Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 5, pp: (14-23), Month: September - October 2019, Available at: www.noveltyjournals.com

Virulence Spectrum of Wheat Stem Rust (*Puccinia graminis f. sp. tritici*) in Arsi Zones

Worku Abebe

Ethiopian Institute of Agricultural Research, Holleta Agricultural Research Center (HARC), P.O. Box 2003, Holeta, Ethiopia

E-mail: workuabebe2005@gmail.com

Abstract: **Stem rust is the most devastating of all wheat diseases under favorable conditions; because it attacks the above ground parts of the host plant. It remains the most important disease of wheat worldwide, because of its wide distribution, its capacity to form new pathotypes that can attack previously resistant varieties, its potential to develop rapidly under optimal environmental conditions that result in serious yield loss. Until recently, stem rust of wheat has been successfully controlled through genetic resistance. However, in recent years, it has gained significance as new virulence trait (Ug99) evolved in its populations, demonstrating the vulnerability of broadly used wheat cultivars across the globe. The emergence of Ug99 pathotype in Uganda in 1998 and its subsequent geographical expansion within Africa, to the Middle East and the appearance of its variants illustrate the imminent threat to wheat production.**

Ethiopia is one of the East African countries where most of its wheat varieties have become susceptible to the previously known prevalent virulences of stem rust and the newly evolved pathotype Ug99. This scientific information showed the importance of replacing the currently susceptible Ethiopian wheat varieties by effective and durable resistant varieties through breeding programs. However, the development of effective and durable resistant varieties against wheat stem rust pathogen population requires knowledge of virulences and virulence spectra present in the pathogen population. Therefore, it is important to study the virulences and virulence spectra of wheat stem rust pathogen population in Ethiopia, particularly in Arsi where almost all of the two zones are known wheat-growing areas in the country. Hence, this study was initiated with the objective to determine virulence and virulence spectra of wheat stem rust pathogen population in the study area. A total of 36 stem rust samples were collected from randomly selected farmers' fields in Arsi and West Arsi zones and identification of pathotypes as well as their virulence spectrum was studied in a greenhouse using complete randomized design (CRD) in two replications and 13 pathotypes were identified from 31 isolate collections. Pathotype designation was done using the international system of nomenclature for wheat stem rust pathogen population. Reaction of the differential lines tested against the stem rust isolates showed that all of the resistance genes in wheat stem rust differential lines were susceptible to one or the other isolates tested against them and most of the Pathotypes identified during the study were virulent on most of the wheat stem rust differential lines. For example, pathotype TTTTT was virulent on all of the 20 wheat stem rust differential lines and hence, can pose a serious threat to the wheat production in the study area. Likewise, three pathotypes (TPTTT, TTTRT and TTTST); other three pathotypes (PTTRT, TTKRT and TTTRP); other five pathotypes (TTTQP, TTPQT, PTKRT, TTKSK & PTTRP); and one pathotype (TPHTP); ranked; second, third, fourth & fifth orders in their virulence spectrum level being virulent on 19, 18, 17 & 16 of the 20 resistance genes of the differentials, respectively. Therefore, percent virulence spectrum of the Pathotypes on resistance genes of the differentials that ranked first, second, third, fourth & fifth in their virulence spectrum level was 100, 95, 90, 85 & 80% respectively. The total percent virulence spectrum of the Pathotypes ranged 80-100%. These findings indicate that the virulence spectrum of the wheat stem rust pathogen population in the survey area was broad enough to pose serious problem on wheat production in the area of study. The findings of the present study can provide information about the current status of virulence spectra and variability of wheat stem rust pathogen population in Arsi & West Arsi zones and this information can be used by wheat breeders to develop durable wheat stem rust resistant varieties.

Keywords: **Wheat; Stem rust; Differential lines; Virulence spectrum.**

1. INTRODUCTION

Stem rust is the most devastating of all wheat diseases under favorable conditions; because it attacks the above ground parts of the host plant, such as leaves, leaf sheaths, stems, spikes, and even owns (Roelfs *et al.,* 1992). It remains the most important disease of wheat worldwide, because of its wide distribution, its capacity to form new pathotypes that can attack previously resistant varieties, its potential to develop rapidly under optimal environmental conditions that result in serious yield loss (Watkins, 2005). It has been a major blight on wheat production since the rise of agriculture (Mackenzie, 2007). The damage to the host plants is caused by the loss of photosynthetic areas (Bushnell, 1984; Shtienberg; 1990). Nutrients are transported to and accumulated at the infection site and their movement from the infection site to other parts of the plant is reduced (Siddiqui, 1980; Bushnell, 1984). This creates the insufficiency of nutrients to the other organs of the plant and results in imbalance efficiency among the organs and weakens the vigor of the plant parts that are not directly attacked by the disease (Agrios, 2005). In severe rusted plants, shoot desiccation and death may occur due to inadequate capacity of the roots to provide water to the above ground parts of the host plants (Singh, 2005).

Until recently, stem rust of wheat has been successfully controlled through genetic resistance (Singh *et al., 2002*). However, in recent years, it has gained significance as new virulence traits that have evolved in stem rust populations, demonstrating the vulnerability of broadly used wheat cultivars across the globe (Pretorius *et al.,* 2000). The emergence of the Ug99 race in Uganda in 1998, its subsequent geographical expansion within Africa, to the Middle East, and the appearance of Ug99 variants illustrate the imminent threat to wheat production (Singh *et al., 2011*). Estimates suggest that 90% of wheat varieties in the world are susceptible to Ug99, justifying elevated concerns about food security (Singh *et al.,* 2011).

In Ethiopia, variability study on stem rust pathogen population began in the late 1950s and early 1960s (Temam *et al.,* 1985). Presence of races 15, 40 and 88 was reported in Bale, Arsi, Sidamo and Harargie during this period. From 1950 to 1982, a total of 43 stem rust races were identified in Ethiopia. From 1982 to 1988, stem rust composition did not change much and since 1988, 60 races were identified (Sorokina *et al*., 1988). Multi-locational testing of differential lines carried out since 1988 (Temesgen *et al*., 1996) revealed that the reaction of differential lines varied from year to year (Mengistu and Yeshi, 1992; Temesgen *et al*., 1996) indicating the presence of variable virulent races of the pathogen population in the country. During the epidemic of stem rust in 1992, the popular cultivar Enkoy lost its resistance due to race BFR (Temesgen *et al*., 1996) which attacks Sr-gene: 8a, 8b, 9a, 9b, 9g, 13, 15, 17, 28, and 36. Serbessa (2003) identified a total of 17 races of stem rust from Arsi and Bale regions in which race BBDL was the most frequent. Likewise Belayneh *et al*. (2005) identified 39 different races between 2001 and 2002. Similarly, Belayneh *et al. (*2008) identified 22 races where the predominant races were races TTKSR (Ug99), TTHSR and RRTTR with frequencies of 26·6, 17·7 and 11·1%, respectively.

Recent virulence studies conducted in major wheat growing areas of Ethiopia indicated that most of the pathotypes identified during the study were virulent for most of the wheat differentials (Belayneh and Emebet, 2005; Belayneh *et al.,* 2008). Another report showed that Ethiopia is one of the East African countries where most of its wheat varieties have become susceptible to the previously known prevalent virulences of stem rust and the newly evolved pathotype Ug99 (CIMMYT, 2005). This scientific information showed the importance of replacing the currently susceptible Ethiopian wheat varieties by effective and durable resistant varieties through breeding programs. However, the development of effective and durable resistant varieties against wheat stem rust pathogen population requires knowledge of virulences and virulence spectra present in the pathogen population. Therefore, it is important to study the virulences and virulence spectra of wheat stem rust pathogen population in Ethiopia, particularly in Arsi where almost all of the two zones are known wheat-growing areas in the country. Hence, this study was initiated with the objective to determine virulences and virulence spectra of wheat stem rust pathogen population in the study area.

2. MATERIALS AND METHODS

2.1. Description of Sampling Area

Wheat stem rust samples were collected from four districts of Arsi and west Arsi zones**:** Namely Gedeb Asasa, Arsi Negelie, Arsi Robie and Tiyo (around Kulumsa) districts; and all greenhouse studies were carried out at Kulumsa Agricultural Research Centre. Tiyo and Arsi Robie districts are found in Arsi zone whereas Gedeb Asasa and Arsi

Negelie districts are found in west Arsi zone. Gedeb Asasa is located at $7^0$12' N and 39^0 20' E, with an elevation of 2300 masl. The mean annual rainfall of the area is 650 mm while the mean minimum and maximum air temperature is 5.6 and 23.6^{0}C, respectively. The geographic location of Arsi Negelie is 7^0 33' N, and 38^0 66' E, at an altitude of 1950 masl. The district has mean annual rainfall of 985 mm. with a mean minimum and maximum air temperature of 15 and 25.4^0 C, respectively. Arsi Robe is located at 7^0 80' N, and 39° 70' E, with an elevation of 2400 masl. and a mean annual rain fall of 900 mm. Its mean minimum and maximum air temperature is 9.2 and 22.5^0 C, respectively. Tiyo is the district in which Kulumsa Agricultural Research Center (KARC) is located. It is located at 8^0 N and 39^0 12'E, at an altitude of 2200 masl. It has a mean annual rainfall of 820 mm and its minimum and maximum air temperature is 10.1 and 22.7^0 C, respectively.

2.2. Wheat stem rust samples collection

A total of 36 stem rust samples were collected from randomly selected farmers' fields. Stems of wheat plants infected with stem rust were cut into small piece of 5 to 10 cm and placed in paper bags. Then the samples collected in the paper bags were tagged with the name of the zone, district, variety, collector name and date of collection. Thereafter, the paper bags were put above a dried silica gel desiccant in plastic bags just right on the field. This was done to keep stem and/or leaf sheath dry to avoid damage of sample tissues and loss of viability of spores due to high moisture content. Then the samples were transported to Kulumsa Agricultural Research Center and were air dried and placed in a refrigerator at 4°C until the sample collection was completed.

2.3. Experimental Design

The experiment was conducted in a complete randomized design (CRD) with two replications

2.4. Experimental Materials and their Sources

In this study 20 wheat stem rust differential host lines (Table 1) together with the check variety, plastic pots, metal cages and other important greenhouse materials were used and all of them were obtained from Kulumsa Agricultural Research Centre (KARC), Ethiopia. Kulumsa Agricultural Research Center is located at 169 Km South-east of Addis Ababa at 080 01'10''N latitude and 390 09'11'' E longitudes and at elevation of 2200masl. The average annual rainfall of the area is 809mm and the maximum and minimum annual mean temperatures are 23.8^{0}C and 9.89^{0}C, respectively. Kulumsa Agricultural Research Center is a center of excellence for wheat production research in the country.

2.5. Wheat Stem Rust Variability Study in a Green House

2.5.1. Preparation of Mono-Pustule Isolates

Five to six seeds of susceptible wheat cultivar, "Morocco", were planted in 7 cm x 7 cm x 6 cm dimension plastic pots containing sterilized mixture of soil, sand and compost in a ratio of 2:1:1, respectively. These growth medium was sterilized using soil sterilizer at a temperature of 180^{0}C for an hour to avoid soil borne seedling diseases. The seedlings were grown in rust free room to protect them from contamination. When seedlings reach the age of seven day, wheat stem rust urediospores from each sample collection were suspended separately in sterile distilled water. Tween 20 was used as wetting agent to maintain the urediospores in suspension (Roelfs *et al.,* 1992), in order to disperse the spores more or less uniformly in the suspension. Before the inoculation, leaves of the seven days-old seedlings were rubbed gently between clean moistened fingers to remove the waxy layer from the surface which hinders the penetration of the pathogen into the host tissues. After rubbing, the leaves were sprayed with sterile distilled water using hand sprayer. Following this, the prepared urediospores suspensions were gently sprayed on seedlings using atomizer to produce distinctly separated or isolated mono-pustules on the leaves of inoculated wheat seedlings. Urediospores from each field sample collection were inoculated separately, on leaves of single pot seedlings. The inoculation was carried out in an isolation hood in aseptic condition. Soon after inoculation, seedlings were again gently sprayed with sterile distilled water to create artificial dew.

Thereafter, the inoculated seedlings were separately incubated in a small cage, made of metal frame and tray, covered by clear moisture proof polyethylene bag with small layer of water at the bottom to maintain saturated condition for maximum spore germination and penetration of the host tissues (Roelfs *et al*., 1992). Additionally, the inner walls of the plastic cover of the cage were sprayed with sterile distilled water prior to incubation, to maximize the amount of humidity during incubation period of the inoculated seedlings. In this way, seedlings were incubated for 24 hours at 18-22^{0}C in a dark condition. The dark condition was created by placing the dew-chamber under tables and covering each cage with

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 5, pp: (14-23), Month: September - October 2019, Available at: www.noveltyjournals.com

moistened (soaked) Sacs. During the inoculation procedures, material and hands were disinfected with 96 percent ethyl alcohol, followed by washing with detergent after each operation. Washing of materials and hands with detergent was done to avoid any harmful or negative effect of ethyl alcohol on the seedlings.

After 24 hours of dark-dew condition, the dew-chamber was half-open to remove the dew gradually to avoid a sudden drying effect of the dewed seedlings. Thereafter, the cages were top-covered by fine mesh cloth, tightly held in position by rubber bands, before they were transferred to greenhouse benches. Covering the cages was done to avoid spore contaminants that can come into and out of the cages. After these procedures, seedlings were transferred to greenhouse benches and supplementary fluorescent light was provided them for 3-4 hours in controlled condition. The supplementary light was used to finish infection, because many penetration pegs fail to develop from the appersorium unless stimulated by supplementary light for a three to four hours period while the seedlings were slowly dried after the dew period (Roelfs *et al.,* 1992). In this way, the inoculated seedlings were kept on the greenhouse bench until urediospores developed. While the inoculated seedlings were on the greenhouse bench, they were closely inspected for appearance of symptoms. After the appearance of symptoms, one seedling leaf per pot, bearing a single well-isolated uredium from each sample was selected and the rest were removed. Then, pots with single leaf, bearing a single uredium for each field sample was separately placed in cages and top-covered again with clean, fine mesh cloth tightly tied with rubber bands to avoid spore contamination. Fourteen days after inoculation, urediospores were collected separately from each uredium representing each field sample collection to make an isolate for each field sample. Such urediospores collection was carried out continually every two days until each uredium provides enough spores for inoculation of spore multiplication process. The urediospores of mono-pustule isolates were collected by tapping rusted leaves on a piece of glyssine sheet and were sieved using piece of sieve cloth to separate them from plant debris or dead tissues. Then, the collected urediospores of each uredium were separately transferred to petri-dishs and placed over a dried Silica gel desiccant in a desiccator after which sealed with Vaseline. Finally, urediospores of mono-pustule isolates were stored in a refrigerator at 4°C until used. With these sorts of procedures, a total of 31 mono-pustule isolates were obtained from 36 total field samples collected.

2.5.2. Multiplication of Mono-Pustule Isolates

Urediospores of the mono-pustule isolates were multiplied on the variety “Morocco”. The multiplication of urediospores was done to produce sufficient amounts of spores to inoculate wheat stem rust differential host lines. Seedling raising, inoculation, incubation and other subsequent procedures were carried out in the same way to that of the procedures mentioned in 2.5.1. of the earlier, except that in section 2.5.1., seedlings of a single pot were inoculated and placed separately in a separate single cage while in this section the available number of seeds and pots were used to multiply each isolate separately and more than one pots of seedlings, inoculated with the same isolate spores were placed in a single large size Cage.

2.5.3. Determination of Pathotypes and their Virulence Spectra

Thirty-one mono-pustule isolates obtained from 36 original field sample collections were used to determine the pathotypes and their virulence spectra of wheat stem rust pathogen population in the study area. Identification of the Pathotypes and their virulence spectra of the 31 isolates was done using the international wheat stem rust differential host lines with specific resistance genes (Table 1) and check variety, “Morocco”.

Table 1: Code for the 20 differential hosts for *Pgt* in ordered sets of five

		infection types produced on near isogenic Sr lines			
	Host set 1	Sr5	Sr21	Sr9e	Sr7b
	Host set 2	Sr11	Sr6	Sr8a	Sr9g
	Host set 3	Sr36	Sr9b	Sr30	Sr17
	Host set 4	Sr9a	Sr9d	Sr10	SrTmp
Pgt code[a]	Host set 5	Sr7a	Sr8b	Sr13	SrMcN
B		L[b]	L	L	L
C		L	L	L	H

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 5, pp: (14-23), Month: September - October 2019, Available at: www.noveltyjournals.com

D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

Adopted from Roelfs & Martens (1988); Fetch & Dunsmore (2004).[b]L = low/resistant infection type (0 to 2); H = high/susceptible infection type (3 to 4).

2.5.3.1. Seedling Raising in a Greenhouse

Seedlings were raised in a similar way to that of procedures described in section 2.5.1. For this study, a set of internationally recommended, modified North American wheat stem rust differential host lines (Fetch and Dunsmore, 2004) and "Morocco" as a susceptible check were used. The differential host lines possessed resistance genes Sr5, Sr6, Sr7a, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr13, Sr17, Sr21, Sr30, Sr36, SrTmp and Sr-McNair. (Table 1)

2.5.3.2. Seedling Inoculation

The methods and procedures used to prepare urediospores suspensions of the mono-pustule isolates were similar to that of the previous one carried out in this study (2.5.1). All the 20 wheat stem rust differential host lines and the susceptible check variety, "Morocco", were inoculated separately with each mono-pustule isolate of the pathogen population to identify pathotypes and to determine the virulence spectra of the 31 isolates that represent wheat stem rust pathogen population in the study area. Methods and procedures of seedling inoculation, incubation and other subsequent procedures were done in the same way to that of the previous ones mentioned in section 2.5.1.

2.5.3.3. Disease Assessment and Data Collection

Stem rust infection types were scored 14 days after inoculation using the 0-4 scale of Stakman *et al*., 1962 in the following way**:** 0=No uredinia or macroscopic signs of infection (Immune), ;= No uredinia, but hypersensitive necrotic or chlorotic flecks present (Nearly immune), 1= Small uredinia surrounded by necrosis (very resistant), 2=small to medium uredinia often surrounded by chlorosis or necrosis; green islands may be surrounded by chlorotic or necrotic border (Moderately resistant), 3= medium–sized uredinia that may be associated with chlorosis (moderately susceptible) and 4= large uredinia without chlorosis (susceptible). Infection types were classified into low infection types (0-2) and high infection types (3-4). Therefore, in the above procedures, it was possible to detect the virulences matching to resistance genes via the differential set. Pathotype identification and determination of their virulence spectra were done according to the reaction of the tested isolates to different resistant genes in the wheat stem rust differential host lines. Pathotype designation was done by grouping differential lines into five subsets (Table 1) in the order of: (I) Sr5, Sr21, Sr9e, Sr7b, (II) Sr11, Sr6, Sr8a, Sr9g, (III) Sr36, Sr9b, Sr30, Sr17, (IV) Sr9a, Sr9d, Sr10, SrTmp, and (V) Sr7a, Sr8b, Sr13, SrMcN. Each isolate was assigned to a five letter-Pathotype code based on its reaction on the differential lines (Roelfs and Martens, 1988; Fetch and Dunsmore, 2004). For example, a low infection type on the four lines in a set was assigned the letter 'B', while a high infection type on the four lines was assigned a letter 'T'. Hence, if an isolate produced a low

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 5, pp: (14-23), Month: September - October 2019, Available at: www.noveltyjournals.com

infection type on the 20 differential lines, the pathotype would be designated with a five letter Pathotype code 'BBBBB'. Similarly, an isolate producing a high infection type on 20 of the differential lines would have a Pathotype code 'TTTTT'. If an isolate produced a low infection type on Sr36, SrTmp and Sr7a, but a high infection type on the remaining 17 differential lines, the pathotype was designated as 'TTKSK'.

2.5.3.4. Data Analysis

The infection types were recorded and used for data analysis. The analyzed data were used to determine pathotypes and their virulence spectra of stem rust isolates tested against the differential lines used in the study.

3. RESULT AND DISCUSSION

3.1. Wheat Stem Rust Pathogen Variability Study

Though the collected wheat stem rust samples were 36 in number, five of them did not yield viable urediospores during inoculation for spore maintenance. Hence, for this study only 31 isolates of stem rust were used (Table 2) and nine, seven, eight and seven of them were collected from Gedeb Asasa, Arsi Negelie, Tiyo and Arsi Robie districts, respectively. Out of the 31 isolates studied, 13 Pathotypes (Table 3) of the pathogen population were identified.

All resistance genes in wheat stem rust differential lines showed susceptible reaction to one or the other isolates tested against them (Table 2). Twelve isolates showed virulence reaction on all of the host resistance genes of the differential lines used in the study. This can be an indicative for the presence of broad virulence spectrum of wheat stem rust pathogen population in the study area in 2008 main cropping season. These isolates with broadest virulence spectrum of the pathogen population were found distributed in all districts under the study area. Generally 12, 5, 6, 8, 1 isolate(s) showed virulence reaction on 20, 19, 18, 17 and 16 resistance genes of the host differential lines, respectively.

Concerning the frequency of virulent isolates on each resistance gene of the differential lines (Table 2), it has been found that all isolates (100%) displayed virulence on resistance genes Sr5, Sr11, Sr9a, Sr9d, Sr9e, Sr8a, Sr13, Sr7b, Sr9g, Sr17 and SrMcN while 30 of the isolates (96.8%) revealed virulence on resistance genes Sr7a, Sr9b and Sr30, followed by 28 isolates (90.3%) on Sr6 and SrTmp, 27 isolates (87.1%) on Sr36, 24 isolates (77.4%) on Sr21 and Sr8b and 17 isolates (54.8%) on Sr10, respectively.

Table 2: Virulence frequency of stem rust isolates on each resistance genes of wheat stem rust differential lines

	Sr- genes	Number of virulent stem rust isolates	Virulence frequency of stem rust isolates (%)
1	Sr5	31	100
2	Sr11	31	100
3	Sr9a	31	100
4	Sr9d	31	100
5	Sr9e	31	100
6	Sr8a	31	100
7	Sr13	31	100
8	Sr7b	31	100
9	Sr9g	31	100
10	Sr17	31	100
11	SrMcN	31	100
12	Sr7a	30	96.8
13	Sr9b	30	96.8
14	Sr30	30	96.8
15	Sr6	28	90.3
16	SrTmp	28	90.3
17	Sr36	27	87.1
18	Sr21	24	77.4
19	Sr8b	24	77.4
20	Sr10	17	54.8

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 5, pp: (14-23), Month: September - October 2019, Available at: www.noveltyjournals.com

3.2 Designation of Stem Rust Pathotypes

Using the international system of nomenclature for wheat stem rust (Roelfs and Martens, 1988) 13 pathotypes were identified from 31 isolates collected (Table 3). Out of the total 31 isolates, seven isolates each belonged to seven different Pathotypes (Table 3). Similarly six isolates were belonged to three Pathotypes. Six other isolates were grouped into two Pathotypes in which three of them were belonged to each of the two Pathotypes and other 12 isolates belonged to a single Pathotype. The pathotype that comprises of these 12 isolates was a pathotype with the broadest virulence spectrum as well as with the widest spectrum of distribution being virulent to all of the differentials used and being widely found in each district of the study area. Most of the Pathotypes identified during the study were virulent on most of the wheat stem rust differential lines (Table 4 and 5). For example, pathotype TTTTT that comprised of 12 isolates was virulent on all of the 20 wheat stem rust differential lines. Hence, it can be said that this pathotype can pose a serious threat to the wheat production in the study area.

Table 3: Pathotypes identified and frequency of stem rust isolates in each pathotype

No	Pathotypes	Frequency of isolates
1	PTKRT	1
2	PTTRP	3
3	PTTRT	3
4	TPHTP	1
5	TPTTT	2
6	TTKRT	1
7	TTKSK(Ug99)	1
8	TTPQT	1
9	TTTQP	1
10	TTTRP	2
11	TTTRT	2
12	TTTST	1
13	TTTTT	12
Total	13	31

Table 4: % Virulence spectrum of each pathotype of stem rust and number of ineffective Sr-genes

No	Pathotypes	Number of defeated Sr genes	% Virulence spectrum of pathotypes on Sr genes
1	PTKRT	17	85
2	PTTRP	17	85
3	PTTRT	18	90
4	TPHTP	16	80
5	TPTTT	19	95
6	TTKRT	18	85
7	TTKSK (Ug99)	17	85
8	TTPQT	17	85
9	TTTQP	17	85
10	TTTRP	18	90
11	TTTRT	19	95
12	TTTST	19	95
13	TTTTT	20	100

For example, pathotype TTTTT that comprised of 12 isolate was virulent on all of the 20 wheat stem rust differential lines. Hence, it can be said that this pathotype can pose a serious threat to the wheat production in the study area. This is because apart from its broadest virulence spectrum, it has also wide spectrum of distribution being prevalent in all of the study area. Pathotype TPTTT, TTTRT and TTTST were virulent on 19 of the 20 resistance genes of the differentials whereas pathotype PTTRT, TTKRT and TTTRP were virulent on 18 of the 20 resistance genes of the differentials, followed by pathotype PTKRT, PTTRP, TTKSK (Ug99), TTPQT and TTTQP that were virulent on 17 of the 20 resistance genes of the differentials and pathotype TPHTP that was virulent on 16 of the 20 resistance genes of the differentials. Therefore, the virulence spectrum of the Pathotype TTTTT was 100% on resistance genes of the differentials. Pathotype TPTTT, TTTRT and TTTST took second order in their virulence spectrum level being virulent on 19 of the 20 resistance genes of the differentials. Pathotype PTTRT, TTKRT and TTTRP, were ranked third in their order of virulence spectrum level, being virulent on 18 of the 20 resistance genes of the differentials. Pathotype TTTQP, TTPQT, PTKRT, TTKSK and PTTRP, were ranked fourth in their virulence spectrum levels, being virulent on 17 of the 20 resistance genes of the differentials, followed by Pathotype TPHTP that was ranked least in its virulence spectrum level, being virulent on 16 of the 20 resistance genes of the host differential lines.

Table 5: Virulence spectra of 13 pathotypes of stem rust and types of ineffective Sr-genes

Pathotypes	Virulence spectrum																				No	%
PTKRT	5	9e	7b	11	6	8a	9g	9b	30	17	9a	9d	Tmp	7a	8b	13	McN				1	3.2
PTTRP	5	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	Tmp	7a	13	McN				3	9.7
PTTRT	5	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	Tmp	7a	8b	13	McN			3	9.7
TPHTP	5	21	9e	7b	11	8a	9g	9b	17	9a	9d	10	Tmp	7a	13	McN					1	3.2
TPTTT	5	21	9e	7b	11	8a	9g	36	9b	30	17	9a	9d	10	Tmp	7a	8b	13	McN		2	6.5
TTKRT	5	21	9e	7b	11	6	8a	9g	9b	30	17	9a	9d	Tmp	7a	8b	13	McN			1	3.2
TTKSK(Ug99)	5	21	9e	7b	11	6	8a	9g	9b	30	17	9a	9d	10	8b	13	McN				1	3.2
TTPQT	5	21	9e	7b	11	6	8a	9g	36	30	17	9a	9d	7a	8b	13	McN				1	3.2
TTTQP	5	21	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	7a	13	McN				1	3.2
TTTRP	5	21	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	Tmp	7a	13	McN			2	6.5
TTTRT	5	21	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	Tmp	7a	8b	13	McN		2	6.5
TTTST	5	21	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	10	7a	8b	13	McN		1	3.2
TTTTT	5	21	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	10	Tmp	7a	8b	13	McN	12	38.7
Total																					31	100

The common pathotype identified from isolates of all the four districts of the study area was only pathotype TTTTT. The presence of only one common pathotype in the isolates of the four districts may indicate the relatively high pathotype diversity of the study area. This might be attributed to the presence of wide range of geographic distribution and agro-climatic conditions among the surveyed districts and/or physical barriers that may prevent short distance movements of urediospores or physical barriers that may prevent exchange of spores among the surveyed districts.

Variation in range of virulence spectrum was observed among the pathotypes of the four districts. Pathotypes of Tiyo district overcome 18 to 20 (90-100%) resistance genes of the host differential lines whereas the pathotypes of Gedeb Asasa and Arsi Robie districts overcome 17 to 20 (85-100%) resistance genes of the differentials. Similarly, the pathotypes of Arsi Negelie district defeated 16 to 20 (80-100) resistance genes of the differentials. Based on the above virulence analysis, it can be concluded that virulence spectrum of pathotypes identified from isolates of Tiyo district was the widest or broadest (90-100%) of all the districts of the study area, followed by pathotypes identified from isolates of Gedeb Asasa and Arsi Robie districts (85-100%). The narrowest virulence spectrum (80-100%) was analyzed from pathotypes of Arsi Negelie district.

In General, the virulence spectrum of the total pathotypes identified from isolates of the study area was 80-100%. That is to say the pathotypes identified from isolates of all the four surveyed districts made 80-100% of resistance genes of the differentials ineffective (susceptible). These findings indicate that the virulence spectrum of the wheat stem rust pathogen population in the survey area was broad enough to pose serious problem on wheat production in the study area. These findings agree with the study of virulences of wheat stem rust pathogen population conducted for four consecutive years

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 5, pp: (14-23), Month: September - October 2019, Available at: www.noveltyjournals.com

(2001-2004) by Belayneh and Emebet (2005) in all wheat-growing regions of Ethiopia including Arsi zones. The findings of the present study can provide information about the current status of virulence spectra and variability of wheat stem rust pathogen population in the study area and this information can be used by wheat breeders to develop durable wheat stem rust resistant varieties.

REFERENCES

[1] Agrios, G.N.2005. Plant Pathology. 5thEd. Department of Plant Pathology, University of Florida. 922p.

[2] Belayneh, A. and Emebet F. 2005. Physiological races and virulence diversity of Puccinia graminis. f.sp.tritici on wheat in Ethiopia. Ethiopian Agricultural Research Organization: Plant Protection Research center. Ambo, Ethiopia Available at http://e jour-fup. uniti/ index. Php/pm/article/view/1809/1744.

[3] Belayneh, A., V. Lind, W. Friedt and F. Ordon. 2008. Virulence analysis of Puccinia graminis f.sp. tritic i population in Ethiopia with special consideration of Ug99. J. British Soc. Plant pathol. 58(2): 362-369

[4] Bushnell, W.R. 1984. Structural and physiological alteration in susceptible host tissue. pp 477-499 In: Bushnell, W.R. and Roelf, A.P. (eds). The Cereal rusts. Vol. I: Origins, Specificity, Structure and Physiology. Academic press, Orlando.

[5] CIMMYT. 2005. Sounding the alarm on global stem rust: An assessment of race Ug99 in Kenya and Ethiopia and the potential for impact in neighboring regions and beyond. Mexico City, CIMMYT.

[6] Fetch TG, Dunsmore KM, 2004. Physiological specialization of Puccinia graminis on wheat, barley, and oat in Canada in 2001. Canadian J. of Plant Patholo. 26, 148–55.

[7] Mackenzie, D. 2007. Scientists warn of wheat disease: Billions at risk from wheat super-blight. Flutrackers. Com. Available at http://www.flutrackers. com/showthread php?+=59428.

[8] Mengistu Hulluka and Yeshi Andnew. 1992. Stability of the Reaction of Wheat Differential Lines to Stem Rusts at Debre- Zeit, Ethiopia. The Seventh Regional Wheat Workshop for Eastern, Central, and Southern Africa. Nauru, Kenya: CIMMYT, pp 190-195.

[9] Pretorius Z.A, Singh R.P., Wagoire W.W., Payne T.S. 2000. Detection of virulence towheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis. 84:203.

[10] Roelfs, A.P, R.P.Singh, and E.E. Saari. 1992. Rust diseases of wheat: Concepts and methods of disease Management. Mexico, D. F, CIMMYT.pp 68.

[11] Roelfs, A.P. and J. W. Martens. 1988. An International system of nomenclature for Puccinia graminis. f.sp.tritici. Ann.Rev. Phytopath.78:526-533.

[12] Serbessa , N. 2003. Wheat Stem Rust (Puccinia graminis f.sp. tritici) Intensity and Pathogenic Variability in Arsi and Bale zones of Ethiopia. M.Sc. Thesis. The School of Graduate Studies of Alemaya University, Ethiopia.

[13] Shtienberg, D. 1990. Effect of foliar disease of wheat on the physiological processes affecting yields under semi-arid condition. Plant Path. 40: 533-541.

[14] Siddiqui, M.Q. 1980. Some effects of rust infection and moisture stress on growth, diffusive resistance and distribution pattern of labeled assimilates in sun flower. Aust. J. Agric. Res. 31: 719-726.

[15] Singh, R.P, Huerta-Espino, A.P.Roelfs. 2002. The Wheat Rusts. FAO Corporate document repository.

[16] Singh, R.S.2005. Plant Diseases. 8th Ed. Department of Plant Pathology. University of Agriculture and Technology Pantngar, Oxford and IBH publishing Co. PVT. LTD. New Delhi, India. 720p.

[17] Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, and Govindan V (2011). The Emergence of Ug99 Races of the Stem Rust Fungus is a threat to World Wheat Production. Annual Review of Phytopathology, 49:465-81.

[18] Sorokina, G., Gorshkov, A. and Madumarov, T. 1988. Working out methods to enable an improvement in resistance of cereals to the major fungal disease. Scientific phytopathological laboratory (SPL), progress report for the period 1988. Ambo, Ethiopia

[19] Stakman, E. C, Stewart, D. M., and Loegering, W. Q. 1962. Identification of physiologic for races of Puccinia graminis var. tritici. U.S. Agric. stem and leaf rust. Research Service. ARS. E 617.

[20] Temam, H., D.A. Solomatin and Masresha A. 1985. Races of Puccinia graminis f.sp. tritici in Ethiopia. In: Tsedeke Abate (Eds). A Review of Crop Protection Research in Ethiopia. Proceeding of the First Ethiopian Crop Protection Symposium, 4-7 February, 1985. Addis Ababa, Ethiopia.

[21] Temesgen, K. Ayele,B.and Bekele, G. 1996. The current status of stem rust virulence and utilization of bread wheat resistance in Ethiopia. In: D.G. Taner, T.S Payne, and O.S Abdella (eds). Proceedings of the Ninth Regional wheat workshop for eastern, central and south Africa, Addis Ababa, Ethiopia. 494-499.