RESEARCH ARTICLE

Virulence of wheat leaf rust and reaction of cultivars to virulent races in Tigray, Northern Ethiopia

Tesfay Gebrekirstos[1], Getaneh Woldeab[2] , Thangavel Selvaraj[3] and Arvind Chavhan[4*]

[1]Tigray Agricultural Research Institute (TARI), Mekelle Agricultural Research Center P.O. Box 258, Mekelle, Ethiopia.
[2]Ethiopian Institute of Agricultural Research (EIAR), Ambo Plant Protection Research Center P.O. Box 37, Ambo, Ethiopia. | [3]Ambo University P.O. Box 19, Ambo, Ethiopia.
[4]Digambarrao Bindu College, Bhokar, Nanded, 431801, MS, India,
*Corresponding author: myhopera2002@yahoo.com/tesfaygebre2009@gmail.com

Manuscript details:

Received: 07.03.2017
Accepted: 24.05.2017
Published : 30.06.2017

Cite this article as:
Tesfay Gebrekirstos, Getaneh Woldeab, Thangavel Selvaraj and Arvind Chavhan (2017) Virulence of wheat leaf rust and reaction of cultivars to virulent races in Tigray, Northern Ethiopia, *International J. of Life Sciences*, 5 (2): 189-197.

Acknowledgements
The author gratefully acknowledges Dr. Getaneh Woldeab for his technical support. The East African Agricultural Productivity Project and Tigray Agricultural Research Institute are acknowledged for providing full financial support to the first author

ABSTRACT

This study was carried out to determine the virulence of *P. triticina* and evaluate the reaction of wheat cultivars to virulent races. Race analysis was carried out by inoculating isolates on to the 16 differential hosts. A total of 22 races were identified of which; PHTT, PHRT, THTT and FHRT were predominant races with frequencies of 20, 15, 10 and 10% respectively. The remaining 18 races were confined to specific locations with a frequency of 2.5% each. The broadest virulence spectrum was recorded from TKTT race, making 15 Lr genes ineffective. About 81% of Lr genes were ineffective to 55% of *P. triticina* isolates. Races TKTT, THTT and PHTT were used to evaluate the resistance of ten wheat cultivars in greenhouse. Bread wheat cultivars, Mekelle-3, Mekelle-4, Picaflor, Dashin and Local cultivar showed susceptible reaction to TKTT, THTT and PHTT races. Unlike bread wheat varieties, durum varieties, Ude and Dembi were resistant to these races. The result of this study showed most bread wheat varieties did not have adequate resistance for leaf rust. Hence, gene pyramiding of Lr9, Lr24 and Lr2a has paramount importance as the additive effects of several genes offer the variety a wider base for leaf rust resistance.

Key words: Race, *Puccinia triticina*, *Lr* genes and differential hosts

INTRODUCTION

Wheat (*Triticum* Spp.) is one of the major cereal crops grown in the highlands of Ethiopia and this region is regarded as the second largest wheat producer in Sub-Saharan Africa (White *et al*., 2001). 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 in terms of production after maize (Zea mays L.) with the total production of 2.54 million tons at the national level and third in terms of area coverage with the total area of 1.5 million hectare after maize and tef (*Eragrostis tef*) (CSA, 2009).

In Tigray region, wheat has been selected as one of the target crops in the strategic goal of attaining regional food self-sufficiency. However, productivity of wheat in Ethiopia in general and Tigray in particular is very low. The low productivity of the crop is attributed to number of factors including biotic (diseases, insects and weeds), abiotic (drought, acidity, depleted soil fertility, and extreme temperatures) and low adoption of new agricultural technologies (Ayele *et al*., 2008). Among these factors, wheat leaf rust, caused by the fungal pathogen *Puccinia triticina* Eriks and Henn. is one of the most important foliar diseases of wheat in our country. Yield loss due to wheat leaf rust reached 75% in susceptible varieties at hot spot areas of Ethiopia (Mengistu *et al*., 1991). The high virulence diversity and evolution rate of the pathogen makes a lot of wheat cultivars at risk in our country. For instance, out of the 26 wheat cultivars released in the period 1970 to 1993, only three retained their resistance to leaf rust (Geleta and Tanner, 1995).

Use of resistant variety is the most economical and environmentally friendly method of controlling wheat leaf rust (Afzal *et al.,* 2009). The development of resistant varieties, however, requires race analysis and identification of effective resistant genes. Moreover, the improved wheat varieties and local landraces which are under production has to be tested their resistance to the newly races of wheat leaf rust. Hence, this study was initiated to determine the virulence of wheat leaf rust and reaction of cultivars to the virulent races.

MATERIALS AND METHODS

Collection of wheat leaf rust samples

Samples of infected leaves (one sample per field) were collected at 5-10 km interval from trial plots and farmers' fields of Hintalo-wajirate, Saharti-samre, Enderta, Degua-temben and Wukro districts. Accordingly, 11, 7, 10, 2 and 10 samples were collected from Wukro, Enderta, Saharti-samre, Degua-temben and Hintalo-wajirate districts respectively. Infected leaves were cut using scissors from the mother plant and placed in paper bags in order to dry the leaf. This technique help the samples easily air dry so as the spores cannot germinate before processing in the greenhouse. The samples collected in the paper bags were labeled with all necessary information and transported to Ambo Plant Protection Research Center Laboratory for races analysis and cultivar evaluation studies.

Multiplication of P. triticina *inoculums*

Seedlings of the universally rust susceptible variety "Morocco" which does not carry known leaf rust resistance genes (Roelfs *et al*., 1982) were raised in 8 cm diameter pots containing stem sterilized soil, sand and manure in the ratio of 2:1:1 mixture respectively. Leaves with fully expanded primary leaves and second leaves beginning to grow, were rubbed gently with clean and moistened fingers. Green house inoculations were done using the methods and procedures developed by Stakman *et al*. (1962). Spores from the leaf rust infected samples were isolated with scalpels and collected on to a watch glass which contain distilled water to make spore suspension, and then rubbed on seedlings of Morocco with clean moistened fingers. Plants were then moistened with fine droplets of distilled water produced with an atomizer and incubated in a dark dew chamber for 24 hours at 18-22^{o}C and 90% relative humidity. Then, the seedlings were transferred from the dew chamber to glass compartments where conditions were regulated at 12 hours photoperiod, 18-25^{o}C and 60-70% of relative humidity to provide suitable condition for infection. The remaining rust spore samples were kept in refrigerator at 4^{o}C to substitute samples which failed to produce infection on the universally susceptible variety.

Multiplication of Mono pustules

After seven days of inoculation, when the flecks/chlorosis were clearly visible, leaves containing single flecks were selected from the base of the leaves and the remaining leaves within the pots were removed using scissors. Only 2-3 leaves which contains mono pustule were covered separately with cellophane bags (145 X235 mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004). After 12-14 days of inoculation, when the mono pustule was well developed, each mono pustule collected using power operated vacuum aspirator and stored separately in gelatin capsule. A suspension, prepared by mixing mono pustule urediospores with distilled water was inoculated on seven day- old seedlings of 'Morocco' for multiplication of each mono pustule on separate pots. After inoculation, seedlings were placed in dew chamber for 24 hours at 18-22^{o}C and with relative humidity of 90%. Then after, seedlings were transferred to growth chamber where conditions were

regulated at 12 hours photoperiod, 18-25°C and relative humidity of 60-70% following the procedures mentioned earlier. After 12-14 days of inoculation, spores from each mono pustule were collected in separate test tubes and stored at 4°C until they were inoculated on the standard differential sets. This procedure was repeated until sufficient amount of spores were produced to inoculate wheat leaf rust differential sets. In this way, a total of 40 mono pustule or isolates were developed from 40 wheat leaf rust samples.

Inoculation of isolates to differential sets

Six seeds of the sixteen wheat leaf rust differentials with known resistance genes (Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a and Lr18) and susceptible variety Morocco were grown in 3 cm diameter pots separately in greenhouse. The susceptible variety Morocco was used to ascertain the viability of spores inoculated to the differential hosts. The single pustule derived spores (approximately 3-5 mg of spores per ml of liquid suspension) was suspended in distilled water and sprayed onto seven-day-old seedlings using atomizers.

After inoculation, plants were moistened with fine droplets of distilled water produced with an atomizer and placed in dew chamber for 24 hours at18-22°C and Relative humidity of 90%. Up on removal from the dew chamber, plants were placed in separate glass compartments in a greenhouse to avoid contamination. Greenhouse temperature were maintained between 18-25°C. Natural day light was supplemented for 12 hours/day with 120 μ $E.M^{-2}$ S^{-1} photo synthetically active radiations emitted by cool white fluorescent tubes arranged above plants.

Phenotyping of differential sets

Phenotyping of differentials was based on the reaction of the inoculated differential hosts. Leaf rust infection types (ITs) were scored 12-14 days after inoculation using the 0-4 scale (Long and Kolmer, 1989). Infection types were grouped in to two, where, low (resistance) = (0, 0; (fleck), 1, 1+, 2 and 2+) and high (susceptible) = (3-, 3+ and 4).

Designation of races

Race designation was done by grouping the sixteen differential hosts into four sets in the following order: (i) *Lr1, Lr2a, Lr2c, Lr3*; (ii) *Lr9, Lr16, Lr24, Lr26*; (iii) *Lr3ka, Lr11, Lr17, Lr30* and (iv) *LrB, Lr10, Lr14a, Lr18*. Each isolate was assigned a four letter race code based on its reaction on the differential hosts (Long and Kolmer, 1989).

Table 1: Nomenclature of *P. triticina* races on 16 differential hosts in ordered sets of four

	Host set	Infection type (ITs) produced on differential *Lr* lines			
	Host set 1	1	2a	2c	3
Pt code	Host set 2	9	16	24	26
	Host set 3	3ka	11	17	30
	Host set 4	B	10	14a	18
B		L	L	L	L
C		L	L	L	H
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

Source: Long and Kolmer, 1989

Response of wheat cultivars to virulent races of *P. triticina* **at seedling stage in greenhouse**, Spores of prevalent and virulent *P.triticina* races identified from the Southeastern zone of Tigray were multiplied on Morocco and collected in separate test tubes to inoculate wheat cultivars. The seedlings of ten wheat cultivars mainly cultivated in the Tigray region were evaluated against the virulent and prevalent races (TKTT, THTT and PHTT) of leaf rust. Seven-day-old seedlings were inoculated with the spores (approximately 3-5 mg of spores per 1 ml of liquid suspension) of the selected races and incubated following the procedures and methods mentioned earlier. Data on infection types was recorded 12-14 days after inoculation using the standard diseases scoring scale 0-4 (Long and Kolmer, 1989). Eight bread wheat varieties (Mekelle-1, Mekelle-2, Mekelle-3, Mekelle-4, Picaflor, Digalu, Dashin, and local cultivar) and two durum wheat varieties (Ude and Dembi) were evaluated their response against the most prevalent and virulent races of *P.triticina*

RESULTS AND DISCUSSION

Distribution and diversity of *P. triticina* races across districts

Though most of the races were confined to specific districts, some had wider spatial distributions. Four races (FHRT, PHRT, PHTT and THTT) were predominant, representing 55% of the isolates analyzed. Races PHTT and PHRT were the most predominant with frequencies of 20 and 15% respectively, followed by THTT and FHRT with a frequency of 10% each. These races were detected from three to four districts of the study area (Table 2), which indicated that they were widespread throughout Southeastern and Eastern zones of Tigray. PHTT was detected eight times in the population of wheat leaf rust collected from Wukro, H/wejirat and Enderta districts while, PHRT detected six times from Saharti-samre, Dedua-temben, Wukro and Enderta samples of wheat leaf rust. On top of this, PHRT was identified as the most distributed race and adapted to

Table 2. Distribution of *Puccinia triticina* races across districts of Eastern and Southeastern zones of Tigray

	Districts						
Races	Wukro	Enderta	S/samre	D/Temben	H/wejirat	Isolates	Frequency (%)
BBBT	-	-	1	-	-	1	2.5
BBQR	-	-	1	-	-	1	2.5
CBBT	-	1	-	-	-	1	2.5
FGRT	-	-	1	-	-	1	2.5
FGTT	-	-	1	-	-	1	2.5
FHRT	1	1	-	-	2	4	10
FHTT	1	-	-	-	-	1	2.5
LBBM	-	-	-	-	1	1	2.5
LBDC	-	-	1	-	-	1	2.5
MBBR	-	1	-	-	-	1	2.5
MCST	-	-	-	-	1	1	2.5
MGJT	-	1	-	-	-	1	2.5
MHTT	-	-	-	-	1	1	2.5
PCRR	-	-	-	-	1	1	2.5
PGRT	1	-	-	-	-	1	2.5
PHRT	1	1	2	2	-	6	15
PHTT	4	1	-	-	3	8	20
PJTT	-	-	1	-	-	1	2.5
RCJT	-	1	-	-	-	1	2.5
RHTT	1	-	-	-	-	1	2.5
THTT	1	-	2	-	1	4	10
TKTT	1	-	-	-	-	1	2.5
Total	11	7	10	2	10	40	100

wide agro ecologies of the study area. Races, THTT and FHRT also isolated four times each from districts of Wukro, Saharti-samre and Hintalo-wejirat and Wukro, Enderta and Hintalo-wejirat respectively.

The predominance of races of *P.triticina* in these districts provides evidence of clonal lineages and short distance migration of this pathogen within the study area. On the other hand, approximately 82% of the races including the most virulent race TKTT, were confined to specific locations and detected only once with a frequency of 2.5% each (Table 2).

The distribution and diversity of *P.triticina* races indicated that, genetic similarity among isolates of within and between districts of the study area was existed. The three adjacent districts (Wukro, Hintalo-wejirat and Enderta) had two similar races, FHRT and PHTT out of eight, seven and seven races detected, in that order. Likewise, Wukro and S/samre districts had two races in common, PHRT and THTT out of eight each respectively. Their geographic proximity, absence of barriers and cultivation of similar bread wheat cultivars among these districts might have played significant role for race similarity.

Degua-temben district on the other hand is geographically isolated by mountains from other districts. Thus, the possibility of migration of urediospores of wheat leaf rust to and from this district is restricted and low diversity among *P.triticina* population is expected in this district.

In contrast, the 'within district' comparison had also indicated that, isolates collected from Enderta showed genetic diversity among the populations of wheat leaf rust. The seven isolates collected from this district yielded seven races (RCJT, PHTT, CBBT, FHRT, MBBR, MGJT and PHRT) (Table 2). The high level of race diversity in this district might be resulted from the windy nature of this area. This area was identified as the second windiest place in Ethiopia (http:// www.eepco.gov.et > home> projects> Ashegoda wind farm /2013/ November).

Hence, the movement of *P.triticina* urediospores via wind from their sources to or from this area is a common phenomenon in rusts in general and leaf rust in particular. This circumstance might be resulted, more heterogeneity in the population of wheat leaf rust and finally the chance of detecting different races in this district become increased**.**

Virulence spectrum of *P. triticina* races

Virulence spectrum was determined by the number of differential lines that the isolate showed virulence. In this case, an isolate having virulence on more leaf rust resistance genes was considered to have wider spectrum compared to those isolates with virulence to relatively lower number of differential lines (Sewalem *et al*., 2008). In view of this, approximately 73% of the races had virulence spectra raging from 9 to 15 *Lr* genes. The widest virulence spectrum was recorded from TKTT race making 15 *Lr* genes ineffective (Table 3). Though, this race was not widely distributed, it seems to be important in that it attacks all the members of the differential hosts except *Lr9*. In addition, this race has a potential to cause heavy infection on many bread wheat varieties grown in areas where this race was discovered. Similarly, races THTT was also the second most virulent race making 14 *Lr* genes susceptible. The virulence spectrum of *P.triticina* indicated that, some races showed the same virulence spectrum on the *Lr* genes. For instance, three races (RHTT, PHTT and PJTT), (FHTT, MHTT and PHRT) and (FGTT, FHRT and PGRT) were virulent equally to 13 (81.3%), 12 (75%) and 11 (68.8%) of *Lr* genes respectively. Likewise, races FGRT, MCST, PCRR and RCJT had the same virulent spectrum, each produced virulence on 10 or 62.5% of *Lr* genes. Race MGJT was virulent on 9 or 56.3% of the *Lr* genes tested. This indicated that, unless wheat varieties have combined *Lr* genes through pyramiding, the mentioned races above have a potential to cause heavy infection during wheat production in the region in general and the study area in particular.

In contrast, the remaining six races (BBBT, BBQR, CBBT, LBBM, LBDC and MBBR) or 27% of the races had narrow virulence spectra ranging from 3 to 5 *Lr* genes. The "L" group races, LBBM and LBDC were the least virulent, producing compatible reaction only on *Lr1, LrB* and *Lr18* and *Lr1, Lr17* and *Lr18* respectively. Races BBBT, BBQR, CBBT and MBBR were also the least virulent, producing susceptible reactions on four, five, five and five leaf rust resistant genes in that order. Approximately 55% of the races identified in Eastern and Southeastern zones of Tigray varied from one another by single gene changes. For instance, races FGTT and FHTT were similar to FGRT and FHRT with additional virulence each to *Lr17,* respectively. In the

same way, races PHRT, PHTT, THTT and TKTT were similar to PGRT, PHRT, RHTT and THTT with additional virulence to *Lr26, Lr17, Lr2c* and *Lr24,* respectively. This slight difference in virulence between these races of leaf rust may resulted from the continuous evolution of leaf rust through one or more of the mechanisms of variation (mutation, migration, recombination and selection pressure on race specific resistance). This idea is in line with the report of Green (1975) who stated that, single step changes in virulence were resulted from the main process of evolutionary change in wheat leaf rust Populations.

The present study indicated that, the identified races of *P.triticina* did not show similarities with the previously identified races in Ethiopia. This could be due to variation over location and time, as races are prevalent in specific season and region depends on the type of wheat cultivars grown (Singh, 1991), and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*., 1992). Similar report was also provided by Mengistu and Yeshi (1992) they stated that, a comparison between the races identified in the present study with the earlier reports revealed differences.

Generally, the virulence spectrum of the pathogen in this study area confirmed the presence of wider range of virulence among the population of wheat leaf rust races. This might be linked with the fact that, the large population size of leaf rust leads to greater probability of mutants and more diversity of virulence/ avirulence combination existed in the crop (Schafer and Roelfs , 1985).

Virulence frequency of *P. triticina* isolates to *Lr* genes

The result on virulence frequency of *P. triticina* indicated that, majority of the resistance genes were found ineffective by most of the isolates tested in this study. Approximately, 81% of the *Lr* genes were ineffective to more than 55% of the isolates. High virulence (≥72.5%) has been exhibited on *Lr* genes *Lr1, Lr2c, Lr3, Lr16, Lr3ka, Lr11, Lr30, LrB, Lr10, Lr26,* and *Lr14a.* There was 100% frequency of virulence for leaf rust resistant genes *Lr18*. The *Lr*17 has an intermediate virulence frequency of 55%, while the remaining genes, *Lr9, Lr24* and *Lr2a* were found to have between 0-17.5% of virulence frequencies (Table 4). Some *Lr* genes such as, *Lr2c* and *Lr26, Lr16* and *Lr30* and *Lr14a* and *Lr11* had the same virulence frequency of 72.5%, 77.5% and 87.5%, respectively.

Table 3. Virulence /avirulence spectrum of *P. triticina* races collected from Eastern and Southeastern zones of Tigray

No	Races	Virulence (Ineffective *Lr* genes)	AVirulence (effective *Lr* genes)	Virulence factor
1	BBBT	LrB, 10, 14a, 18	Lr1, 2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 17, 30	4
2	BBQR	Lr3ka, 11, B, 10, 18	Lr1, 2a, 2c, 3, 9, 16, 24, 26, 17, 30, 14a	5
3	CBBT	Lr3, B, 10, 14a, 18	Lr1, 2a, 2c, 9, 16, 24, 26, 3ka, 11, 17, 30	5
4	FGRT	Lr 2c, 3, 16, 3ka, 11, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24, 26, 17,	10
5	FGTT	Lr2c, 3,16, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24, 26	11
6	FHRT	Lr2c, 3, 16, 26, 3ka, 11, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24, 17	11
7	FHTT	Lr2c, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24	12
8	LBBM	Lr1,B,18	Lr2a , 2c, 3, 9, 16, 24, 26, 3ka, 11,17, 30, 10, 14a	3
9	LBDC	Lr1,17,18	Lr2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 30, B, 10, 14a	3
10	MBBR	Lr1, 3, B,10,18	Lr2a, 2c, 9, 16, 24, 26, 3ka, 11, 17, 30, 14a	5
11	MCST	Lr1, 3, 26,3ka,11,17, B,10,14a,18	Lr2a, 2c, 9, 16, 24, 30	10
12	MGJT	Lr1, 3, 16, 11, 17, B,10,14a,18	Lr2a, 2c, 9, 24, 26, 3ka, 30	9
13	MHTT	Lr1, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2a, 2c, 9, 24	12
14	PCRR	Lr1, 2c,3, 26,3ka,11,30,B,10,18	Lr2a, 9, 16, 24, 17,14a	10
15	PGRT	Lr1, 2c, 3,16, 3ka, 11, 30, B, 10, 14a, 18	Lr2a, 9, 24, 26, 17	11
16	PHRT	Lr1, 2c, 3, 16, 26, 3ka, 11, 30, B, 10, 14a, 18	Lr2a, 9, 24, 17	12
17	PHTT	Lr1, 2c, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2a, 9, 24	13
18	PJTT	Lr1, 2c, 3, 16, 24, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2a, 9, 26	13
19	RCJT	Lr1, 2a, 3, 26, 11,17, B, 10, 14a, 18	Lr2c, 9, 16, 24, 3ka, 30	10
20	RHTT	Lr1, 2a, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2c, 9, 24	13
21	THTT	Lr1,2a, 2c,3,16,26,3ka,11,17,30,B,10,14a,18	Lr9, 24	14
22	TKTT	Lr1,2a, 2c,3,16,24,26,3ka,11,17,30,B,10,14a,18	Lr9	15

Table 4. Virulence frequency of *P. triticina* isolates on sixteen *Lr* genes

Lr gene	Number of Virulent isolates	Virulence frequency (%)	*Lr* gene	Number of Virulent isolates	Virulence frequency (%)
Lr1	30	75	Lr3ka	33	82.5
Lr2a	7	17.5	Lr11	35	87.5
Lr2c	29	72.5	Lr17	22	55
Lr3	36	90	Lr30	31	77.5
Lr9	0	0	Lr B	39	97.5
Lr16	31	77.5	Lr10	38	95
Lr24	2	5	Lr14a	35	87.5
Lr26	29	72.5	Lr18	40	100

The *Lr18* displayed consistently high infection type to all isolates of *P.triticina* collected from Eastern and Southeastern zones of Tigray. All the identified races including the least virulent races, LBBM and LBDC were virulent on this gene and showed susceptible reaction just like the universally susceptible variety "Morocco". Different authors have reported similar results on the ineffectiveness of the *Lr18*.

For instance, Torabi *et al*. (2001) reported that, the host with *Lr18* appeared to be ineffective to all isolates at seedling in the green house, but it showed considerable resistance at adult plant. Singh *et al*. (1991) also reported that, virulence status in the pathogen for this gene could not be determined. Similarly, there was also 97.5% frequency of virulence for *LrB.* This gene was found to be effective only to the least virulent race, LBDC isolated from the local cultivar in Saharti- samre district.

The ineffectiveness of the genes *Lr11* and *Lr17* at seedling stage were expected as they were reported to be adult plant resistant genes (Mesterhazy *et al*., 2000; Kolmer, 2003). Moreover, the ineffectiveness of *Lr1, Lr2c, Lr3* and *Lr10* might be due to these genes have been used in wheat cultivation for many years (Long, 1986), during which virulence to these genes become common and most races identified in recent years are virulent to these genes. Likewise, virulence for *Lr26, Lr16, Lr30, Lr3ka* and *Lr14a* was very common by most isolates of leaf rust with virulence frequencies of 72.5, 77.5, 77.5, 82.5 and 87.5% respectively.

This virulence could be result from the fact that, leaf rust differential lines have single and specific resistant genes, and race specific resistant genes have been proven to be very vulnerable to selection and increase virulent races in rust population (Kilpatrick, 1975).

Table 5. Response of wheat cultivars to dominant and virulent races of *P. triticina* at seedling stage in greenhouse

Cultivar	**Races**		
	TKTT	**THTT**	**PHTT**
Mekelle -1	3	3	2+
Mekelle- 2	3	3-	2
Mekelle- 3	3	3	3
Mekelle -4	3	3	3
Picaflor	3	3+	3
Digalu	3-	2+	2
Dashin	3+	3+	3
Ude	1+	2	2-
Dembi	2-	2-	2-
Local cultivar (Shahan)	4+	4	4
Morocco(Susceptible check)	4+	4	4

"+"=slightly larger than the normal uredinia; "-"= slightly smaller than the normal uredinia

On the other hand, *Lr9, Lr24,* and *Lr2a* were found to be effective to most of wheat leaf rust populations (Table 4). The leaf rust resistant gene, *Lr9* derived from *Aegiolops umbellulata*, demonstrated an incompatible host-pathogen interaction to all isolates of leaf rust. This implied that, no virulence was observed on *Lr9* (virulence frequency=0%) in all the districts of collection. In Ethiopia, this gene was also found effective to wheat leaf rust isolates collected in 2004 from Ethiopia and Germany (Sewalem *et al*., 2008). Similarly, *Lr24* was found to confer resistance to 95% of the tested leaf rust isolates. Virulence on *Lr*2a was also rare and found to be effective to 82.5% of leaf rust isolates.

Response of wheat varieties to *P. triticina* races at seedling stage in greenhouse

The reaction of wheat varieties to *P. triticina* races in the green house revealed that none of the varieties were immune (no sign of infection to the naked eye) while the infection type varied from 1 (small uredia surrounded by necrotic area) to 4 (large uredia without chlorosis). In general, both durum wheat varieties showed better resistance than bread wheat. This might be associated with the fact that, most of the durum wheat genotypes were developed from local landraces as Ethiopia is the centre of genetic diversity of this species. In effect, indigenous pathogens with high complimentary genetic diversity might co-exist with a wider range of durum wheat genotypes (Tesemma and Bechere, 1998). This idea is also in agreement with previous reports which stated that, most of the commercial durum wheat cultivars exhibited stable resistance to wheat rusts across seasons in hot spot areas of Ethiopia and they could be exploited in wheat breeding programs (Efrem *et al.,* 1995).

In contrast, as bread wheat is not indigenous to Ethiopia, cultivars are developed through selection and crossing programs using genetic materials introduced from abroad, mainly from CIMMYT. As a result, bread wheat cultivars in Ethiopia have a narrow genetic base (Hailu, 1991). The narrow genetic base makes bread wheat varieties highly selected and break their resistance by the new races after short period of releasing.

CONCLUSION

To conclude, all the tested bread wheat varieties do not have adequate resistances for leaf rust populations, indicating the need for incorporating more effective genes in to the target wheat cultivars. However, durum wheat varieties, Ude and Dembi showed resistance to leaf rust population in the field and greenhouse. Hence, they could be important sources of leaf rust resistant genes for this area.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Afzal SN, Haque I, Ahmedani MS, Munir M, Firdous SS, Rauf A, Ahmad I, Rattu AR and Fayyaz M (2009) Resistance potential of wheat germplasm (*Triticum aestivum* L.) against stripe rust disease under rain fed climate of Pakistan. Pak. J. Bot. 41(3): 1463-1475.

Ayele B, Eshetu B, Berhanu B, Bekelle H, Melaku D, Asnakech T, Amare A, Kiros M and Fekedu A (2008) Review on two decades of research on diseases of small cereal crops. In Abraham Tadesse (eds.) increasing crop production through improved plant protector Volume I. Proceedings of (4th Annual conference of the Plant protection Society of Ethiopia) 19-22 Dec.2006 Addis Ababa, Ethiopia.PP.598.

International Center for Maize and Wheat Improvement, 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.

Central Statistical Authority, CSA (2009) Country level Agricultural products producer price index, (Ag-PPI). Addis Ababa, Ethiopia. Pp 20-25.

Efrem B, Tesfaye T, Mengistu H and Yeshi A (1995) Breeding durum wheat for resistance to leaf rust and stem rusts in Ethiopia. In: Danial DL(ed.). Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. October 2-6, Njoro, Kenya. CIMMYT. Pp. 125-132.

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

Geleta B and Tanner G (1995) Status of cereal production and pathology research in Ethiopia. P.42-50. In D.L. Daniel (ed.). Breeding for diseases resistance with emphasis on durability. Regional wheat work shop for eastern, Central and Southern Africa, Njoro, Kenya.2-6 Oct.1994. Landbouwuniv. plant breeding Dep., Wageningen, The Netherlands.

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

Hailu G (1991) Bread wheat breeding and genetic research in Ethiopia. *In* Hailu, G., Tanner, D.G. and Mangistu, H. (eds). Wheat production and research in Ethiopia: A

historical perspectives. Addis Ababa. IAR / CIMMYT. pp. 73-93.

Kilpatric RA (1975) New wheat cultivars and longevity of rust resistances, 1971-1975. U.S. Dep. Agric. Res. Serv.

Kolmer JA (2003) Postulation of leaf rust resistance genes in selected soft red winter wheat. Crop Sci.43:1266-1274

Long DL, Schfer J F, Roelfs AP and Roberts JJ (1986) Virulence and epidemiology *P. recondita f.sp.tritici* in the United States in 1985. Plant dis.70:1107-1110.

Long and Kolmer JA (1989) A North American System of nomenclature for *Puccinia triticina.* Phytopathology *79:525-529*

Mengistu H, Getaneh WA, Rebeka D and Ayele B (1991) Wheat pathology research in Ethiopia. *In*: wheat research in Ethiopia. A historical perspective. Addis Ababa, IAR /CIMMYT.

Mengistu H and Yeshi A (1992) Variation within indigenous durum wheat germplasm for responses to stem and leaf rust races in Ethiopia. In: Tonner, D.G. and Mwangi, W.(eds.) 7th Regional wheat workshop for Eastern, Central and South Africa. Nakuru, Kenya, CIMMYT Crop development department variety registration, Addis Ababa, Ethiopia.

Mesterhazy A, Bartos P, Goyeau H, Niks RE, Csosza M, Andersen O, Casulli F, Ittu M, Jones E, Menisterski J, Manninger K, Pasquini M, Rubiales D, Schachermayr G, Strzembricka A, Szunics, L, Todorova M, Unger O, Vanco B, Vida G and Walther U (2000) European virulence survey for leaf rust in wheat. Agronomy, 20:793-804.

Roelfs AP (1982) Effects of barberry eradication on stem rust in the United States. Plant Dis. 66:177-181.

Roelfs AP, Singh RP and Saari EE (1992) Rust diseases of wheat. Concept and methods of disease management. Mexico, DF: CIMMYT.81. pp. 6.

Schafer JFand Roelfs AP (1985) Estimated relation between numbers of urediospores of *P. graminis f.sp. tritici* and rates of occurrences of virulence. Phytopathology, 75:749-750.

Sewalem AM, Heinz WD, Klaus P and Erich CO (2008) Postulation of seedling leaf rust resistance genes in selected Ethiopian and German bread wheat cultivars. Crop Science society of America, 48: 507-516.

Singh RP (1991) Pathogenecity variation of *Puccinia recondita f.sp.tritici* and *P.graminis f.sp. tritici* in wheat growing areas of Mexico during 1988-1989. Plant disease 75:790-794.

Stakman EC and Levine MN (1962) Analytical key for the identification of physiologic races of *Puccinia graminis tritici*. (Processed) division of cereal crops and dis., USDA, Minnestota Agric. Exp.Sta.pp.7.

Torabi M, Nazari K and Afshari F (2001) Genetics of pathogenecity of *Puccinia recondita f.sp. tritici,* the causal agent of leaf rust of wheat. Iran.J. Agric.Sci. Abstract in English, 32: 635.

Tesemma Tand Bechere E (1998) Developing elite durum wheat selections (composites) for Ethiopian peasant farm use: Raising productivity while keeping diversity alive. *Euphytica* 102: 323 - 328.

White JW, Tanner DG and Corbett JD (2001) An agro-climatologically characterization of bread wheat production in Ethiopia. CIMMYT, Mexico NRG-GIS ser.0101.