International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2018; 6 (1):301-310
ISSN:2320-7817(p) | 2320-964X(o)
UGC Approved Journal No 48951

Original Article Open Access

Prevalence and physiologic races of wheat leaf rust in Tigray, Ethiopia

Tesfay Gebrekirstos Gebremariam

Tigray Agricultural Research Institute (TARI), Mekelle Agricultural Research Center P.O. Box 258, Mekelle, Ethiopia.

Manuscript details:

Received : 07.12.2017
Accepted : 15.04.2018
Published : 24.04.2018

Editor: Dr. Arvind Chavhan

Cite this article as:
Tesfay Gebrekirstos Gebremariam (2018) Prevalence and physiologic races of wheat leaf rust in Tigray, Ethiopia, *Int. J. of. Life Sciences*, Volume 6(2): 301-310.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Wheat Leaf rust (Puccinia triticina Eriks and Henn.) is one of the most important foliar diseases of wheat in Tigray region. It cause up to 75% yield loss during epidemic years. Hence, this study was conducted to determine the distribution, intensity and to detect the virulence diversity of the pathogen in Eastern and Southeastern zones of Tigray. The findings of this paper were based on leaf rust surveys to compute the prevalence and intensity of the diseases; race analysis by inoculating isolates on to the 16 differential hosts. During the survey, 108 wheat fields were assessed in five districts, of which 88% of the fields were affected with leaf rust. The overall mean incidence and severity of the disease were 48.4 and 18.2%, respectively. A total of 22 races were identified from 40 isolates, 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 and detected once with a frequency of 2.5% each. The broadest virulence spectrum was recorded from TKTT race, making 15 Lr gens ineffective. Most of the Lr genes were ineffective to one or more of the tested isolates except Lr9. High virulence was observed on Lr3, Lr10, LrB and Lr18 with frequencies of 90, 95, 97.5 and 100%, in that order. In contrast, Lr9, Lr24 and Lr2a were found effective to 100%, 95% and 82.5% of the tested isolates, respectively. Hence, the use of Lr9, Lr24 and Lr2a genes singly or in combination through gene pyramiding has paramount importance as the additive effects of several genes offer the variety a wider base for leaf rust resistance along with periodic race survey to track further virulence evolution.

Keywords: Predominant races, effective genes, isolates, Lr genes

INTRODUCTION

Wheat (*Triticum aesvivum* L.) is one of the major crops cultivated in Ethiopia. During the last 14 years, the area covered by wheat has increased from 0.77 million ha in 1997 to 1.55 million ha in 2011, and it now ranks third among the crops next to teff (*Eragrostis teff)* and maize (*Zea mays* L) (CSA, 2011).

In Tigray region, wheat has been selected as one of the target crops in the strategic goal of attaining regional food self-sufficiency. In this region, wheat covers over 0.1 million hectare with total production of 1.93 million quintals annually (CSA, 2011). Eastern and Southeastern zones of Tigray are the major wheat growing areas and recognized as wheat belts in the region. However, the production and productivity of wheat in Ethiopia in general and Tigray region in particular is still very low. The low productivity of the crop is attributed to number of factors including biotic (diseases, insects and weeds), abiotic 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, and its recurrent outbreaks have threatened wheat production in the country (Bedabo, 2002). 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). In addition, 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 race (s) after short period of releasing. Yield loss due to wheat leaf rust reached 75% in susceptible varieties at hot spot areas of Ethiopia (Mengistu *et al*., 1991).

Wheat leaf rust can be effectively managed by growing resistant varieties. However, the development of resistant varieties requires knowledge of the pathogen distribution, virulence diversity and race distribution in particular region, and which resistance genes are effective against these races. Hence, this study was initiated to determine the distribution and virulence of wheat leaf rust.

METHODOLOGY

Survey of wheat leaf rust in wheat growing areas of Eastern and Southeastern Zones of Tigray:
Field leaf rust survey was conducted on 108 farmers' fields in 2013 main growing season, in the major wheat growing areas of Eastern and Southeastern zones of Tigray. Private farms in five districts (Wukro, Hintalo-wejirat, Saharti-samre, Degua-temben and Enderta) were included in the assessment. The survey was conducted at 5-10 km intervals on wheat fields along the main and accessible roadsides. Wheat fields were surveyed at critical growth stage of the crop (flag leaf stage) during which leaf rust reached its maximum severity level (Seck, 1985). Leaf rust assessment was made along the two diagonals (in ''X'' fashion) of the field at five points using $0.5m^2$ quadrant.

The incidence of leaf rust was calculated by using the number of infected plants and expressed as a percentage of the total number of plants assessed. Similarly, severity of leaf rust was examined visually on the whole plants within the quadrants and recorded as the percentage of plant parts or tissue affected (percentage of rust infection of the plant), and plant response (type of infection) using modified Cobb's scoring scale of rust disease under field conditions (Peterson *et al.,* 1948). The prevalence of the disease was computed using the number of fields affected divided by total number of fields assessed and expressed in percentage. The prevalence, incidence and severity data were analyzed by using descriptive statistical analysis (means) over districts and varieties.

Collection of wheat leaf rust samples:
A total of 50 leaf rust samples, 10 samples per district were collected from wheat fields and experimental plots of Eastern and Southeastern zones of Tigray. Infected leaves were cut from the mother plant using scissors and placed in an envelope. Samples collected in an envelope were labeled with all necessary information, preserved in an ice box and transported to Ambo Plant Protection Research Center laboratory for race analysis studies. The samples were kept in the refrigerator at 4^oc until used for the virulence analysis. Ten samples were not produce viable spore after inoculating on to the universally susceptible variety "Morocco". Hence, 40 leaf rust samples were used for race analysis.

Isolation and 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 *P. triticina* isolates:

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 contain 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, well developed isolate from each sample was collected using power operated vacuum aspirator and stored separately in gelatin capsule. A suspension, prepared by mixing single isolate urediospores with distilled water was inoculated on seven day- old seedlings of 'Morocco' for multiplication of each isolate 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^{o}C and relative humidity of 60-70% following the procedures mentioned earlier.

After 12-14 days of inoculation, spores from each isolate were collected in separate test tubes and stored at 4^{o}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 isolates or mono pustules 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.

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

Pt code	Host set	Infection type (ITs) produced on differential *Lr* lines			
	Host set 1	1	2a	2c	3
	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

The susceptible variety Morocco (without Lr gene) 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^{o}C and RH 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^{o}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 of 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 of *P.triticina*

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). For instance, low infection type (L) on the four hosts in a set is assigned with the letter 'B', while high infection type (H) on the four hosts is assigned with a letter 'T'. Hence, if an isolate produces low infection type (resistant reaction) on the 16 differential hosts, the race will be assigned with a four letters race code 'BBBB'. In the same way, an isolate which produces a high infection type (susceptible reaction) on the 16 wheat differential hosts have a race code 'TTTT' (Table 1).

RESULTS AND DISCUSSION

Prevalence and intensity of wheat leaf rust in eastern and southeastern zones of Tigray :

In the main crop-growing season of 2013, 108 wheat fields planted with improved and local cultivars were assessed for the intensity of wheat leaf rust. The result of the survey revealed that, the intensity of the disease vary from slight to complete infection of wheat fields depending on the crop type (bread wheat and durum wheat), variety and altitude ranges. The disease was more prevalent at Hintalo-wejirat and Wukro districts with prevalence of 100% and 96.7% respectively, while districts of Enderta and Saharti-samre showed similar distribution, 85% each. In contrast, the lowest prevalence (45.5%) of leaf rust was registered at Degua-temben district. As a whole, leaf rust was observed on 95 (88%) of 108 wheat fields inspected.

The highest mean incidence of the disease was noted in Wukro district with range of 0-100% and a mean value of 63.2% and followed by Saharti-samre, with range of 0-100% and mean incidence of 60%. Enderta and Hintalo-wejirat districts also showed high levels of leaf rust incidences with range and mean values of 0-100% and 57.9% and 5-100% and 53.3%, following the same order. In contrast, the lowest incidence of leaf rust (7.4%) was registered in Degua-temben district. The overall mean incidence for the five districts of Eastern and Southeastern zones of Tigray reached 48.4% (Table 2).

Likewise, leaf rust severity showed similar trend as that of incidence in Wukro, Saharti-samre and Degua-temben districts. The highest severity was recorded in Wukro district with a range of 0-95% and mean value of 37.3%. This was followed by Saharti-samre district, with range of 0-85% and mean severity of 26.1%. While, lowest mean leaf rust severity (4.1%) was recorded at Degua-temben district. The highest severity of 37.3% was recorded where the highest incidence of 63.2% was registered at Wukro district. In a similar trend, the lowest mean severity of 4.1% was recorded where the lowest mean incidence of 7.4% was registered at Degua-temben district (Table 2). Generally, leaf rust was found more important at Wukro, Saharti-samre, Hintalo-wejirat, and Enderta districts. These districts were found with the altitude range of 1912-2399 meter above sea level (Table 2). Hence, this elevation range is suitable for the development of leaf rust. Moreover, the cultivation of wheat in belg season in some areas of these districts could act as a green bridge to carryover the disease from offseason to main season. Earlier study also indicated that the presence of two overlapping seasons (meher and belg) for growing wheat helps in the buildup of inoculum in one season and transferred to the other season as a source of primary inoculum, facilitating the availability of inoculum year after year in the country (Serbessa, 2003).

Table 2. Prevalence and intensity of wheat leaf rust across districts of eastern and southeastern zones of Tigray in 2013 main cropping season

Districts	Altitude range (masl)	Number of fields inspected	Number of fields infected	Prevalence (%)	Incidence (%)		Severity (%)	
					Range	Mean	Range	Mean
Hintalo-wejirat	1966-2198	27	27	100	5-100	53.3	1-75	14.1
Wukro	1927-2399	30	29	96.7	0-100	63.2	0-95	37.3
Saharti-samre	1961-2339	20	17	85	0-100	60	0-85	26.1
Enderta	1912-2292	20	17	85	0-100	57.9	0-25	9.5
Degua-temben	2431-2654	11	5	45.5	0-24	7.4	0-10	4.1
Total /Mean	1912-2654	108	95	88	0-100	48.4	0-95	18.2

However, the low prevalence and intensity of leaf rust in Degua-temben district might be resulted from low temperature occurred during the season. In this district, the temperature was less than 10°C (ENMA, 2013) which is below the optimum level of temperature. This low temperature likely eliminates or reduces the reproduction and spread of the leaf rust. Earlier studies also confirmed that, at 10°C, infection developed very slowly and restricted in size (Dyck and Johnson, 1983).

Prevalence and intensity of wheat leaf rust by wheat type and variety in eastern and southeastern zones of Tigray:

During the survey, the prevalence and intensity of leaf rust varied between durum and bread wheat varieties (Table 3). Though, both durum wheat varieties (Ude and Dembi) showed prevalence of 100%, the intensity of the disease was lower compared to most of bread wheat varieties. The mean range of incidences and severities of leaf rust in these varieties varied between 2.5-5% and 2.5-3.8% respectively. The lowest mean incidence (2.5%) and severity (2.5%) of the disease were recorded on Dembi variety, and followed by Ude with mean incidence of 5% and severity of 3.8%. Moreover, both durum varieties showed resistance response to wheat leaf rust in all durum wheat fields of the study areas (Table 3). 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).

In contrast, higher intensity of leaf rust was recorded in bread wheat varieties at different levels of incidences and severities. The mean incidences and severities of wheat leaf rust on bread wheat varieties ranged between 2.5-72.9 and 2.5-27 respectively. Particularly, the local cultivar locally known as “Shahan” consistently showed susceptible response to the disease in all its fields. As a result, the highest mean incidence of 72.9% and severity of 27% were recorded on this cultivar (Table 3). The long period cultivation and increase in susceptibility from time to time by leaf rust population probably makes the local cultivar highly infected.

Table 3. Prevalence and intensity of leaf rust by wheat type and varieties in Eastern and Southeastern Zones of Tigray in 2013 cropping season.

Varieties	Altitude range (m.a.s.l)	Number of fields Inspected	Number of fields infected	prevalence (%)	Incidence (%)		Severity (%)	
					Range	mean	Range	Mean
Mekelle- 1	1980-2142	6	4	66.7	5-19.1	5.9	1-5	5 R, MR
Mekelle -2	1970-2155	4	2	50	0-15	7.5	0-5	2.5R, MR
Mekelle- 3	1975-2165	3	2	66.7	0-15	7.5	0-10	5R,MR
Mekelle- 4	2012-2178	2	2	100	0-5	2.5	0-5	2.5R,MR
Picaflor	2021-2420	7	6	86	0-65.5	32.3	0-50	12MR,MS
Dashin	1994-2595	18	17	94.4	0-100	43.3	0-65	13.7MR,MS
Digalu	1961-2006	2	2	100	54-75	64.5	5-25	15MR,MS
Shahan (local)	1912-2654	61	55	90.2	0-100	72.9	0-95	27 S
Ude (Durum)	2000-2626	3	3	100	0-10	5	0-10	3.8R
Dembi (Durum)	1973-2614	2	2	100	0-5	2.5	0-5	2.5R

In addition, the wide cultivation of this cultivar in the study area (Table 3) also played a significant role for its susceptibility, as leaf rust is probably more damaging when large areas are sown to single, genetically homogeneous or closely related cultivars (Ahmad *et al*., 2010). This idea is in line with the reports of Mamluk *et al*. (2000) who stated that, majority of the Ethiopian farmers grow local cultivars that are susceptible to the disease. As a whole, 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 race (s) after short period of releasing. 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).

Distribution and diversity of *P. triticina* races in Eastern and Southeastern zones of Tigray:

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 4), which indicated that they were widespread throughout Eastern and Southeastern zones of Tigray. PHTT was detected eight times in the population of wheat leaf rust collected from Wukro, Hintalo-wejirat and Enderta districts while, PHRT detected six times from Saharti-samre, Degua-temben, Wukro and Enderta samples of wheat leaf rust. On top of this, PHRT was identified as the most distributed race and adapted to 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 4).

Table 4. Prevalence of *Puccinia triticina* races across districts of Eastern and Southeastern zones of Tigray in 2013 cropping season

	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

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 Saharti-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 4). 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 its sources to or from this area is a common phenomenon in rusts in general and leaf rust in particular. This circumstance might be resulted genetic diversity among the population of wheat leaf rust. Hence, the chance of detecting different races in this district becomes 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 5). 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, 12 and 11 *Lr* genes respectively. Likewise, races FGRT, MCST, PCRR and RCJT had the same virulent spectrum, each produced virulence on ten *Lr* genes. Race MGJT was virulent on nine *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 (Table 5). 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* revealed differences with the earlier 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).

Table 5. Virulence frequency of *P. triticina* isolates on 16 *Lr* genes in 2013 cropping season

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

Generally, the virulence spectrum of the pathogen in the 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 6). 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.

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. 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 et al, 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.

On the other hand, *Lr9, Lr24,* and *Lr2a* were found to be effective to most of wheat leaf rust populations (Table 6). 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. This gene was ineffective only by two races, TKTT and PJTT identified from Wukro and Saharti-samre isolates respectively. Virulence on *Lr*2a was also rare and found to be effective to 82.5% of leaf rust isolates. Hence, the use of these genes singly or in combination through gene pyramiding has paramount importance as the additive effects of several genes offer the variety a wider base for leaf rust resistance.

Table 6: virulence (H) and avirulence (L) spectrum of *Puccinia triticina* races on the sixteen leaf rust differential hosts.

Pt code [a]	*Lr genes*																Morocco Sus. Check	Virulence factors
	1	2a	2c	3	9	16	24	26	3ka	11	17	30	B	10	14a	18		
BBBT	L[b]	L	L	L	L	L	L	L	L	L	L	L	H	H	H	H	H	4
BBQR	L	L	L	L	L	L	L	L	H	H	L	L	H	H	L	H	H	5
CBBT	L	L	L	H	L	L	L	L	L	L	L	L	H	H	H	H	H	5
FGRT	L	L	H	H	L	H	L	L	H	H	L	H	H	H	H	H	H	10
FGTT	L	L	H	H	L	H	L	L	H	H	H	H	H	H	H	H	H	11
FHRT	L	L	H	H	L	H	L	H	H	H	L	H	H	H	H	H	H	11
FHTT	L	L	H	H	L	H	L	H	H	H	H	H	H	H	H	H	H	12
LBBM	H	L	L	L	L	L	L	L	L	L	L	L	H	L	L	H	H	3
LBDC	H	L	L	L	L	L	L	L	L	L	H	L	L	L	L	H	H	3
MBBR	H	L	L	H	L	L	L	L	L	L	L	L	H	H	L	H	H	5
MCST	H	L	L	H	L	L	L	H	H	H	H	L	H	H	H	H	H	10
MGJT	H	L	L	H	L	H	L	L	L	H	H	L	H	H	H	H	H	9
MHTT	H	L	L	H	L	H	L	H	H	H	H	H	H	H	H	H	H	12
PCRR	H	L	H	H	L	L	L	H	H	H	L	H	H	H	L	H	H	10
PGRT	H	L	H	H	L	H	L	L	H	H	L	H	H	H	H	H	H	11
PHRT	H	L	H	H	L	H	L	H	H	H	L	H	H	H	H	H	H	12
PHTT	H	L	H	H	L	H	L	H	H	H	H	H	H	H	H	H	H	13
PJTT	H	L	H	H	L	H	H	L	H	H	H	H	H	H	H	H	H	13
RCJT	H	H	L	H	L	L	L	H	L	H	H	L	H	H	H	H	H	10
RHTT	H	H	L	H	L	H	L	H	H	H	H	H	H	H	H	H	H	13
THTT	H	H	H	H	L	H	L	H	H	H	H	H	H	H	H	H	H	14
TKTT	H	H	H	H	L	H	H	H	H	H	H	H	H	H	H	H	H	15

[a]Adopted from Long and Kolmer, (1989). [b]L=low infection type / avirulent race; H= high infection type / virulent

Acknowledgements

I would like to thank the East African Agricultural Productivity Project (EAAPP) and Tigray Agricultural Research Institute (TARI) for providing full financial support to the first author.

REFERENCE

Ahmad S, Afzal M, Noorka IR, Iqbal Z, Akhtar N, Iftkhar Y and Kamran M (2010). Prediction of yield losses in wheat (*Triticum aestivum* L.) caused by yellow rust in relation to Epidemiological factors in Faisalabad. Pak. J. Bot. 42(1): 401-407.

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. 598.

Bedabo A (2002). Breeding bread wheat with multiple disease resistance and high yield for the Ethiopian highlands: Broadening the genetic bases of yellow rust and tan spot resistance. Cuvillier Verge, Goettingen, Germany.

CSA (Central Statistical Authority). (2011). Report on area and crop production forecast for major crops (for private peasant holdings, meher season). Statistical bulletin. Addis Ababa, Ethiopia.

Dyck PL and Johnson R (1983). Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita.* Can. J. Plant Pathol.5: 229-34.

ENMA (Ethiopian National Meteorology Agency). (2013). Atlas of Tigray regional state. Meteorological data in Tigray: 1997-2013.

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. 42-50. In D.L. Daniel (ed.). Breeding for diseases resistance with emphasis on durability. proc. Regional wheat work shop for eastern, Central and Southern Africa, Njoro, Kenya.2-6 Oct.1994. Landbouwuniv. plant breeding Dep., Wageningen, The Netherlands.

Green GJ (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. 73-93.

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

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

Long DL, Schfer JF, 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.

Mamluk OF, El-Daoudi YH, Bekele E, Soth MB, Ahmed MS, Mahir MA and Bahamish HS (2000). Wheat Leaf and Stem Rusts in the Nile Valley and Red Sea Region. ICARDA/NVRSRP, Cairo, Egypt.

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

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.

Peterson RF, Campbell AB and Hannah AE (1948). A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Res. Sect. C, 26: 496-500.

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: 6.

Schafer JF and 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.

Seck M, Teng PS and Roelfs AP (1985). The role of wheat leaves in grain yield and leaf rust losses. Phytopathology 75:1299 (abstract)

Serbessa N (2003). Wheat Stem Rust (*Puccinia graminis* f. sp. *Tritici*) Intensity and pathogenic variability in Arsi and Bale zones of Ethiopia. Pl. Sc. M. Sc. Thesis presented to School of Graduate Studies of Alemaya University, Ethiopia.

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.7.

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

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.