incidence and mean severity, respectively.

In general, the stem rust disease intensity was higher when we go from low altitude to mid altitude and gets maximum at higher altitude in study areas of both Oromia and Amhara

regional states. These results contradict previous report that the stem rust disease was much important on lower to mid altitude range (Alemayehu *et al*., 2015). In Kenya, stem rust had been recorded and known to occur mainly in the low altitude areas of 1800 m.a.s.l (Wanyera *et al*., 2009). Abiyot *et al*., (2014) also reported that the stem rust disease was very important at an altitude below 2300 m.a.s.l. Similarly, Ayele *et al*., (2008), reported that stem rust infection was higher in the altitude ranges of 1600-2500 m.a.s.l. Alemayehu *et al*., (2015) also mentioned that the stem rust intensity had increased from low altitude to mid altitude and decreased at higher altitude. These discrepancies with previous findings show that the stem rust disease has been broadening its probability of occurrence in a higher altitude ranges through times. This might be due to climate change, frequent cultivation of susceptible varieties and emergence of new virulent races. significant cost for control methods.

4.1.3. Intensity of stem rust on different wheat varieties in the study area

In the 464 wheat fields assessed for stem rust disease in both regions, 31 wheat varieties were grown by farmers (Table 1). Among the improved varieties, Ude and Tesfaye were durum wheat, whereas the remaining ones were unknown bread wheat varieties. During this study, the name “Improved Unknown” was given for those varieties that the farmers call “Mirt zer” but they do not know the exact name of the cultivar (Table 8). The varieties Danda’a, Hulluka, Kakaba and Ude have shown trace resistant (Tr) and resistant (R) types of reaction to the pathogen even though the disease was highly prevalent on the varieties. This may probably be due to their relative resistance and/or due to the prior fungicide application before the survey was conducted. The varieties Alemtena, Tesfaye, Hidase and Madda Walabu have shown higher disease level with 100, 100, 31.85 and 25% mean incidence, respectively, and 40, 20, 10.71 and 5% mean severity, respectively, and susceptible (S) type of reaction.

In East Arsi zone, 20 wheat varieties were grown in 137 wheat fields were assessed. The most dominant varieties were Improved unknown, Ogolcho, Hidase, Pavon 76, Kubsa, Danda'a and Digalu, whereas, varieties such as Wane, Shorima, Lemu, Galama, Enkoy, Hogena, Kakaba, K6295-4A and mixed varieties were sown in lower frequency. From these varieties, Hidase and Improved unknown have shown relatively susceptible reaction type with 0.71% and 0.36% mean incidence and 0.35% and 0.23% mean severity, respectively, whereas, the others had zero percent mean incidence and severity. This might be due to the absence of enough inoculum in the area, or the pathogen might be avirulent to the varieties. Danda'a, Digalu, Hidase, Hulluka, Improved unknown, Kubsa and Ogolcho were grown in 48 wheat fields in West Arsi zone. The most dominant ones were Ogolcho, Hidase, Improved unknown, and Kubsa. These varieties exhibited differential reactions to the disease. For instance, the variety Hidase showed relatively susceptible reaction with 2.5 % mean incidence and 2.10% mean severity followed by Ogolcho with 0.23% mean incidence and 0.18 mean severity when compared with the others. However, the remaining varieties displayed resistance reaction with zero percent mean severity. In general, the varieties Danda'a, Hulluka, Kubsa, Digalu and Pavon 76 showed relatively resistant type of reaction when compared with the other varieties, whereas, the varieties such as Hidase, Ogolcho and the durum ones (Tesfaye and Alemtena) showed relatively susceptible reaction to the disease.

Table 8. Intensity of stem rust on different varieties in Oromia region.

Zone	Varieties	NFI	Mean Prevalence (%)	Incidence (%)		Severity (%)		Host response
				Range	Mean	Range	Mean	
	Alemtena	1	100	0	100	0	40	S
Bale	Danda'a	4	75	0 – 30	0.75	0	0.15	Tr
	Hidase	7	100	0	31.85	0-30	10.71	Tr,S
	Hulluka	2	50	0 – 10	0.5	0	0.1	Tr
	Imp.Unkno	4	50	0	12.75	0-10	2.55	Tr,S
	Kakaba	1	100	0 – 10	1	0	0.2	Tr
	Madawalabu	2	50	0 – 10	25	0-10	5	S
	Ogolcho	16	75	0 – 10	2.56	0-10	0.76	Tr,S
	Tesfaye	1	100	0	100	0	20	S
	Ude	1	100	0	1	0	0.2	Tr
	Total	**39**	**80**	**0-30**	**27.54**	**0-30**	**7.96**	**Tr,S**
	Batu	1	0	0	0	0	0	-
	Danda'a	8	0	0	0	0	0	-
	Dashen	1	100	0	15	0	2	Mr
	Digalu	7	0	0	0	0	0	-
	Enkoy	2	0	0	0	0	0	-
	Galama	1	0	0	0	0	0	-
	Hidase	14	13.33	0-5	0.71	0-3	0.36	Mr,MrMs
	Hogena	2	0	0	0	0	0	-
East	Hulluka	4	0	0	0	0	0	-
arsi	Imp.Unkn	28	7.14	0-5	0.35	0- 4.5	0.23	Mr,Mss
	K6295-4A	4	0	0	0	0	0	-
	Kakaba	3	0	0	0	0	0	-
	Kubsa	13	0	0	0	0	0	-
	Lemu	6	0	0	0	0	0	-
	Madda walabu	6	0	0	0	0	0	-
	Mixed	2	0	0	0	0	0	-
	Ogolcho	18	0	0	0	0	0	-
	Pavon-76	13	0	0	0	0	0	-
	Shorima	2	0	0	0	0	0	-
	Wane	2	0	0	0	0	0	-
Total		**137**	**6.02**	**0-5**	**0.8**	**0-4**	**0.13**	**Mr,Mss**
	Danda'a	2	0	0	0	0	0	-
	Digalu	2	0	0	0	0	0	-
	Hidase	10	50	0-5	2.5	0-4	2.10	Ms
West	Hulluka	2	0	0	0	0	0	-
Arsi	Imp.Unkn	6	0	0	0	0	0	-
	Kubsa	4	0	0	0	0	0	-
	Ogolcho	22	4.54	0-5	0.23	0-4	0.18	Ms
Total		**50**	**7.79**	**0-5**	**0.4**	**0-5**	**0.32**	**Ms**

NFI: Number of Fields Inspected, Imp.Unkno: Improved Unknown, Tr: Trace, Ms: Moderately susceptible, Mr: Moderately resistant, S: Susceptible

In East Gojjam zone, a total of 70 wheat fields were surveyed. The varieties grown were Improved unknown, Danda'a and local unknown. The Improved unknown variety covered 64 wheat fields with a minimum stem rust prevalence of 4.68% and the disease was high on the variety Danda'a with 100% prevalence, and its mean incidence and mean severity were 5% and 15.25%, respectively. It was followed by Local Unknown with 0.5% mean incidence and 0.5% mean severity. On the other hand, the Improved unknown variety showed 0.39% mean incidence and 0.56% mean severity. The varieties showed Ms, and S type of reactions (Table 9).

In North Wello, 39 wheat fields were surveyed. Danda'a, Digalu, Hidase Improved Unknown, Kakaba, local Unknown, PBW343 and Sorra were sown in the area. Among these, the varieties Danda'a, Hidase and Improved unknown were the most dominant ones. The variety Digalu was planted only on one field and showed susceptible (S) type reaction (10% incidence and 2% severity) when compared with other varieties. Danda'a was the second widely grown variety in which stem rust was most prevalent with mean incidence of 1.43% and mean severity of 0.14%. However, the other varieties have showed relatively resistant types of response with zero (0%) mean severity and incidence (Table 9). In South wello, nine varieties (Danda'a, ETBW7690, Gundile local, Hidase, Impro unknown, Kakaba, Kursht (local), Local Unknown and Pavon 76) were grown in 56 fields. Among them Danda'a, Pavon76, Kursht and local unknown were the most widely grown varieties in the area.

Table 9. Intensity of stem rust on different varieties in Amhara region.

Zone	Variety	NFI	Mean Prevalence (%)	Incidence (%)		Severity (%)		Host response
				Range	Mean	Range	Mean	
	Danda'a	4	100	0	5	2-50	15.25	Ms, S
East	Imp. Unk	64	4.68	0-10	0.39	0-16	0.56	Ms, S
Gojjam	Local Unk	2	50	0-1	0.5	0-1	0.5	Ms
Total		**70**	**51.56**	**0-10**	**1.96**	**0-50**	**5.43**	**Ms, S**
	Danda'a	14	7.14	0-20	1.43	0-2	0.14	Mr
	Digalu	1	100	0	10	0	2	Mr
	Hidase	6	0	0	0	0	0	-
	Impr.Unkn	5	0	0	0	0	0	-
North	Kakaba	3	0	0	0	0	0	-
Wello	Local Unkn	3	0	0	0	0	0	-
	Pavon76	1	0	0	0	0	0	-
	PBW343	4	0	0	0	0	0	-
	Sorra	2	0	0	0	0	0	-
Total		**39**	**11.90**	**0-20**	**1.27**	**0-2**	**0.23**	**Mr**
	Akbir	1	0	0	0	0	0	-
	Danda'a	1	100	0	5	0	5	S
South	Impr.Unkn	44	0	0	0	0	0	-
Gondar	Local Unkn	1	0	0	0	0	0	-
	Triticale	5	60	0-60	17	0-50	11.20	MrMs
Total		**52**	**32**	**0-60**	**4.4**	**0-50**	**3.24**	**MrMs**
	Danda'a	30	26.66	0-80	7.26	0-30	2.61	Tr, Mr, S
	ETBW 7690	1	0	0	0	0	0	-
	Gundile(local)	1	0	0	0	0	0	-
	Hidase	1	100	0	100	0	32	Ms
South	Impr.Unkn	1	0	0	0	0	0	-
Wello	Kakaba	1	0	0	0	0	0	-
	Kursh(local)	8	0	0	0	0	0	-
	Local unkn	3	0	0	0	0	0	-
	Pavon76	9	33.33	0-10	2.78	0-20	4.22	Mr, Ms, S
Total		**55**	**17.77**	**0-80**	**12.22**	**0-30**	**4.31**	**Tr, Mr, Ms**
West	Impr.Inkn	21	14.28	0-50	2.71	0-20	2	Ms, S
Gojjam	Pavon 76	2	100	0-5	12.50	0-5	7.50	S
Total		**23**	**57.14**	**0-50**	**7.60**	**0-20**	**4.75**	**Ms, S**

Imp.Unk: Improved unknown, Local Unk: Local Unknown, TNFI: Total Number of Fields Inspected, Tr: Trace, Ms: Moderately susceptible, Mr: Moderately resistant, S: Susceptible

Stem rust was high on one field of variety Hidase with 100% incidence and severity of 32% moderately susceptible (MS) reaction. The disease was also high on Variety Pavon 76 with 33.33 % mean prevalence and Danda'a with 26.66 % mean prevalence. The variety Pavon 76 was relatively susceptible with 2.78 % mean incidence and 4.22 % mean severity even though the mean incidence was smaller compared with Danda'a. The disease was not scored on the remaining varieties. These might be probably due to the race composition appeared in the area and the varieties reaction to that specific race.

In south Gondar, 52 wheat fields growing five varieties (Akbir, Danda'a, Improved Unknown, local unknown and Triticale) were assessed. Among these, Improved Unknown and Triticale were the most dominant varieties and covered 44 and 5 fields, respectively. The highest stem rust was scored on the variety Danda'a with 100% mean prevalence followed by Triticale, which had 60% prevalence and MR MS reaction type. But the highest mean incidence (17%) and mean severity (11.20%) were recorded on Triticale compared with others followed by Danda'a which had 5% mean incidence and 5% mean severity. In West Gojjam, 23 wheat fields were surveyed and two varieties were grown mainly, such as Improved Unknown and Pavon 76. Stem rust disease was prevalent on the variety Pavon 76 with 100% prevalence and with 12.50% mean incidence and 7.50% mean severity showing Susceptible (S) reaction when compared with Improved unknown, which had 14.28% mean prevalence, 2.71 % mean incidence and 2% mean severity with MS reaction.

In general, most varieties released recently in the country succumbed to stem rust disease shortly after their introduction. In most cases, the failures have been due to the virulence present in the pathogen population and deployment of qualitative type of resistance in wide array of wheat cultivars (Netsanet *et al*., 2017).

4.2. Phenotypic race analysis and virulence structure of *Puccinia graminis* f. sp *tritici*

Of the 60 stem rust samples collected during the current work, 48 were viable and the remaining 12 samples did not yield viable spores after inoculation on the susceptible check McNair 701 in the Greenhouse. As a result, the race analysis was performed on these 48 viable samples (Table 10). Three races namely TKTTF, TTKSK and TTRTF were identified from the stem rust samples collected from the study areas. Race TKTTF which is also known as "Digalu race" was the most dominant one in the surveyed regions with a frequency of 75%. The remaining two races TTKSK (Ug-99) and TTRTF were detected with frequencies of 4.16 and 22.91%, respectively.

In Oromia, TKTTF was detected in the three zones surveyed (Bale, East Arsi, and West Arsi) and was most frequent in the East Arsi (45%) followed by Bale (33.33%) zone. It was least abundant (8.3%) in West Arsi zone (Table 10). TKTTF was identified from samples collected from varieties Hidase, Ude, Tesfaye, Ogolcho, Senbete, Kubsa, Dashen, Danda'a, Crossing lines, Ravi-18 and some Unknown varieties in the region. Race TTKSK (Ug-99) was detected in East Arsi and West Arsi zones with a frequency of 4.17% and was absent in Bale zone. In the present study, this race was detected from samples collected from the varieties Ogolcho and Kakaba in the region. It accounted for about 8.33% of the *Pgt* population in the surveyed zones in Oromia. On the other hand, race TTRTF was detected at a single location (4.17%) from variety Hidase in East Arsi zone but not detected from samples collected in Bale and West Arsi zones. In Amhara region, from the 24-stem rust infected samples analyzed, two races TKTTF (Digalu race) and TTRTF were detected. The former one was the predominant race and was detected in all surveyed zones. It was obtained most frequently in East Gojjam, North Wello,

and South Wello zones each with frequency of 20.83%, 16.67%, and 12.50%, respectively. However, it was least abundant in South Gondar with 4.17% frequency.

Table 10. Wheat varieties infected by the Pgt races identified in 2017 main cropping season.

Region	Races	Varieties	Number of isolates
Oromia	TKTTF	Hidase	4
		Ude	1
		Tesfaye	1
		Ogolcho	2
		Senbete	1
		Kubsa	2
		Dashen	1
		Danda'a	1
		Crossing line	5
		Ravi-18	1
		Unknown	2
	TTKSK	Ogolcho	1
	TTRTF	Hidase	1
Total			**24**
Amhara	TKTTF	Danda'a	6
		Unknown	5
		Digalu	1
		PBW-343	1
		Pavon-76	
	TTKSK	-	-
	TTRTF	Unknown	4
		Digalu	1
		Danda'a	1
		Pavon-76	1
		Israel	3
		Triticale	1
Total			**24**

Race TKTTF was detected from the varieties Digalu, Danda'a, PBW-343, Pavon-76 and some other unknown varieties. This study revealed that the TTKSK (Ug-99) race was not detected in any of the five zones in Amhara region, whereas, the new race TTRTF was detected in all surveyed zones except West Gojjam. This race was widely detected from samples collected

from South Gondar with frequency of 45.45%. It was identified from samples collected from varieties Digalu, Danda'a, Pavon-76, Israel, Triticale and some Unknown varieties in the region.

TKTTF (Digalu race) was detected in Ethiopia for the first time at a trace level in 2012 main cropping season from samples collected from Arsi and Bale zones and was found to be primary cause of the epidemics in the south eastern parts of the country in 2013 and 2014 cropping seasons (Olivera *et al*, 2015, Hodson, 2015). The epidemic due to this race forced the highly cultivated variety Digalu out of production and caused close to 100% yield loss in some fields (Olivera *et al*., 2015). The race was first identified from Turkey in 2005 and it was widely distributed in the Middle East region and currently it has been confirmed in nine countries (Turkey, Iran, Lebanon, Egypt, Ethiopia, Georgia, Azerbaijan, Eritrea, Yemen) (Olivera *et al*., 2015). Race TTKSK (Ug-99) was first detected in Ethiopia in 2003 in south and central parts of the country (Belayneh *et al*., 2010). This race predominated the *Pgt* population of Ethiopia and threatened wheat production in the country since its first report in 2013. It had virulence on *Sr31* which was resistant for over 40 years. According to Endale *et al*., (2016) the virulent race TTKSK was the most dominant and widely distributed race across the country after 2003 until Digalu race (TKTTF) over took the dominance. Olivera *et al.,* (2015) reported similar result that the race TTKSK was dominant in Arsi and Bale, before the Digalu (TKTTF) race and caused epidemics in 2012. However, in the present study it was detected only from two stem rust samples. This indicated that the race TTKSK was not dominant in the study areas may be due to the release of many resistant varieties against this race.

The race TTRTF is new for the country and had not been reported in Ethiopia in previous years. This race was identified for the first time in Italy (Patpour *et al*., 2016). In 2016, the race TTRTF caused severe epidemics in a place where the disease in the past was insignificant or

absent for the last 50 years in Europe. In Italy, Sicily, this new race damaged cultivated durum and bread wheat and numerous breeding lines to the significant level. Susceptibility of major commercial durum cultivars and breeding lines suggests the need for both durable resistance breeding and systematic surveys coupled to an early warning system (Patpour *et al*., 2016).

Table 11. Summary of samples collected, analyzed and races obtained from the study area.

Region	Zone	No of samples analyzed	TKTTF	Freq. (%)	TTKSK	Freq (%)	TTRTF	Freq. (%)
	Bale	8	8	33.33	-	-	-	-
Oromia	East Arsi	13	11	45.83	1	4.17	1	4.17
	West Arsi	3	2	8.33	1	4.17	-	
	Sub total	**24**	**21**	**87.50**	**2**	**8.33**	**1**	**4.17**
	East Gojjam	6	5	20.83	-	-	1	4.17
	West Gojjam	2	2	8.33	-	-	-	
Amhara	North Wello	6	4	16.67	-	-	2	8.33
	South Wello	4	3	12.50	-	-	1	4.17
	South Gondar	6	1	4.17	-	-	5	20.83
	Sub total	**24**	**15**	**62.50**	**-**	**-**	**9**	**37.50**
	Total	**48**	**36**	**75.00**	**2**	**4.17**	**19**	**39.58**

Freq: Frequency

4.2.1. Virulence Spectra to Sr resistance genes

According to the findings of this study, 85% of the stem rust resistance genes were ineffective to all the races detected and 30% of the resistance genes were found to be effective to one or more of the races identified (Table 12). Each of the races defeated 85% of the resistance genes (Sr). Fourteen differential hosts carrying the resistance genes *Sr5, Sr21, Sr9e, Sr7b, Sr6 Sr8a, Sr9g, Sr9b, Sr17, Sr9a, Sr9d, Sr10, Sr38 and McN* were ineffective to all of the three races detected and a higher virulence frequency of 100% was observed on these *Sr* genes (Table 13).

On the other hand, the differential hosts carrying the resistance genes *Sr11* was found to be effective against the race TKTTF but not against TTKSK and TTRTF. Similarly, differential host with *Sr36* was ineffective to race TKTTF and TTKSK although the gene was effective for the race TTRTF. The virulence study showed that the differential host carrying the resistance gene *Sr Tmp* was not effective against the race TKTTF and TTRTF but it was effective against the race TTKSK. The differential host carrying stem rust resistance gene *Sr31* was found to be effective against both TKTTF and TTRTF races, but not against TTKSK. The most important resistant gene Sr24, which the majority of Ethiopian commercial variety possess (Belayneh *et al*., 2010) was found to be effective against all races obtained from the study area. In general, the result of this study revealed that there is a wider variation in virulence spectrum among the races and supports the report by Belayneh *et al.,* (2010); Teklay *et al*., (2012); Abiyot *et al.,* (2014); and Endale *et al*., (2016) which indicated that no virulent race was detected against *Sr24* gene in Ethiopia.

Table 12. Virulence spectra of the Pgt races identified in Oromia and Amhara regions in 2017

Race	Virulence	Avirulence
TKTTF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*11, 24,31*
TTKSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*	*36, Tmp, 24,*
TTRTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, McN*	*30, 24, 31*

The present study found that the Sr 24 is the only stem rust resistance gene that remain resistant for the three races TKTTF, TTKSK and TTRTF. Virulence on the resistance gene SrTmp is considered as the main factor behind the complete susceptibility of the variety "Digalu" to this race. 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 (Belayneh and Emebet, 2005; Belayneh *et al*., 2010).

The co-evolution of *Puccinia graminis* f.sp. *tritici* along with wheat being the reason for high virulence diversity in Ethiopian *Pgt* populations. This might be due to variation over location and time as the races found in a specific season and region depend on the type of wheat varieties grown and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*.,1992). Virulence diversities within *Puccinia graminis* f.sp. *tritici* were also reported from other countries such as South Africa, Mexico, USA and Canada (Jin, 2005).

The race spectrum in Ethiopia was definitely different from those in other parts of the world. For instance, surveys in Canada, USA, Russia and South Africa detected fewer races (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 Tigary (Serbessa, 2003; Belayneh *et al.,* 2010 and Teklay *et al*., 2012). However, with only three races from eight administrative zones, the present study is contrary to the previous works that have been done in Ethiopia. According to Belayneh *et al*., (2010) and Teklay *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 TKTTF and TTRTF identified in the present study varied by single gene changes. For instance, TKTTF was similar to TTRTF with avirulence to *Sr24* and *Sr31,* respectively. However, a single gene change in virulence occurred on *Sr 30 and Sr 11*. This entails *Sr 30* was broken by the race TKTTF but remains resistant to the race TTRTF and *Sr 11* gave up its resistance to the race TTRTF but remains resistant to the race TKTTF. This study therefore confirms the report of Teklay *et al*., (2012) which stated that the stem rust resistance gene 24 (*Sr 24)* is amongst the

effective genes to all stem rust collected from the northern Ethiopia. However, virulence to *Sr24* gene was reported in Kenya in 2006 (Wanyera *et al.,* 2010). A variant of Ug99 group that added virulence on stem rust gene *Sr24* (Ug99+*Sr24* virulence, called TTKST) has further increased the vulnerability of wheat to stem rust worldwide (Jin *et al*., 2008). The breakdown of the *Sr31* resistant gene in Ethiopia is reported by many authors previously which serve as evidence for the existence of Ug99 (TTKSK) (Belayneh *et al*., 2010; Teklay *et al*., 2012; Endale *et al*., 2016).

Table 13. Virulence frequency of Pgt races collected from the study area to single gene wheat differentials.

Stem rust resistance gene	Virulence frequency (%)	Stem rust resistance gene	Virulence frequency (%)
Sr5	100	Sr 30	66.67
Sr 21	100	Sr 17	100
Sr 9e	100	Sr 9a	100
Sr 7b	100	Sr9d	100
Sr 11	66.67	Sr 10	100
Sr 6	100	Sr Tmp	66.67
Sr 8a	100	Sr 24	0
Sr 9g	100	Sr 31	33.33
Sr 36	66.67	Sr 38	100
Sr 9b	100	McN	100

Sr: Stem rust

4.3. Genotypic analysis & pgt population diversity

After filtering, 765 SNP loci, 45 genotyped isolates belonged to 37, 7 and 1 isolates the races TKTTF, TTRTF and TTKSK (Ug-99) race groups via genotyping, respectively and were used for the analysis, A phylogenetic analysis divided the isolates into three well supported clades with bootstrap values of 100% (Fig. 7). The three clades used to cluster the isolates from the study area were: Clade – I, which contains the TTKSK (Ug-99) race group and reference isolates which were similar with these race group (Table 14). Clade III-B, a clade in which a new race TTRTF was clustered in and Clade IV, which was further sub divided into three

subclades (Clade IV -A, Clade IV-B and Clade nd). From the 37 isolates of TKTTF (Clade IV), 27 isolates were clustered under sub clade IV-B, 4 isolates were clustered under clade IV-A and 6 isolates were grouped under Clade nd.

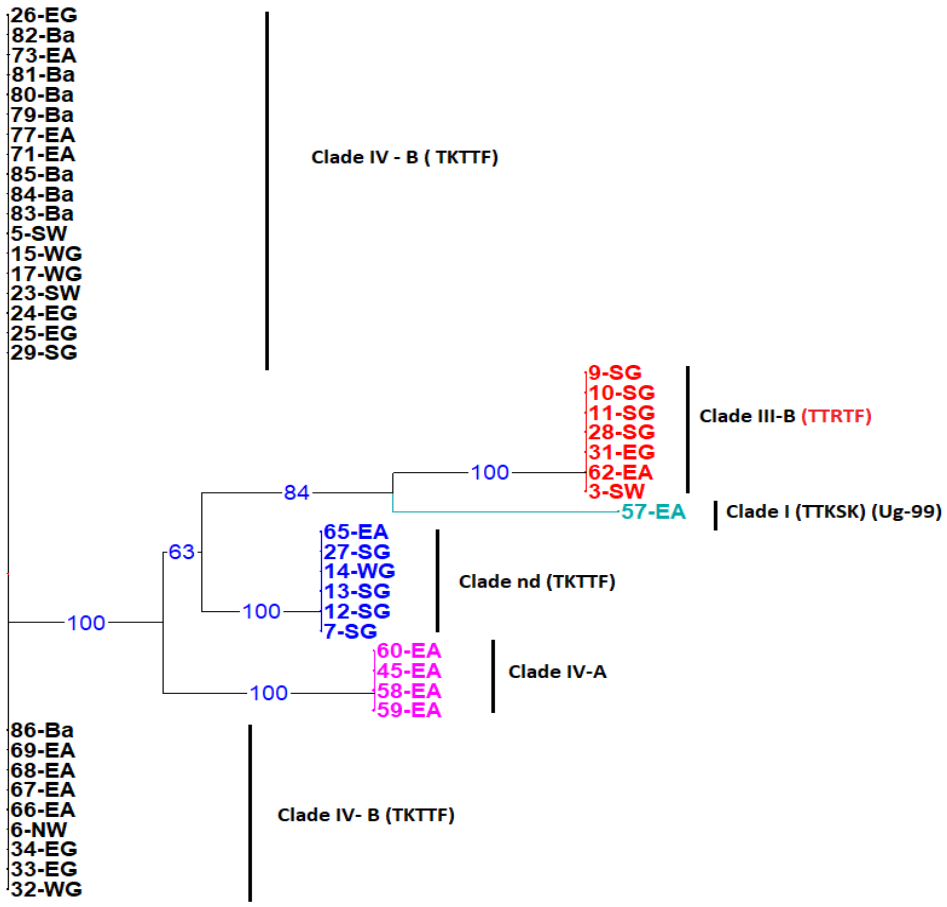


Figure 7. Neighbor-joining phylogenetic tree of 45 isolates of Pgt from the study area.Ba: Bale; EA: East Arsi; EG: East Gojjam;SG: South Gondar; WG: West Gojjam; NW: North Wello

There is only phenotypic difference between Clade IV-A and Clade IV -B, for instance, in Clade IV-A, the infection was avirulent to Sr 7a, Sr 33, Sr45, Sr Tt-3, however, infection types on Sr 7a, Sr 33, Sr45, Sr Tt-3 is unknown. Similar scenario has been observed in Germay (Firpo *et al*., 2017) that from the collected samples, two isolates showed phenotypic differences even though they belong to race TKTTF group. According to Firpo *et al*. (2017), the reference isolates and an extended or additional stem rust resistance gene were used to phenotype and

genotype then they were able to identify to which clade the isolates belongs to. The majority of the isolates in the current study were phenotyped and genotyped as TKTTF race group and clustered into Clade IV. Whereas, only one (2.22%) isolate which was collected from East Arsi (Coded as 57-EA) was categorized under Clade -I in which race TTKSK (Ug-99) belongs to (Fig. 7). This may imply the replacement of race TTKSK by TKTTF and other races.

In addition, the same race phenotype is often found in more than one genotype subclade. This supports the idea that changes in virulence occur frequently, resulting in isolates with common race phenotypes but different genetic backgrounds. This makes it difficult to predict evolution of lineages based solely on race phenotypes (Newcomb *et al*., 2015). The new race TTRTF was clustered under a clade III-B, and seven isolates (4 from South Gondar, 1 from East Gojjam, one from East Arsi and one from South Wello) were genotyped and confirmed that new for the country and not reported before. However, TTRTF race was first reported in Georgia and Sicily in 2014, and then it was detected in Azerbajan, Egypt, Eritrea, Iraq and Italy (Firpo *et al*., 2017). These studies reveal that, it is interesting to note that Pgt isolates of clade III-B have been observed in Ethiopia for the first time, however been detected in Georgia and Sicily.

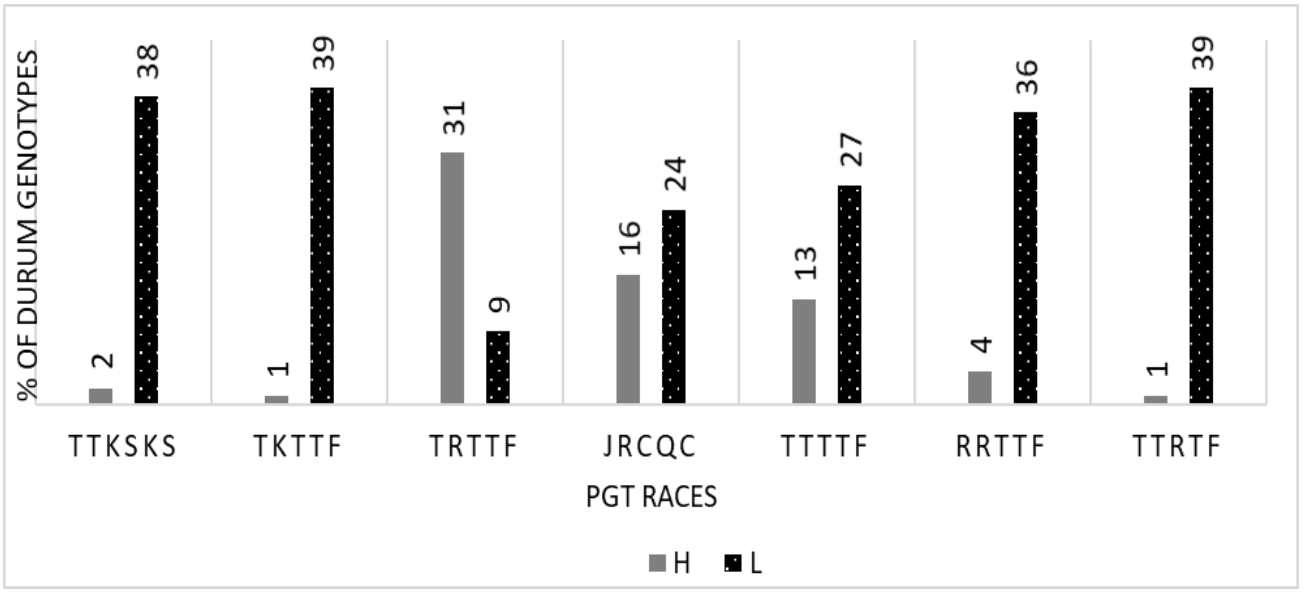
The isolates under Clade (nd) were found to be TKTTF race group, these race groups were not categorized in any clades so far according to (Pablo *et al*., 2015) in Ethiopia in previous years. Even though they showed similar characteristics with the other groups of TKTTF race, there was genetic difference among the population (Newcomb *et al*., 2016).

4.4. Seedling resistance for major stem rust races

4.4.1. Durum wheat

The result of the present study showed that the tested durum wheat genotypes differed in their resistance to different races and high level of variability was observed in response to each of the pgt races used in this study (Appendix 8). Among the 40 durum wheat materials tested against

seven wheat stem rust races, five genotypes namely, Arendato, Boohai, Denbi, Kokate and CD13 DZOS F6 SR 2013 MS DZ LS-15 were susceptible to more than 57% of the races used for screening. A high IT on the tested materials indicates that they did not have any of the resistance gene for which the test isolate (race) was avirulent (Singh 2003, Belayneh *et al.*, 2010). These does not mean that the test materials would continue to be susceptible in adult stage, however, they may have resistance to the races not detected during this study period or carry adult plant resistance. Netsanet (2014) described Ethiopian wheat lines showing susceptible IT at seedling stage while maintaining low rust severity at adult stage in the field. Thirty-one (77.5%) durum genotypes showed susceptible or compatible infection (3 to 4 IT's) types to the race TRTTF while 16 (40%) showed susceptibility to race JRCQC. Likewise, 13 (32.5%) of the durum materials were susceptible to the race TTTTF (Fig. 8). These results are in agreement with the reported by Abiyot *et al.,* (2015). On the other hand, 39 (97.5%) and 38 (95%) durum wheat materials were resistant to the races TTRTF and TKTTF, respectively. Similarly, 37 (92.5%) durum wheat genotypes had incompatible IT's with the race TTKSK while 36 (90%) were resistant against the race RRTTF. Five durum wheat genotypes namely Bichena, Tob-6, Assasa, Kokate and DW/NVT-LMA showed complete resistance to all the pgt races used (Appendix 8).



High; L: Low ; Pgt**:** *Puccinia graminis tritici*;
Figure 8. Frequency distribution of infection types (IT's) of durum wheat materials at the seedling stage.

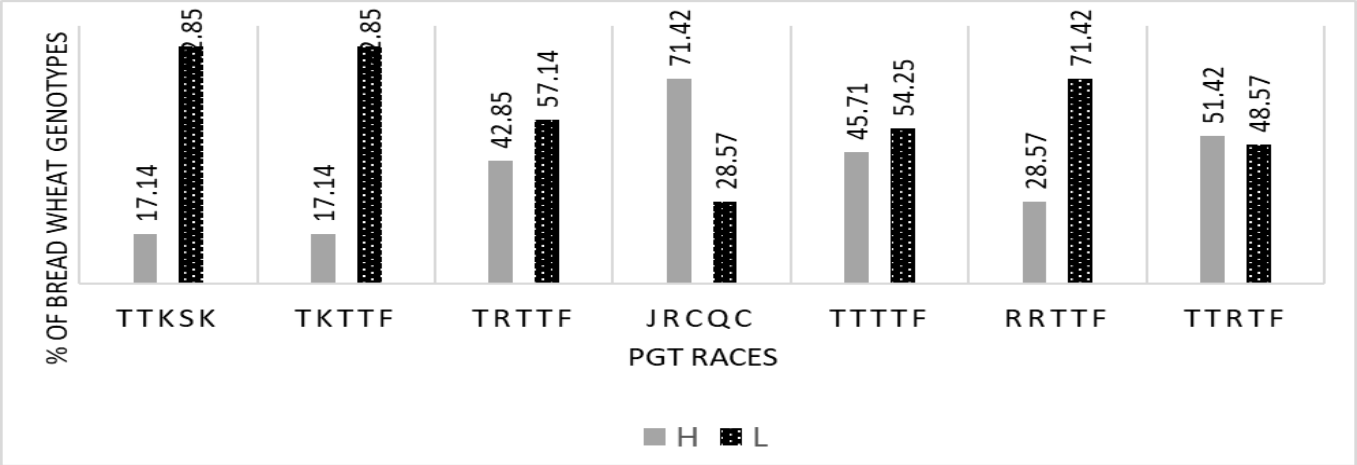
The resistance reaction in these genotypes indicated that the presence of seedling or major gene resistance towards these virulent races. This result agrees with Ogutu *et al*., (2017) who reported cultivars that exhibited low infection types at seedling stage could be either due to the presence of one or more major stem rust genes. 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 major gene (Cheruiyot *et al.,* 2015).

4.4.2. Bread wheat

From the 35 bread wheat genotypes, 25 (71.42%) were susceptible to JRCQC race, and 18 (51.42%) of the materials showed compatible infections to the race TTRTF (Fig. 9). About 16 (45.71%) and 15 (42.85%) bread wheat genotypes were also susceptible to the races TTTTF and TRTTF, respectively. ETH-SH15418 and McNair were susceptible to all races at seedling stage. Susceptibleness of these genotypes reveals that they do not possess major gene resistance

against the test races. However, cultivars that exhibited susceptible infection types in seedlings may display minor gene resistance at adult plant stage. According to Cheruiyot *et al*. (2015) in the absence of major gene resistance varieties only with adult plant resistance will still be susceptible to stem rust at the seedling stage. On the other hand, majority of the bread wheat genotypes tested 29, (82.85%) showed incompatible or resistance infection types to TTKSK and TKTTF races, which were virulent over known commercial varieties in the country. TTKSK and TKTTF had been major threats of wheat production in Ethiopia for the last several years and the wheat improvement programs of the country have released many varieties which were resistant against these important races (DRRW, 2010). Some of the bread wheat genotypes tested in the present study were among the resistant lines released for these races. Hence, majority of the bread wheat lines tested in this study had resistant infection types to TTKSK and TKTTF. Likewise, 25 (71.42%) bread wheat genotypes showed resistance to the race RRTTF. Six genotypes namely, Sulla, Hidase, Wane, 36ESWY (from the commercial varieties) and ETBW 8459, 36ESWYT (from advanced breeding lines) were resistant against all the races. These could be due to the fact that these varieties carry effective race-specific or seedling resistance genes to the virulent races. Race specific resistance functions against certain stem rust races or biotypes but not against others (Babiker *et al*., 2009; Sheikh *et al*., 2017) and is relatively simply inherited. This is the reason why seedling type of resistance has been used for years in wheat breeding programs and is often deployed over a broad area (Hulbert and Pumphrey, 2014). However, in almost all cases, the pathogen overcomes effectiveness of the genes because the pathogen has the ability to change through sexual recombination and mutation (Ayliffe *et al.*, 2008; Hulbert and 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*) is present in proximity to wheat production areas of Ethiopia and the pathogen is able to complete its life cycle in the country (Endale *et al*., 2016).



Where H: High; L: Low; pgt: *Puccinia graminis tritici*;

Figure 9. Frequency distribution of infection types (IT's) of Bread wheat materials at the seedling stage.

According to Ambika and Meenakshi (2018), changing temperature and rainfall patterns have also 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 major gene resistance to it. Netsanet *et al*., (2017) reported that most wheat varieties released in the country were succumbed to stem rust disease shortly after their introduction. In most cases, the failures have been due to deployment of qualitative/race specific type of resistance in wide array of wheat cultivars, the virulence present in the pathogen population and continuous cultivation of susceptible commercial varieties. In general, the most principal and excellent management strategy that provide adequate protection without the need for chemicals to control wheat stem rust has been host resistance, which is effective and affordable to small-scale farmers. Several efforts were made towards resistant cultivars development in Ethiopia and several bread and durum wheat cultivars with various levels of rust resistance were released for production.

However, the resistance to stem rust of most of the released bread wheat and durum wheat varieties has been broken soon after their release. This experience in the country 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). Combining seedling resistance with adult plant resistance in the field will provide valuable indications to select resistant varieties.

5. SUMMARY AND CONCLUSION

Wheat rust pathogens have reduced global wheat production since the domestication of the crop and continue to threaten the world's wheat supply. Wheat stem rust is airborne diseases of wheat caused by *Puccinia graminis* f. sp. *tritici* which causes one of the most potentially destructive wheat diseases, seriously threatening and remains a constraint to the world's wheat production due to variability of virulence in the pathogen population, the rapid evolution of new races, the ability of urediniospores to spread over long distances by wind, and an exponential reproduction capacity. The objectives of these studies were to assess wheat fields in selected major wheat growing areas and study the distribution of the Sr diseases, diversity of physiological races, virulence spectrum of wheat stem rust races and identify their prevalence in the country, and screen bread and durum wheat genotypes for their major gene resistance against the major virulent (dominant) races (*Puccinia graminis* f. sp. *tritici)* found in Ethiopia.

The results revealed that the distribution and extent of damage caused by the wheat stem rust across the study areas. According to this study, the highest disease prevalence of 81.66% was scored in Bale zone with the corresponding higher severity of 14.64%, followed by west Arsi with a mean prevalence of 9.18%. However, the disease was least prevalent in East Arsi zone with a mean prevalence of 2.27%. In the study areas of Amhara, the mean prevalence of the disease varied considerably. The disease was most prevalent (100%) in West Gojjam zone and least prevalent (0%) in Basoliben, Debre elias, and Goncha siso districts of in East Gojjam zone. The maximum mean incidence (6.52%) was scored in South Wello zone and the minimum mean incidence (0%) was scored in East Gojjam.

Wheat stem rust intensity appears to be increasing with elevation across the survey areas This shows probably the pathogen is coming to adapt a high-altitude environment where yellow rust was expected to be common, and this in turn tell us the climate change is favoring the pathogen to adapt the new environment where it was not been there before.

For physiological race analysis, 48 viable stem rust samples were analyzed on 20 stem rust differentials and three races (TKTTF, TTKSK and TTRTF) were identified. TKTTF was a common and predominant race detected from all the three zones of Oromia and Amhara with a frequency of 75%. This has broken the resistance of a highly cultivable variety Digalu. TTKSK was one of the most dominant races in the country in previous years but now it has been dominated race TKTTF, which was detected in the study areas of both Oromia and Amhara in the current study. TTKSK was obtained from East Arsi (Amhara region) and West Arsi (Oromia region) but it was absent in Bale. According to current results, race TKTTF (Digalu race group) has replace the TTKSK (Ug-99) race group of *Pgt*, which is detected at frequency of only about 8.33%. TTRTF race was detected from a single location in East Arsi zone, and this is the first work to report this race in Ethiopia. Which is confirmed through molecular techniques and phenotypic analysis at USDA, Cereal disease laboratory and APPRC laboratory, respectively. This race was detected from a sample collected from a variety Hidase, which was known to be moderately resistant earlier; this reveals the new race is coming leading to a rapid race shift in the country.

A phylogenetic analysis divided the isolates into three well supported clades with bootstrap values of 100% The three clades used to cluster the isolates from the study area were: Clade – I, which contains the TTKSK (Ug-99) race group and reference isolates which were similar with these race group Clade III-B, a clade in which a new race TTRTF was clustered in and

Clade IV, which was further sub divided into three subclades (Clade IV -A, Clade IV-B and Clade nd). From the 37 isolates of TKTTF (Clade IV), 27 isolates were clustered under sub clade IV-B, 4 isolates were clustered under clade IV-A and 6 isolates were grouped under Clade nd. The reference isolates and an extended or additional stem rust resistance gene were used to phenotype and genotype then they were able to identify to which clade the isolates belong to. The majority of the isolates in the current study were phenotyped and genotyped as TKTTF race group and clustered into Clade IV. The physiological races (TTKSK, TTRTF and TKTTF) detected at green house using differentials sets and molecular genotyping result was similar and found to be same.The race TTRTF is new for the country and had not been reported in Ethiopia in previous years. This race was identified for the first time in Italy (Patpour *et al*., 2016).

A total of 76 wheat genotypes (40 durum, 35 bread wheat genotypes and one check) were screened for resistance to *Pgt*. From the 40 durum wheat genotypes tested for their seedling resistance, five genotypes namely Bichena, Tob-6, Assasa, Kokate and DW/NVT-LMA were identified to possess major gene resistance against the virulent races used in the current study. However, genotypes such as Arendato, Boohai, Denbi, Kokate and CD13 DZOS F6 SR 2013 MS DZ LS-15 had susceptible infection types to the majority of the races, revealing that they do not exhibit seedling resistance gene. In similar manner, from the 35 bread wheat genotypes, six genotypes namely, Sulla, Hidase, Wane, 36ESWY (from the commercial varieties) and ETBW 8459, 36ESWYT (from advanced breeding lines) were resistant against all the races, whereas, ETH-SH15418 and McNair were susceptible to all races at seedling stage in the greenhouse. Susceptibleness of these genotypes described above reveals that they do not possess major gene resistance against the test races.

Based on current findings, this study recommends

1. The present study has confirmed the presence of TTRTF as newly emerged race in Ethiopia. However, there might be a probability of occurrence of other new races in pocket areas or areas that were not covered during the present study. Therefore, it is strongly advisable to study the genotypic diversity of *Pgt* population on a wider scale
2. Additional race analysis work with additional Sr genes need to be done to confirm the correlation between phenotypic and genotypic variations and conducting impact assessment of the disease regularly would help to establish early warning system in Ethiopia and this can be achieved by Establishing trap nurseries are necessary to monitor the pathogenic variation or the race shift in Ethiopia.
3. Additional studies are also recommended to screen the wheat genotypes tested in the greenhouse under field conditions and, wheat stem rust survey should be conducted in a wider altitude ranges in order to know the actual disease distribution and variability of races for effective decision making before investing and incurring
4. To build strong surveillance network, which accompanies both surveys and intensive sampling, surveillance partnership within pathologists, breeders, modelers, epidemiologists, molecular geneticists and policy makers is crucial in this regard.

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7. APPENDICES

Appendix 1. List of bread wheat cultivars and elite lines that were evaluated for major stem rust races at seedling stage.

No	Genotypes	Types of wheat	Cross
1	Arendato	DW	DZ 04-118
2	Boohai	DW	COO "S" / CANDEAL II CD 3062- BS OGR
3	Bichena	DW	ILUUMILO / COCORIT 71 DZ 393-4
4	Tob-66	DW	RCBBCI/LD-357//DUCK''S''/YEL''S''TOB-66
5	Assasa	DW	CHO "S"/ TARUS//YAV "S" 3/FG" S" /4/ FGS/CR "S" /5/ DZ 2085
6	Robe	DW	HORA/ CIT "S" // JO 'S' / GS 'S' /3/ SOME 'S' /4 / HORA RESPINEGRO// CM 9908 /3/ RAHUM DZ 1640
7	Ginchi	DW	BOOHAI/ULNV-DZ1050
8	Ude	DW	CHEN / ALTAR- 84// JO69 CD95294-9M-030Y-040 PAP-2Y-OB
9	Denbi	DW	AJAIA/BUASHEN
10	Hitosa	DW	CHEN/ALTAR-84
11	Mukiye	DW	STJ3//BCR/LK54/3/TE-3
12	Yoltu	DW	-
13	Tellie	DW	-
14	Magnegna	DW	Gerardo/3/ Boohai
15	Kokate	DW	DZ-2016-1BZR-10205-OAK-2AK (23)
16	Flakit	DW	-
17	Mettaya	DW	-
18	Mossobo	DW	-
19	Tate	DW	DACK/KIWI/OSTE/3/CHEN 84//4/MEXI/5/5
20	Bekelcha	DW	98 OSN GEDILFA /GUEROU
21	Oda	DW	DZ046881/IMLO//CIT71/3/RCHI/LD357//IMLO/4/YE MEN/CIT'S'//PLC'S'/3/ TAGANROY
22	Ilani	DW	IMILO/RAHUM//A4#72/3/GERARDO
23	Utuba	DW	-
24	Kokate	DW	-
25	DW/PVT-OltMASetI	DW	-
26	F2-D/off/173/2010-81-2-3H	DW	F2-D/off/173/2010-81-2-3H
27	F2-D/off/149/2010-80-1-1H	DW	F2-D/off/149/2010-80-1-1H
28	45IDSN 2013 MSDZLS	DW	45IDSN 2013 MSDZLS
29	CD13DZOS F6SR 2013 MS DZLS-5	DW	CD13DZOS F6SR 2013 MS DZLS-5
30	CD13DZOS F6SR 2013 MS DZLS-7	DW	CD13DZOS F6SR 2013 MS DZLS-7
31	F2-D/off/295/2010-87-1-5H	DW	F2-D/off/295/2010-87-1-5H

32	CD13DZOS F6SR 2013 MS DZLS-10	DW	CD13DZOS F6SR 2013 MS DZLS-10
33	CD13DZOS F6SR 2013 MS DZLS-13	DW	CD13DZOS F6SR 2013 MS DZLS-13
34	CD13DZOS F6SR 2013 MS DZLS-15	DW	CD13DZOS F6SR 2013 MS DZLS-15
35	CD13DZOS F6SR 2013 MS DZLS-18	DW	CD13DZOS F6SR 2013 MS DZLS-18
36	CD13DZOS F6SR 2013 MS DZLS-21	DW	CD13DZOS F6SR 2013 MS DZLS-21
37	DW/NVT-LMA	DW	-
38	DSP/off/759/2010-45-1H-4H	DW	DSP/off/759/2010-45-1H-4H
39	43rdIDSNmeh 82/2011	DW	43rdIDSNmeh 82/2011
40	DW/NVT/LMA16/17	DW	-
41	DW/NVT-LMA	DW	-
42	Ogolcho	BW	WORRAKATA/2*PASTOR
43	Sulla	BW	HAR710
44	Hoggana	BW	PYN/BAU//MILAN= (FLAG 5)
45	Hidase	BW	YANAC/3/PRL/SARA//TSI/VEE#5/4/CROC-1/AE.SQUARROSA (224) //OPATTA
46	Kingbird	BW	TAM200/TUI/6/PAVON76//CARIANCA422/ANAHUAC75/5/BOBWHITE/ CROW//BUCKBUCK/PAVON-F-76/3/YECORA-F-70/4/TRAP-1.
47	Wane	BW	SOKOLL/EXCALIBUR
48	Werer	BW	SOKOLL/3/PASTOR//HXL7573/ 2*BAU*2/4/PAURAQ
49	Mangudo	BW	MRF_1STJ2/3/1718BT24//KARIM
50	36ESWYT	BW	SOKOLL/3/PASTOR//HXL7573/ 2*BAU*2/4/PAURAQ
51	23SAWYT	BW	CHIBIA//PRLII/CM65531/3/MISR2*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07
52	ETBW 8311	BW	-
53	ETBW 8065	BW	-
54	ETBW 8427	BW	-
55	ETBW 8459	BW	-
56	ETBW 9464	BW	-
57	ETBW 9466	BW	-
58	14HTWYT	BW	MILAN//PRL/2*PASTOR/4/CROC_1/AE.SQUARROSA(213)//PGO/3/BAV92/5/PAURAQ
59	ETBW 9470	BW	-
60	ETBW 7598	BW	-
61	ETBW 9282	BW	-
62	ETBW 9292	BW	-
63	ETBW 9486	BW	-
64	3WYCYT	BW	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PARUS/PASTOR
65	ETBW 9549	BW	KFA/2*KACHU/3/KINGBIRD#1//INQALAB 91*2/TUKURU/4/KFA/2*KACHU

66	ETBW 9550	BW	KFA/2*KACHU*2//WAXBI
67	ETBW 8862	BW	C80.1/3*BATAVIA//2*WBLL1/3/C80.1/3*QT4522//2*PASTOR/4/WHEAR/SOKOLL
68	36ESWYT	BW	SOKOLL/WBLL1/4/D67.2/PARANA
69	ETBW 8804	BW	TURACO/CHIL/6/SERI 82/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI
70	ETBW 8996	BW	FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA/4/FRET2/5/DANPHE#1/11/CROC_1/AE.SQUARROSA (213)//PGO/10/ATTILA*2/9/KT/BAGE//FN/U/3/BZA/4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA
71	ETBW 8583	BW	MINO/898.97/4/PFAU/SERI.1B//AMAD/3/KRONSTAD F2004
72	ETBW 8668	BW	BAVIS*2/3/ATTILA/BAV92//PASTOR
73	ETBW 9585	BW	KFA/2*KACHU*2//QUELEA
74	ETH-SH15418	BW	TACUPETOF2001/SAUAL//BLOUK
75	ETH-SH15436	BW	PBW343*2/KUKUNA*2//FRTL/PIFED/3/KFA/2*KACHU
76	ETH-SH15424	BW	WATTAN92*2/3/KINGBIRD

Appendix 2. Chi square test statistics for all surveyed zones for stem rust incidence and severity.

	Value	Degree of freedom (DF)	Probability
Pearson Chi-Square	404.338	77	P<.0001
Likelihood Ratio	234.839	77	P<.0001
N of Valid Cases	464		

N: Number of, DF: Degree Freedom

Appendix 3. Chi square test statistics for all surveyed zones in terms of Sr severity.

	Value	Degree of freedom (DF)	Probability
Pearson Chi-Square	926.383	540	P<.0001
Likelihood Ratio	273.508	540	P<.0001
N of Valid Cases	464		

N: Number of, DF: Degree of Freedom

Appendix 4. Wheat rust disease survey

Please return completed forms to the National Focal Point in your country

Global Cereal Rust Monitoring Form

Surveyor name: _____

Country/Institution: _____

Date of survey (dd/mm/yy): _____ / _____ / _____

Location name: _____

Latitude (decimal degrees): **N S** | | | . | | | | | | |

Longitude (decimal degrees): **E W** | | | | . | | | | | | |

Elevation: _____ meters

Survey site: Farmer field ☐ Weed ☐ Trial Site ☐

Crop: **Bread wheat** **Durum wheat** **Barley** **Triticale** **Oats** **Other**

Growth stage: **Tillering** **Boot** **Flowering** **Milk** **Dough** **Maturity**

Field area size: _____ ha Variety: _____

Disease	Stem Rust	Leaf Rust	Yellow Rust	None
Incidence at field level	L M H	L M H	L M H	
Severity (on infected plants)	L M H	L M H	L M H	
Reaction (R,MR,MS,MSS,S)				

L (low) = less than 20% *M (moderate) = 20 - 40%* *H (high) = more than 40%*

Stem Rust sample collected: **Y N** Sample ID number:

Comments / Observations:

INSTRUCTIONS

1. *write your name, country, and institute on the blank lines*
2. *write the date and location name of the survey stop*
3. *use the GPS to determine the coordinates of the survey location; write the entire decimal degrees for both latitude and longitude, circle N (or S) and E (or W), write the elevation*
4. *tick the appropriate box describing the survey site*
5. *circle the crop and its growth stage*
6. *write the field area (hectares) and crop variety name*
7. *circle each disease that is present and indicate the incidence and severity for each by circling the appropriate letter (LMH); if no diseases are present, circle* None
8. *indicate if a sample was collected by circling* Y *or* N
9. *if you collect a sample, write an ID number on the envelope and the same on the form*
10. *return the completed form to the National Focal Point in your country*

Appendix 5. Wheat stem rust pathogen sampling for DNA extraction

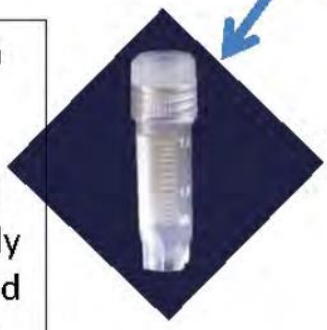
DNA Sampling Protocol

[NB: Clean scissors + hands with Ethanol after each sample]

1. Select a single, large, isolated pustule. Cut the stem either side of the pustule. [NB: **Only 1 pustule per tube**]

2. Remove the core stem tissue

3. Place pustule (and sheath tissue) into a cryovial. Add 70% Ethanol (or absolute alcohol) to ¾ fill the cryovial (ensure pustule is completely covered). Label tube + record unique code on survey form



+

4. Store in ethanol for 7 days. Decant the ethanol, air dry (with tube cap off) for 12-24 hours. When dry, seal the tube, place in zip lock bag and send via courier to Cereals Disease Lab, Minnesota

Appendix 6. Wheat stem rust race analysis differentials scoring sheet.

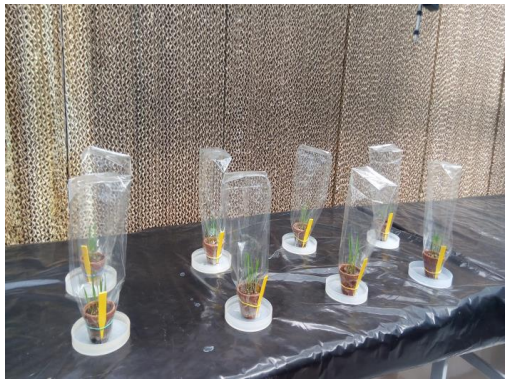
Region_____ Zone _____ District _____ Kebele _____

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+**						
II	4	ISr7b-Ra	05 Aberdeen	**7b**	**2**						
	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**						

Appendix 7. Seedlings of universal susceptible line (McNair 70) used for multiplication**.**

Seven-day old seedlings of Mc NaMc Nair



Inoculated with sr pathogen and isolated with cellophane bag

Seeds of differential lines used to detect races

Seven-day old seedlings of differential lines after being inoculated with sr pathogen

Differential lines ready for scoring after 14 days of inoculation

Appendix 8. Phenotypic response of bread durum and bread wheat genotypes to seven stem rust races (TTKSK, TKTTF, TRTTF, JRCQC, TTTTF, RRTTF, and TTRTF).

No	Genotype	TTKSK	TKTTF	TRTTF	JRCQC	TTTTF	RRTTF	TTRTF	Type
1	Arendato	3-	;2	3	3	3-	3	2+	CV
2	Boohai	3-	;1	3	3-	3-	;1+	;1+	CV
3	Bichena	;1+	;1	;1	;1+	;2	;2-	2-	CV
4	Tob-6(Arsi Robe)	;1+	;1	;1	;1+	2	;1+	1+	CV
5	Assasa(DZ 1640)	;1	0	2-	;1	2	;2	2+	CV
6	Robe (DZ 1640)	;2	0	3	3-	;1+	2+	2+	CV
7	Ginchi (DZ 1050)	2	;	3-	2-	;1+	;1	2	CV
8	Ude (CD 95294)	;2	;1	3	2	2	2+	2	CV
9	Denbi	;2-	0	3-	3-	3-	;3-	2	CV
10	Hitosa (CHENAL TAR -84)	2-	2	3	3-	3-	;2+	2-	CV
11	Mukiye	;1+	2+	3-	3-	2-	2	1+	CV
12	Yoltu/4/B/R9096	2	0	3-	2	2-	2	2+	CV
13	Tellie 50(DZ 1605)	2-	2	3-	2-	3-	;1+	2+	CV
14	Magnegna (DZ 2023)	2	;1	3-	3-	3-	3-	2	CV
15	Kokate (DZ 2016)	;2-	;1	;2-	;2	;1+	;2	1+	CV
16	Flakit (EN 25)	;2	2-	3-	3-	3-	;2-	2	CV
17	Mettaya (DZ 2212)	;1+	2-	3-	2-	2	2	2-	CV
18	Mossobo (DZ2178)	2+	;1	3	2+	2-	2-	;1	CV
19	Tate (CD 94523)	2-	;1	2	3-	2-	;1	1+	CV
20	Bekelcha	;1+	;1	3-	;2-	3-	2-	1+	CV

21	Oda (2227)	;2-	;1	3-	3-	3-	;1	2	CV
22	Ilani (DZ 2234)	2	;1	3-	3-	3-	;1	1+	CV
23	Utuba (Dsp. 2009.oFF. F4	;1	;1	3-	;1	;1	3-	1	CV
24	DW/PVT-OltMASetI	;2	2+	3-	;2-	;2+	;1	2+	ABL
25	F2-D/off/173/2010-81-2-3H	;2-	;1	2	2-	3-	;2	2+	ABL
26	F2-D/off/149/2010-80-1-1H	2-	;1	3-	;2	3-	2-	1+	ABL
27	45IDSN 2013 MSDZLS	;1+	;1	2	3	2+	2	1+	ABL
28	CD13DZOS F6SR 2013 MS DZLS-5	;2	;1	3	;2-	2+	2	;2	ABL
29	CD13DZOS F6SR 2013 MS DZLS-7	;1	2	2+	;1	;2-	;1	3-	ABL
30	F2-D/off/295/2010-87-1-5H	;2	0	3	;1	;1	;2	2+	ABL
31	CD13DZOS F6SR 2013 MS DZLS-10	;2	;1+	3-	;2	;2-	2-	2	ABL
32	CD13DZOS F6SR 2013 MS DZLS-13	;1+	;1+	3	;2	;2-	;1+	1+	ABL
33	CD13DZOS F6SR 2013 MS DZLS-15	;2-	3-	3	;3-	3-	;1+	2+	ABL
34	CD13DZOS F6SR 2013 MS DZLS-18	2-	;1	3	3-	2-	;1	2	ABL
35	CD13DZOS F6SR 2013 MS DZLS-21	;2-	2	3	3-	2	;1+	2+	ABL
36	DW/NVT-LMA	;1	;1	;2-	;2-	;1	;1+	1+	ABL
37	DSP/off/759/2010-45-1H-4H	3-	3-	3	;2+	;2-	;1+	2-	ABL
38	43rdIDSNmeh 82/2011	;2-	;1	3-	3-	2+	2	;1	ABL

39	DW/NVT/LMA16/17	;2-	;1	3	;1	;1	;2-	2	ABL
40	DW/NVT-LMA	;1+	2	3	2-	;1+	;2	2	ABL
41	Ogolcho	2	2-	3	;2-	;1	;1+	2+	BW
42	Sulla	;1	0	;1+	;1+	;1+	;1+	;1+	BW
43	Hoggana	;2	;1	2	3	;	;3-	1+	BW
44	Hidase	;1	;1	;1+	2-	;1+	;1+	;1+	BW
45	Kingbird	;1+	;1	3-	3-	3-	2+	2	BW
46	Wane	;1	;1	2+	;1	2-	;1+	1+	BW
47	36ESWYT	;1	;1	;2-	;1+	;1+	;1+	1	BW
48	Werer (Mamouri- 1)	2+	2	3-	3-	3-	2+	3-	BW
49	Mangudo	2-	2	3	3-	3-	;3	3-	BW
50	23SAWYT	;1+	2	2+	3-	;1+	2	3-	BW
51	ETBW 8311	2-	2+	2+	3-	2-	3-	3-	BW
52	ETBW 8065	2	2	2+	3-	2	;1	;	BW
53	ETBW 8427	2	2+	3	3	3-	;2+	;	BW
54	ETBW 8459	;1	;1	;1+	;1	;1+	;1+	;	BW
55	ETBW 9464	;1	2	;2	3-	2	3	3-	BW
56	ETBW 9466	2	2+	3-	3-	3-	;2	3-	BW
57	14HTWYT	;1+	2-	;2	3-	2+	;2-	3-	BW

58	ETBW 9470	3-	2	3-	3	3-	;1+	3-	BW
59	ETBW 7598	;1	;1	;2	3-	3-	;1+	3-	BW
60	ETBW 9282	;2-	;1	;	;1	2+	3-	2	BW
61	ETBW 9292	3-	3-	3-	3-	2+	3-	;1+	BW
62	ETBW 9486	;1	;1	;2-	3-	3-	3-	2+	BW
63	3WYCYT	;1	;1	;1	;1+	3-	;1+	3-	BW
64	ETBW 9462	;2-	2-	3-	3	;1	;2+	3-	BW
65	ETBW 9463	;1+	2-	;1	3-	3-	;1+	3-	BW
66	ETBW 8862	3-	2	3-	3	3-	3	;1+	BW
67	36ESWYT	;	;1	;	;	;	;1	;	BW
68	ETBW 8804	3-	3-	3-	3	;	3-	;1	BW
69	ETBW 8996	2	3-	3	3	3-	2-	3-	BW
70	ETBW 8583	;2	3-	3-	3-	3-	;2+	3-	BW
71	ETBW 8668	;2+	;2	;2	3	3-	;1+	3-	BW
72	ETBW 9585	;1	;1	;1	3-	2+	;1+	2+	BW
73	ETH-SH15418	3-	3-	3	3	3-	3	3-	BW
74	ETH-SH15436	;1	;1+	;2	;2	3-	;1+	3-	BW
75	ETH-SH15424	3-	3-	3	3-	;1+	;2+	3-	BW
76	Universal Susceptible check (McNair 701)	3	3	3	3	3	3	3-	

8. BIOGRAPHY

The author, Tsega'ab Tesfaye, was born from his mother Bezabish Atebo and his father Tesfaye Wanore in Durame on May 30,1989. He attended his elementary and junior school education at Durame kutir Ande school and secondary school education at Durame senior and preparatory school in Kembata Tembaro zone, Durame town. After completion of his highschool, he applied for the scholarship at Menschen fiir Menschen Agricultural collage for his higher education study at Harar and joined the university.in the department of Crop production, He graduated with Bsc in crop production in July 21,2012. Soon after he joined the Job opportunity creation agency office at Durame agricultural office and served as area representative coordinator for one year. After he got an opportunity to join Ethiopian Institute of Agricultural Research office (EIAR), Ambo plant protection research center (now Ambo Agricultural Research center, where he served as junior researcher, now Assistant researcher I for four years.Then after he joined the school of graduate studies of Hawassa University in October 2017 to pursue his post graduate study in Crop Protection. Now a time he is waiting for successful completion of his Msc degree in Crop protection in 2019