Analysis of *Puccinia graminis* f.sp. *tritici* Virulence and Reaction of Wheat Varieties to Virulent Races in Tigray

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Abstract

Wheat stem rust caused by Puccinia graminis f.sp. tritici is amongst the biotic factors which cause up to 100% yield loss during epidemic years. The highland of Ethiopia is considered as a hot spot area for the development of stem rust. Hence, this study was carried out to detect the virulence diversity of *P. graminis* f. sp. tritici in Southern Tigray, and evaluate the seedling reaction of commonly grown wheat varieties to virulent stem rust races. The findings of this research were based on race analysis through inoculation of stem rust populations, isolation and multiplication of single-pustule of the pathogen and race determination by inoculating on the standard set of stem rust differential hosts; and testing eleven wheat varieties to three virulent races at seedling stage in a greenhouse. The phenotypic characterization of *P. graminis* f. sp. tritici resulted in identification of 20 races from 32 isolates, which included the most prevalent races TTSNK, RRJJC and HRJJC with a frequency of 9.4% each and the most virulent races TTKSK and TTSSK each making 85% of Sr genes ineffective. Among 20 wheat stem rust differential hosts, four were found effective for 75% and more of the races identified. Differential host carrying Sr24 was effective to all, while gene SrTmp was effective to 90% of the races followed by Sr17 and Sr31 (75% each). In contrast, differential hosts carrying SrMcN, Sr9b, Sr9g and Sr10 were ineffective to 96.9, 93.8, 87.5 and 81.2% of the isolates tested, respectively. On the other hand, of the eight bread wheat varieties evaluated against three virulent races, more than 85% were susceptible to one or more of these races. Varieties Tura, Shina and Kubsa were susceptible to the three (TTKSK, TTSNK and RRTTF) races and TTSNK was virulent to all tested except variety Digalu. In contrast, the durum wheat varieties Gerardo, Asasa and local landrace were resistant to all the races tested. Thus, use of resistant Sr genes(Sr24 and SrTmp) and landraces in single variety through gene pyramiding gives the variety with wider base of stem rust resistance.

Key words: *Puccinia graminis*, races, stem rust, Triticum spp., virulence, wheat

Introduction

Wheat (*Triticum aestivum*) is one of the major crops cultivated in Ethiopia. It is among the cereal crops that contribute significantly to food security in the country. It is the main staple food for about 36% of the Ethiopian population (CIMMYT, 2005). Wheat ranks second both in terms of volume of production and productivity after maize, with the total volume of production of 2.54 million tones at the national level and it ranks third in terms of area coverage with the total area of 1.5 million ha after maize and tef (CSA, 2009). In Tigray region, wheat is a priority cereal crop for food security.

However, productivity of wheat in Ethiopia in general and Tigray in particular is very low. The low productivity is attributed to a number of factors including biotic (diseases, insects and weeds), abiotic and low adoption of new agricultural technologies. Among these factors, wheat stem rust also known as black rust caused by the fungus *Puccinia graminis* f. sp. *tritici* has been the most devastating of all wheat rusts in Ethiopia causing up to complete devastation of wheat crops over wide areas during epidemic years. The high virulence diversity and evolution rate of the pathogen is a threat for wheat production in the country (Belayneh *et al.*,2009). According to Leppik (1970), the highland of Ethiopia is considered a hot spot area for the development of stem rust diversity. Furthermore, studies that were carried out in Ethiopia showed that most races identified were virulent on most varieties grown in the country (Belayenh and Embet, 2005; Belayneh *et al.,* 2009) and are among the most virulent in the world (Van Ginkel *et al.,* 1989).

Wheat stem rust can effectively be controlled by growing resistant varieties. The development of resistant varieties, however, requires a knowledge of the virulence diversity and race distribution in a particular region. In addition, virulence surveys are important for studying the evolution of new races and forecasting the virulence shifts in a population. Investigations on stem rust physiologic race variability and their virulence on *Sr* genes have been going on for several years in Ethiopia, which did not cover all parts of the country like Tigray. In addition, tests to identify new sources of resistance and potential varieties for their current level of resistance to the new races are, at present, a necessity. Hence, this study was initiated to determine the virulence diversity of the pathogen and to test the variety's reaction to virulent races in Tigray.

Materials and Methods

Identification of Physiological Races of *P. graminis* f. sp. *tritici*

Collection of wheat stem rust samples

Stem rust infected samples were collected at 5-10 km interval from wheat fields and trial plots in south Tigray. Stem rust samples were collected from wheat farms in five districts, Alamata, Raya-Azebo, Ofla, Enda-Mekoni, and Emba-Alage in south Tigray. Stems and/or leaf sheath of wheat plants infected with stem rust were cut into small pieces of 5-10 cm using scissors and placed in paper bags after the leaf sheath was separated from the stem in order to keep leaf sheath dry. The samples collected in the paper bags were labeled and transported to Ambo Plant Protection Research Center's (APPRC) greenhouse for analysis.

Isolation and multiplication of single-pustules

Seedlings of the universally rust susceptible variety "Morocco" which does not carry known stem rust resistance genes was raised in suitable 8 cm diameter pots. Seven-day-old seedlings or when the primary leaves were fully expanded and the second leaves begining to grow, the leaves were rubbed gently with clean moistened fingers to remove the waxy layer. Greenhouse inoculations were done using the methods and procedures developed by Stakman *et al.*, (1962). Part of the stem rust infected sample was scrubbed with scalpels on a watch glass and suspended in distilled water to

make rust spore suspension, and then it was rubbed on the seedlings of Morocco. The plants were then moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 18 hours dark conditions at 18-22°C followed by exposure to light for 3-4 hours to provide condition for infection and seedlings were allowed to dry for about 1-2 hours. Then, the seedlings were transferred from dew chamber to glass compartments in the greenhouse where conditions were regulated at 12 hours photoperiod, at temperature of 18 –25°C and RH of 60-70%. The remaining rust spore samples were kept in the refrigerator at 4°C and was used to substitute samples which failed to produce infection on the universally susceptible variety in greenhouse.

After seven to ten days of inoculation (when the flecks/symptoms was clearly visible) leaves containing single fleck that produce single pustule was selected from the base of the leaves and the remaining seedlings within the pots were removed using scissors. A single leaf which contain single pustule was separately covered with cellophane bags (145 X 235 mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004).

After two weeks of inoculation each monopustule was sucked using power operated machine called vacuum pump and collected in gelatine capsule separately. A suspension, prepared by mixing monopustule urediospores with lightweight mineral oil (Soltrol 130), was inoculated on seven-day-old seedlings of the susceptible variety 'Morocco' for multiplication of each monopustule on a separate pot. Immediately after inoculation, the seedlings were placed in a humid chamber in dark condition at 18-22°C for 18 hours and light for 3-4 hours, after which they were transferred to a greenhouse where the temperature varied between 18 and 25°C and RH of 60-70% following the procedures mentioned earlier. In order to avoid cross contamination, seedlings were covered with cellophane bags and tied up at the base with a rubber band. About 14-15 days after inoculation, the spores of each monopustule/isolate were collected in separate test tubes and stored at 4°C. This procedure was repeated until sufficient amount of spores are produced to inoculate the set of stem rust differential hosts. By doing this a total of 32 monopustules/isolates were developed from 16 wheat stem rust samples.

Inoculation of wheat stem rust differential hosts

Five seeds each of the twenty wheat stem rust differential hosts with known resistance genes (*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr31, Sr17, Sr21, Sr30, Sr36, Sr38, Sr24, SrTmp,* and *SrMcN*) and one susceptible variety (Morocco) were grown in 3cm diameter pots separately in a greenhouse. The single pustule isolate spores (approximately 3-5 mg of spores/ml of liquid suspension) was suspended in distilled water and sprayed/ inoculated onto seven-day-old seedlings using atomizers and/or vacuum pump and maintained under the conditions described in section 2.1.2 . Upon removal from the dew chamber, plants inoculated with each isolate were placed in separate glass compartments in a greenhouse to avoid contamination and produce infection. Greenhouse temperature was maintained between 18°C and 25°C. Natural day light was supplemented for additional 4 hours/day with 120µ $E.M^2$ S^1 photo synthetically active radiations emitted by cool white fluorescent tubes arranged directly above plants.

Phenotyping differential sets

Stem rust infection types/IT/ were scored 14 days after inoculation using the 0–4 scoring scale of Stakman *et al.* (1962). Infection types were grouped in to two, where, Low (Resistance) = (0, 0; (fleck), 1, 1+, 2 and 2+) and High (Susceptible) = (3-, 3+ & 4).

Designation of races

Race designation was done by grouping the differential hosts into five subsets (Roelfs & Martens, 1988; Jin *et al.*, 2008; Belayneh *et al.*, 2009). Each isolate was assigned a five letter race code based on its reaction on the differential hosts. For instance, low IT on the four hosts in a set is assigned with the letter 'B', while high IT on the four hosts is assigned with a letter 'T'. Hence, if an isolate produces low infection type (resistant reaction) on the 20

differential hosts, the race will be designated with a five letter race code 'BBBBB'. In the same way, an isolate which produces a high IT (susceptible reaction) on the 20 wheat differential hosts has a race code 'TTTTT' (Belayneh *et al.*, 2009). If an isolate produces a low IT on *Sr36, SrTmp*, and *Sr24,* but a high infection type on the remaining 17 differential hosts, the race will be designated as TTKSK (Ug99) (Jin *et al.*, 2008). The frequency of each race was calculated as a percentage of the total number of isolates analyzed.

The Response of Wheat Varieties to Stem Rust Races at Seedling Stage

The spores of prevalent and virulent stem rust race(s) identified from Southern zone of Tigray were multiplied on the universally susceptible variety Morocco and collected in separate test tubes to inoculate wheat varieties . The seedlings of eight bread and three durum wheat varieties (Table 4) mainly cultivated in Tigray region were evaluated against the selected virulent stem rust races (TTKSK, TTSNK and RRTTF). Seven-day-old seedlings (the first leaf is fully expanded and the second leaf is just emerged), were inoculated with spores (approximately 3-5 mg of spores/ml of liquid suspension) of virulent races and incubated. A Complete Randomized Design (CRD) with three replications was used. Data on infection types were recorded 14 days after inoculation according to the host response.

Results and Discussion

Physiological Races and Virulence Diversity of *P. graminis* f. sp. *tritici* in South Tigray

Of the 22 rust samples collected from farmers' field and research experimental plots of Southern zone of Tigray in 2010 growing season, six samples did not yield viable spores at the time of inoculation in the laboratory. Of the remaining 16 viable samples, a total of 32 single monopustules or isolates (two isolates per sample) were developed using the methods and procedures developed by Stakman *et al.*, (1962). Hence, 32 isolates were used for race analysis. Using the international system of nomenclature for *P. graminis* f. sp. *tritici* (Roelfs and Martens, 1988; Jin *et al.*, 2008), 20 races were identified from the 32 isolates based on their reaction on 20 differential hosts. This showed that most of the monopustules from individual fields varied in their race groups, and only some belonged to the same race group. The highest race composition was detected from Raya-Azebo district accounting for 65% of the races identified. The worldwide important races like RRTTF, TTSNK, TTKSK and TTSSK were detected from Raya-Azebo district on variety Dashen making wheat production under threat in the area. The remaining 35% of the races were detected from Raya-Alamata, Ofla and Enda-Mekoni districts (Table 1).

Table 1: Prevalence of *P. graminis* f. sp. *tritici* races across district

District	Race	Isolates tested	Remark
Raya-Alamata	BBBBC, HHSTF and JRGSC	4	
Raya-Azebo	BBBLC, BHJBC, CCGBC, GMHJC, HRJJC, JTGDB, RRTTF, SKQNH, SPSSF, TCQJH , TTKSK, TTSNK and TTSSK	22	
Ofla	DBHQC and DBHSC	2	
Enda-Mekoni	GKJSF and RRJJC	4	
Emba-Alaje	-	-	No viable spore

Of the 20 races, the most frequent and predominant races identified were TTSNK, RRJJC and HRJJC with a frequency of 9.4% each. The second most frequent and dominant races were BHJBC, GMHSC, HHSTF, RRTTF, SPSSF, and SKGNH, with a frequency of 6.3% each. Whereas, the remaining races were detected only once each with frequency of 3.1% (Table 1).

The 20 races identified in Southern zone of Tigray had wide virulence spectrum (Table 2). The broad virulence spectrum was recorded on races TTKSK and TTSSK making 17 stem rust resistance genes ineffective. The most devastating stem rust race TTKSK (Ug99) virulent on gene *Sr31* was first detected in Uganda in 1999 (Pretorius *et al.*, 2000), and had spread to most of the wheat growing areas of Kenya in 2002 and Ethiopia in 2003. In 2005, reports confirmed its presence in six dispersed locations of Ethiopia (Singh *et al.*, 2008), and spread to most wheat growing regions of the country and became the main threat of wheat production (Belayneh *et al.*, 2009). TTKSK was virulent to 17 *Sr* genes except *Sr36, Sr24,* and *SrTmp*. Furthermore, the new Ug99 variant TTSSK, which is identified in this study, was also detected in Kenya in 2006 and 2007 with virulence to gene *Sr36* indicating the evolution of Ug99 (Singh *et al.*, 2008). This race was virulent to all the resistance genes except *Sr17, SrTmp* and *Sr24*. Likewise , TTSNK and RRTTF were equally virulent to 80% of the stem rust resistance genes tested. On the other hand, eight races or 40% of the races identified were virulent on less than 50% of the 20 *Sr* genes included in the test. Race BBBBC was the least virulent, producing susceptible reaction only on monogenic gene, *SrMcN*. Races such as BBBLC, CCGBC, BHJBC, DBHQC and DBHSC were also less virulent, producing susceptible reactions on only two, four, five, six, and seven wheat differential hosts, respectively.

In general, this study confirmed the presence of wider range of virulence in the area and is inline with previous works conducted in Ethiopia (Belayenh and Emebet, 2005; Belayneh *et al.*, 2009). A comparison of the races identified in the present study with earlier reports revealed differences. This could be due to variation over locations and time, as the races prevalent 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).

Most of the races in Ethiopia varied from one another by single-gene changes (Belayneh *et al.*, 2009). In this study, eight races or 40% of the races identified varied by single-gene changes. For instance, race TTSSK was similar to TTSNK with additional virulence to *Sr9d*. In the same way, races BBBLC, DBHSC, and RRJJC were similar to BBBBC, DBHQC, and HRJJC with additional virulences to *Sr9a, Sr10* and *Sr5*, following the same order mentioned (Table 2). Such single-step changes in virulence were reported to be the main process of evolutionary change in *P. graminis* f. sp. *tritici* populations (Green, 1975; Belayneh *et al.*, 2009).

Table 2: Virulence spectrum and frequency of races of *P. graminis* f. sp. *tritici* collected from Southern zone of Tigray in 2010

Race	Ineffective *Sr* genes	No. of isolates	Frequency (%)
BBBBC	*McN*	1	3.1
BBBLC	*9a, McN*	1	3.1
BHJBC	*6, 9g, 9b, 30, McN*	2	6.3
CCGBC	*7b, 9g, 9b, McN*	1	3.1
DBHQC	*9e, 9b, 17, 9a, 9d, McN*	1	3.1
DBHSC	*9e, 9b, 17, 9a, 10, 9d, McN*	1	3.1
GKJSF	*21, 6, 8a, 9g, 9b, 30, 9a, 9d, 10, 38, McN*	1	3.1
GMHJC	*21, 11, 6, 9g, 9b, 17, 9d, 10, McN*	2	6.3
HHSTF	*21, 7b, 6, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, 38, McN*	2	6.3
HRJJC	*21, 7b, 11, 6, 9g, 9b, 30, 9d, 10, McN*	3	9.4
JRGSC	*21, 9e, 11, 6, 9g, 9b, 9a, 9d, 10, McN*	1	3.1
JTGDB	*21, 9e, 11, 6, 8a, 9g, 9b, 10,*	1	3.1
RRJJC	*5, 21, 7b, 11, 6, 9g, 9b, 30, 9d, 10, McN*	3	9.4
RRTTF	*5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	2	6.3
SKQNH	*5, 21, 9e, 6, 8a, 9g, 36, 9b, 9a, 10, 31, McN*	2	6.3
SPSSF	*5, 21, 9e, 11, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, 38, McN*	2	6.3
TCQJH	*5, 21, 9e, 7b, 9g, 36, 9b, 9d, 10, 31, McN*	1	3.1
TTKSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*	1	3.1
TTSNK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 36, 30, 9a, 10, 31, 38, McN*	3	9.4
TTSSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 36, 30, 9a, 9d, 10, 31, 38, McN*	1	3.1
Total		32	100

Virulence frequency of *P. graminis* f. sp. *tritici* isolates to *Sr* resistance genes

It was evident that the majority of the resistance genes were found ineffective against most of the isolates tested in this study. About 55% of the *Sr* genes were ineffective to more than 60% of the isolates. The differential host carrying the resistance gene McNair 701 (*SrMcN*) was ineffective to 96.9% of the isolates tested. Similarly, six differential hosts carrying resistance genes *Sr9d, Sr21, Sr6, Sr10, Sr9g* and *Sr9b* were ineffective, with virulence frequency of 65.6, 78.1, 75, 81.2, 87.5 and 93.8% to the isolates tested, in that order (Table 3). Belayneh *et al.*, (2009), reported similar finding, McNair 701 was susceptible to all of the races identified. By the same token , according to the same authors seven stem rust resistance genes; *Sr9a, Sr9g, Sr10, Sr7b, Sr9b, Sr9d* and *Sr8b* were ineffective to more than 96% of the isolates collected during 2006-2007 cropping season from different regions of Ethiopia. Earlier studies indicated that virulence to *Sr6, Sr8b, Sr9a, Sr9d* and *Sr11* is common worldwide (Roelfs *et. al.*, 1992).

Table 3: Virulence frequency of *P. graminis* f. sp. *tritici* isolates (32 isolates) on 20 *Sr* genes

Sr gene	Virulence frequency (%)	*Sr* gene	Virulence frequency (%)
5	46.9	*30*	62.5
21	78.1	*17*	21.9
9e	43.8	*9a*	56.25
7b	53.1	*9d*	75.0
11	59.4	*10*	81.5
6	75.0	*Tmp*	12.5
8a	31.3	*24*	0.0
9g	87.5	*31*	25
36	40.6	*38*	37.5
9b	93.8	*McN*	96.9

In contrast, the stem rust resistance gene *Sr24* was found effective to all stem rust isolates collected from Southern Tigray region. This confirms the report of Roelfs *et al.*, (1992), which stated that this gene is amongst the effective genes, which have an adequate and some immediate values to almost all races in the world. But, virulence to *Sr24* was reported in Kenya in 2006. A variant of Ug99 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). Furthermore, *Sr24* was also defeated by another race PTKST which is detected in Ethiopia in 2007, Kenya and South Africa in 2009. This represented the first confirmed occurrence of Ug99 variant with virulence to *Sr24* in Ethiopia (FAO, 2011).

Five resistance genes, *SrTmp, Sr17, Sr31, Sr36* and *Sr38* were found to be effective against most of the stem rust races detected in this study. Of these *Sr* genes, differential hosts carrying *SrTmp, Sr31* and *Sr17* were resistant to 87.5, 75 and 78.1% of the isolates tested, respectively. The diiferential host carrying *Sr17* which was ineffective to more than 95% of the isolates (Belayneh et al., 2009), was found effective to 78.1% of the tested isolates in this study. This could be the genes that allow development of only microscopic or macroscopic hypersensitive reactions and/or specific resistance is due to dominant genes in the host (Roelfs *et. al.*, 1992). Gene *Sr38* was effective for about 62.5% followed by *Sr36* which was effective against 59.4% of the isolates analyzed in this year (Table 3). Belayneh *et al.*, (2009) reported similar finding that *Sr36* and *SrTmp* were effective for 81.6 and 76.3% of the isolates, respectively for the samples collected from Shewa, Arsi, Bale and northwest regions of Ethiopia.

Reaction of Wheat Varieties to *P. graminis* Races at Seedling Stage in Greenhouse

Genetic resistance to wheat stem rust has largely been based on two types of resistance; seedling and adult plant resistance. Seedling resistance genes which also work at the adult plant stage usually confer strong resistance response (Singh *et al.*, 2008). The reaction of wheat varieties to stem rust races in the greenhouse revealed that none of these varieties were immune. Among the tested wheat varieties three (Tura, Shina and Kubsa); five (Dashen, Tura, Shina, Sirbo and Kubsa) and seven (Dashen, Tura, Shina, Sirbo, Hawi, KBG01 and Kubsa) of them produced susceptible reaction to RRTTF, TTKSK and TTSNK races, respectively. whereas, bread wheat variety Digalu was resistant to all the tested races.

Unlike bread wheat varieties, all the three durum wheat varieties Gerardo, Asasa and local were resistant to the three stem rust races tested (Table 4). The local variety "*Tselim Sirnay*" to mean "black" wheat showed resistance to the virulent races tested. According to Mujeeb-Kazi and Rajaram (2002), report landraces are priority, as they may possess a wide range of variation, specific adaptation to the different environments in regions of growth, and resistance or tolerance to diseases in general and stem rust in particular.

Table 4. Reaction of wheat varieties to three virulent stem rust races identified in southern Tigray, 2010.

Variety	Code	Pedigree	Race		
			TTKSK	TTSNK	RRTTF
Dashen	HAR 408	VEE 17/KVZ/BUHO"S" //KAL/BB	3	3	1+
Tura	HAR-1775	ARO YR SEL. 60/89	3-	3-	3-
Hawi	HAR-2501	CHIL/PRL	2	3-	1+
Shina	HAR-1868	GOV9/AZ//MUS"S"/3/R37GHL/21//KAL/BB/4/ANI"S"	2+	3-	0;
KBG-01	FH-1-7-A	300 /SM+501M/HAR 1709	2+	3-	0;
Sirbo	HAR-2192	VS73.600/MRL/3/BOW//YR/TRF (MILLAN)	3	3	2
Digalu	HAR 3116	SHA7/KAUZ	1+	1+	2
Kubsa	HAR1685	NDG9144//KAL/BB/3/YACO"S"/4VEE#5"S"	3-	3-	3-
Gerardo	Durum	VZ466/61-130XLD SX GII"S" CM9605	1+	1	1
Asasa	Dz2085	CHO"S"/TARUS//YAV"S"3/FG"S"/4/ FGS/CR"S"/5/DZ2085	1+	1	2
Local			1+	1	1
Morocco (Sus.ck)			3	3	3-

Resistant IT (0 to 2+), susceptible IT (3- to 4); (-) uredia smaller, (+) uredia larger than normal

A variation in resistance was observed between durum and bread wheat varieties. According to this study, durum wheat showed a broader resistance spectrum than bread wheat. This might be associated with the fact that most of the durum wheat varieties were developed from local landraces, which have co-evolved with indigenous pathogen populations (Belayneh *et al.*, 2009). The results of this study also support this fact and show that durum wheat varieties and the local could be valuable sources of resistance to the stem rust races in the area. This finding was also in agreement with previous reports, which stated that the Ethiopian cultivated tetraploid wheat accessions are resistant or moderately resistant to stem rust, and the landraces are found to be a potential source of resistance to stem rust (Beteselassie *et al.*, 2007).

References

Belayneh, A., Lind, V., Friedt, W., and Ordon, F. 2009. Virulence analysis of *Puccinia graminis* f.sp. *tritici* populations in Ethiopia with special consideration of Ug 99. Plant Pathology 58: 362-369.

Belayneh, A., and Emebet, F. 2005. Physiological races and virulence diversity of *P. graminis* f.sp. *tritici* on wheat in Ethiopia. Phytopathologia Mediterranea 44(3): 313–318.

Beteselassie, N., Fininsa, C., and Badebo, A. 2007. Source of stem rust reistance in Ethiopia tetraploid wheat accessions. African Crop Science Journal 15: 51-57.

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. Prepared by the expert panel on the stem rust outbreak in Eastern Africa. 29 May 2005. CIMMYT, Mexico. pp 1-22.

CSA. 2009. Country Level Agricultural Products Producer Price Index, (Ag-PPI). Addis Ababa, Ethiopia. pp 20-25.

FAO. 2011. Stem Rust Archive. A Golobal wheat rust monitoring system. Available at http://www.fao.org/agriculture/crops/rust/stem/rust-report/stem-report/en/ Accessed: January 14, 2011.

Fetch, TG., and Dunsmore, KM. 2004. Physiological specialization of *P. graminis* on wheat, barley, and oat in Canada in 2001. Canadian Journal of Plant Pathology 26: 148-55.

Green GJ. 1975. Virulence changes in *Puccinia graminis* f. sp. *tritici* in Canada. Canadian Journal of Botany 53: 1377-1386.

Jin, Y., Szabo, LJ., Pretorius, ZA., Singh, RP., Ward, R., and Fetch, TJ. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Disease 92: 923-926.

Leppik EE. 1970. Gene centers of plants as sources of disease resistance. Ann. Rev. of Phytopathol.8:323-344

Mujeeb-Kazi, A., and Rajarm, S. 2002. Transfering alien genes from related species and genera for wheat improvement. *In*: Citrus, B.C., Rajaran, S. and Macpherson Gomez, H. (eds.). Bread wheat improvement and production. Plant Production and Protection Series No. 30. FAO, Rome, pp. 199-216.

Pretorius, ZA., Singh, RP., Wagoire, WW., and Payne, TS. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Phytopathology. 84: 203.

Roelfs AP., and Martens, JW. 1988. An international system of nomenclature for *P. graminis* f. sp. *tritici*. Phytopathology. 78: 526–533.

Roelfs, AP., Singh, RP., and Saari, EE. 1992. Rust Diseases of Wheat: Concept and Methods of Disease Management. Mexico, D.F: CIMMYT. pp 81.

Singh, RP., Hodson, DP., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, SA., and Ward, RW. 2008. Will Stem Rust Destroy the World's Wheat Crop? Advances in Agronomy, 98: 271-309.

Stakman, EC., Stewart, DM., and Loegering, WQ.1962. 'Identification of physiologic races of *Puccinia graminis* var. *tritici*.' USDA ARS, E716. United States Government Printing Office: Washington, DC pp 5-50.

Van Ginkel, M., Getinet, G., and Tesfaye, T. 1989. Stripe, stem and leaf rust races in major wheat producing areas in Ethiopia, IAR News letter of agricultural research 3(4): 6-8.