RESEARCH ARTICLE

Distribution, physiologic races and reaction of wheat cultivars to virulent races of leaf rust (*Puccinia triticina* Eriks and Henn.) in south eastern zone of Tigray, Ethiopia

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ABSTRACT

Leaf rust (*Puccinia triticina* Eriks and Henn.) is one of the most important foliar diseases of wheat (*Triticum aestivum* L.) in South eastern zone of Tigray in Ethiopia. Regular surveying, race identification and searching for resistant genes plays significant role to develop resistant varieties against leaf rust. Hence, this study was carried out to determine the distribution and intensity of wheat leaf rust to identify physiologic races of *P. triticina* and also to evaluate the seedling reaction of commonly grown wheat varieties to virulent races of leaf rust. The results of this study were based on leaf rust survey to compute the prevalence and intensity of the disease; race identification through inoculation of leaf rust populations, isolation, multiplication of mono pustules of the pathogen and determination of races by inoculating on leaf rust differential hosts; and evaluating ten wheat varieties to virulent and dominant races (TKTT, THTT and PHTT) at seedling stage in greenhouse. During the survey, a total of 108 farmers' wheat fields and experimental plots were assessed in five districts of South Eastern Tigray, of which 95 of the fields (88%) were affected with leaf rust. The overall mean incidence and severity of the disease were 48.4 and 18.2%, respectively. The highest intensity of leaf rust was recorded in Wukro wheat fields with incidence of 63.2% and severity of 37.3%. In contrast, the lowest incidence and severity of the disease were recorded in D/ Temben district with mean values of 7.4 and 4.1%, respectively. The characterization of 40 mono pustules of *P. triticina* was resulted for the identification of 22 races. Races PHTT and PHRT were predominant with frequencies of 20 and 15%, respectively, followed by THTT and FHRT with a frequency of 10% each. 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 all *Lr* genes except *Lr* 9 was ineffective. About 81% of the *Lr* genes were ineffective to more than 55% of *P. triticina* isolates. High virulence was observed on *Lr*3, *Lr*10, *Lr* B and *Lr*18 with frequencies of 90, 95, 97.5 and 100%, respectively. However, *Lr* genes 9, 24 and 2a were found effective to 100, 95 and 82.5 of the tested isolates, respectively. The variety evaluation revealed that, Mekelle-3, Mekelle-4, Picaflor, Dashin and local showed susceptible reactions to TKTT, THTT and PHTT races. Mekelle-1 and Mekelle-2 were susceptible to races TKTT and THTT, but resistant to PHTT. Digalu was only susceptible to TKTT race. Unlike bread wheat varieties, the durum varieties, Ude and Dembi were resistant to these races. The results of this study showed that most bread wheat varieties did not have adequate resistance for leaf rust. Hence, the gene pyramiding of *Lr*9, *Lr*24 and *Lr*2a has paramount importance as the additive effects of several genes offer the variety a wider base for leaf rust resistance.

Keywords: Bread wheat, Durum wheat, Leaf Rust, Isolates, *Lr* genes.

INTRODUCTION

Wheat (*Triticum* spp.) is one of the important leading cereal grain, where 40% of the world population uses as a staple food (Anonymous, 2007). It is grown on 255 million hectares worldwide from the equator to the latitudes of 60^0N and 44^0S and at altitude ranges from sea level to 3000 m. a. s. l. Approximately 600 million tons of grain is produced annually, roughly half of which is in developing countries (Aquino *et al*., 2002). Its popularity comes from the versatility of its use in the production of a wide range of food products, such as "Injera", breads, cakes, pastas, cookies etc (Pena, 2002). In addition, it has high nutritive value (>10% protein, 2.4% lipid, and 79% carbohydrates) and it accounts for about 20% of the caloric intake of the human diet (Gooding and Davies, 1997). Wheat is one of the major cereal crops grown in the highlands of Ethiopia; particularly, the Tigray region in Ethiopia is regarded as the second largest wheat producer in Sub-Saharan Africa (White *et al*., 2001). The major wheat production areas are Western Harerghe, Illubabur, Bale, Arsi, Shewa, Sidamo, Tigray, Northern Gondar and Gojam zones (Bekele, 2000). The area under wheat production is estimated to be about 1.5 million hectare and ranks third after maize and teff (CSA, 2009). Wheat is cultivated in a wide altitude range from 1500-3000 m. a. s. l. However, the most suitable agro-ecological zones for wheat production fall between 1900- 2700 m. a. s. l (Hailu, 1991). This crop is used as a staple food for about 36% of the Ethiopian population (CIMMYT, 2005).

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). South eastern zone of Tigray is one of the major wheat growing areas and recognized as wheat belt in the region. It is a pillar crop and covers an area of 49,244 hectares in south eastern zone of the region (BoARD, 2005). Although, the area cultivated under wheat has been increased in the last few years, the production and productivity of the crop in Ethiopia in general and Tigray region in particular is still very low. The national average yield is estimated to 1.7 tons/ha (CSA, 2009). This is by far below the world's average yield which is about 3.3 tons/ha (Curtis *et al*., 2002). The low productivity of the crop is attributed to number of factors including biotic (diseases, insects and weeds), abiotic (drought, acidity, alkaline, extreme temperatures, depleted soil fertility and snow) and low adoption of new agricultural technologies (Ayele *et al*., 2008). Among these factors, plant diseases are the major biotic constraints of wheat production in the world including Ethiopia. The rusts are the most destructive diseases of wheat worldwide (Singh *et al*., 2008). The three ruts; stem (*P. graminis f. sp. tritici*), leaf (*P. triticina*) and stripe rust (*P. striiformis f. sp. tritici)* are foliar and stem diseases that causes significant reduction in yield and quality in different regions of the world (Kuraparthy *et al*., 2007). Wheat rusts have reported as devastating, having the ability to destroy entire susceptible wheat crops in a matter of weeks and resulting in large economical losses (Marsalis and Goldberg, 2006).

Wheat leaf rust, also known as brown rust, caused by the fungal pathogen, *Puccinia triticina* Eriks and Henn., possibly the most widespread of the wheat rusts and occurs in most wheat growing areas of the world (Kolmer, 2005). The wide range adaptability helps this fungus to co-exist with wheat wherever it is grown

(Winzeler *et al.,* 2000). In Ethiopia, wheat leaf rust is one of the most important 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). During 2007-2009 cropping seasons, incidence of 30.2% was recorded for leaf rust in Oromia, Amhara, SNNPP and Tigray regions. Prevalence of leaf rust for the above mentioned locations was 53.3%. In 1976, leaf rust was recorded in all wheat growing regions of the country with severity range of 40-50% in local cultivar, while 100% on susceptible checks at Kulumsa (Madumarov, 1977). In Tigray region, wheat diseases survey was also made in 1994 and 1995 cropping seasons and identified eight fungal diseases (MARC, 1998). Leaf rust and yellow rust were found to be the most important diseases affecting wheat production in the region in general and South eastern zone in particular (MARC, 1998). In Ethiopia, yield loss due to leaf rust reached 75% in susceptible wheat varieties at hot spot areas (Mengistu *et al*., 1991). This yield loss is attributed due to infection of the flag leaf, which is thought to be responsible for greater than 70% of grain filling (Feyissa *et al.*, 2005).

Use of host resistance is the most economical and environmentally friendly method of controlling wheat leaf rust (Afzal *et al.,* 2009). In Ethiopia, wheat production is characterized by high biodiversity in crops and low input systems, and the control of rust diseases largely relies on genetic resistance (Sewalem *et al.,* 2008). However, host resistance may not always be readily available for use against leaf rust, and it requires regular surveying, race identification and continuous search for new sources of resistant genes in the cultivated and wild forms of wheat (Kuraparthy *et al.,* 2007). In Ethiopia, many studies on virulence and race identification of wheat leaf rust were carried out in different times. For instance, according to information obtained from SPL, 57 leaf rust races were identified, among these, race1, 53, 58, 61, 62, and 141 were predominant (Solmatin and Teman, 1985). Similarly, 23 races of wheat leaf rust were analyzed from 31 *P. triticina* isolates collected from Central and South eastern part of Ethiopia (Sewalem *et al*., 2008). During this time, virulence was reported on *Lr* genes *Lr11, Lr12, Lr13, Lr22, Lr33, Lr34, Lr35, LrB* and *LrTc*, whereas no virulent races were identified for genes *Lr9, Lr19, Lr24, Lr26, Lr29, Lr38,* and *LrW*. Mideksa (2011) also reported that, 19 races from 46 isolates of *P. triticina* were identified from two districts of West Shewa zone. In his study, *Lr* genes namely, *Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr3ka* and *Lr30* were effective to most of wheat leaf rust isolates. However, this work was covered mainly Central, West Showa and Southeastern parts of Ethiopia. Other parts of the country like Tigray region in general and Southeastern zone in particular, do not have information on the distribution, physiologic races and response of wheat varieties to leaf rust populations. Therefore, this study was carried out to determine the distribution and intensity of wheat leaf rust to identify the physiologic races of *Puccinia triticina* in South eastern zone of Tigray, Ethiopia and also to evaluate the seedling reaction of commonly grown wheat varieties to virulent races of leaf rust in green house conditions.

MATERIALS AND METHODS

Description of the study area

Tigray region is comprised of diverse topographic features including, about 53% lowland (1400-1800 m. a. s. l), 39% midland (1800-2400 m. a. s. l) and 8% highland (2400-3400 m. a. s. l) and the region is also classified in to three agro ecological zones of 67% dry, 24% moist and 9 % wet (BFED, 2007). The climate of the region is generally sub tropical with an extended dry period of nine to ten months and maximum effective rainy season of 50 to 70 days. The mean annual rain fall ranges from 500-1000 mm (ENMA, 2013). The rainfall pattern is predominantly uni-modal (June to September). Considering rainfall and temperature, more than 90% of the region is categorized as semiarid. The remaining areas of the region can be categorized as dry sub-moist and arid. The region is divided into six zones: Central, West, Northwest, East, Southeast and Southern. South eastern Zone of the region includes five districts viz; Enderta, H/wejirat, S/ Samre, Degua Temben and Wukro (Fig. 1). The major area of this zone is mid highland and highland with temperature range of 10-25.5^{o}C and the annual average rainfall fluctuates between 500-907 mm. This zone is located at 39^{0} 48’East longitude and 13^{o}5’North latitude at an elevation of 1970- 2589 m. a. s. l.

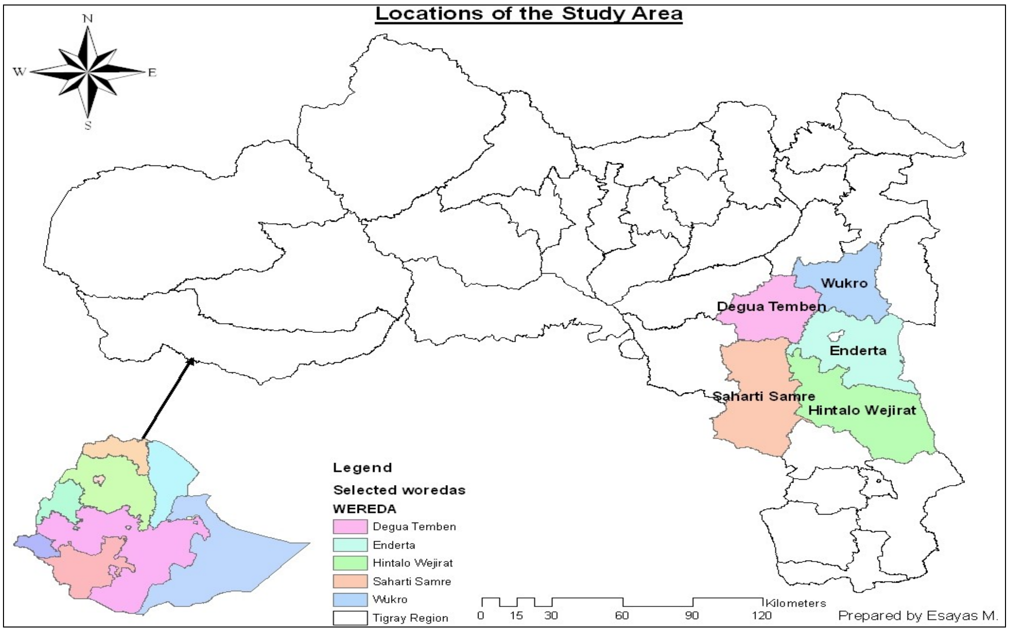

Fig. 1. Map of the five surveyed districts of the South eastern zone of Tigray, Ethiopia.

Survey of leaf rust in south eastern zone of Tigray

Field survey of leaf rust was carried out in the major wheat growing areas of south eastern zone of Tigray in 2013 main growing season. Private farms in five districts (Wukro, H/wejirat, S/samre, D/Temben and Enderta) and experimental plots of MARC were included in the assessment. In each district, the localities were selected based on the data of dominance of wheat production obtained from the BoARD of the respective districts. A total of 10 localities, three from H/wejirat, two each from Wukro, Enderta and S/Samre and one from D/Temben were assessed for wheat leaf rust infection. Wheat fields along the main and accessible road sides were inspected at 5-10 kilometer intervals. A total of 108 wheat fields were surveyed at critical growth stage of the crop (flag leaf stage) during which leaf rust reached its maximum severity level (Seck *et al*., 1985). Leaf rust assessment was made along the two diagonals (in ''X'' fashion) of the field at five points using 0.5m^2 quadrant. In each field, plants within the quadrant were counted as diseased/ infected and healthy / non- infected and the incidence, severity and prevalence of wheat leaf rust were calculated as follows:

$$\text{Incidence (\%)} = \frac{\text{Number of diseased plants X 100}}{\text{Total number of plants assessed}}$$

Severity (%) =Leaf area infected (Peterson *et al*., 1948)

$$\text{Prevalence (\%)} = \frac{\text{Number of leaf rust affected fields X 100}}{\text{Total number of fields assessed}}$$

The severity of leaf rust was examined visually on 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) (Fig. 2). The host response to infection in the field scored using 'R' to indicate resistance or miniature uredinia; 'MR' indicate moderate resistance, expressed as small uredinia; 'MS' to indicate moderately susceptible, expressed as moderate sized uredinia somewhat smaller than the fully compatible type and 'S' to indicate full susceptibility. Moreover, data on geographic information (latitude, longitude and altitude) of each field was recorded using GPS (e Trex Legend GPS system, Garmin). The GPS data were later used to plot surveyed fields on a map using the computer program Arc view 3.0 (ESRI). In addition, for each surveyed wheat field, information like wheat type, variety and growth stage of the crop was collected. Growth stage of the crop was recorded based on Zadoks *et al.,* (1974). The prevalence, incidence and severity data were analyzed by using descriptive statistical analysis (means) over districts, localities, varieties, altitudes and crop growth stages.

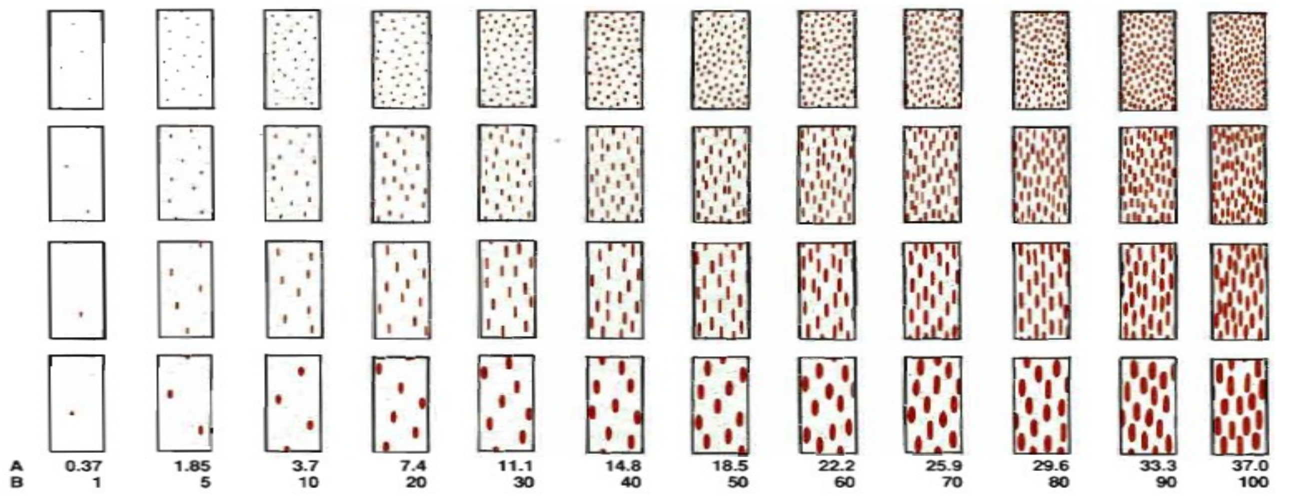

Fig. 2 The modified Cobb Scale: (A) Actual percentage occupied by rust uredinial; (B) Rust severities of the modified Cobb Scale after Peterson *et al*., (1948).

Collection of wheat leaf rust samples

Leaf rust infected samples (five samples per locality) were collected from wheat fields and experimental plots of MARC (Fig 3 a & b) 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 including name of the region, zone, district, variety, GPS data and date of collection. Samples were kept in refrigerator until the survey in all districts was finalized. Then after, samples were preserved in ice box and transported to APPRC laboratory for race analysis and variety evaluation studies. The samples were kept in the refrigerator at 4°c until used for the virulence analysis.

Isolation and multiplication of *P. triticina* inoculum

The inoculum was increased and maintained on universally rust susceptible variety "Morocco" which does not carry any known leaf rust resistant gene (Roelfs *et al*., 1992). Six seedlings of "Morocco" were raised in suitable 8 cm diameter clay pots containing sterilized soil, sand and manure in a ratio of 2:1:1 mixture, respectively. Seven day-old seedlings or when the primary leaves were fully expanded and the second leaves beginning to grow, the leaves were rubbed gently with clean (dis-infected with 97% of alcohol) moistened (with distilled water) fingers to remove the waxy layer from the surfaces of the leaves (Fig. 3 c & d). Green house inoculations were done using the methods and procedures developed by Stakman *et al*. (1962). Bulked spores from the leaf rust infected samples were collected with scalpels and transferred on to a watch glass which contain distilled water to make spore suspension, and then it was rubbed on seedlings of Morocco with clean moistened fingers. The plants were then moistened with fine droplets of distilled water produced with an atomizer and incubated in dew chamber for 24 hours at 18-20°C with 90% RH and seedlings were allowed to dry gradually. Then, the seedlings were transferred from the dew chamber to glass compartments where conditions were regulated at 12 hours photoperiod, at temperature of 18-25°C and RH of 60-70%. The remaining spore samples were kept in refrigerator at 4°C and were used to substitute samples which failed to produce infection on the universally susceptible variety.

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 was collected in a test tubes separately using vacuum pump A suspension, prepared by mixing mono pustule urediospores with light weight mineral oil (SolTrol 130), was inoculated on seven day- old seedlings of 'Morocco' for multiplication purpose. After inoculation, seedlings were placed in dew chamber for 24 hours at 18-22°C

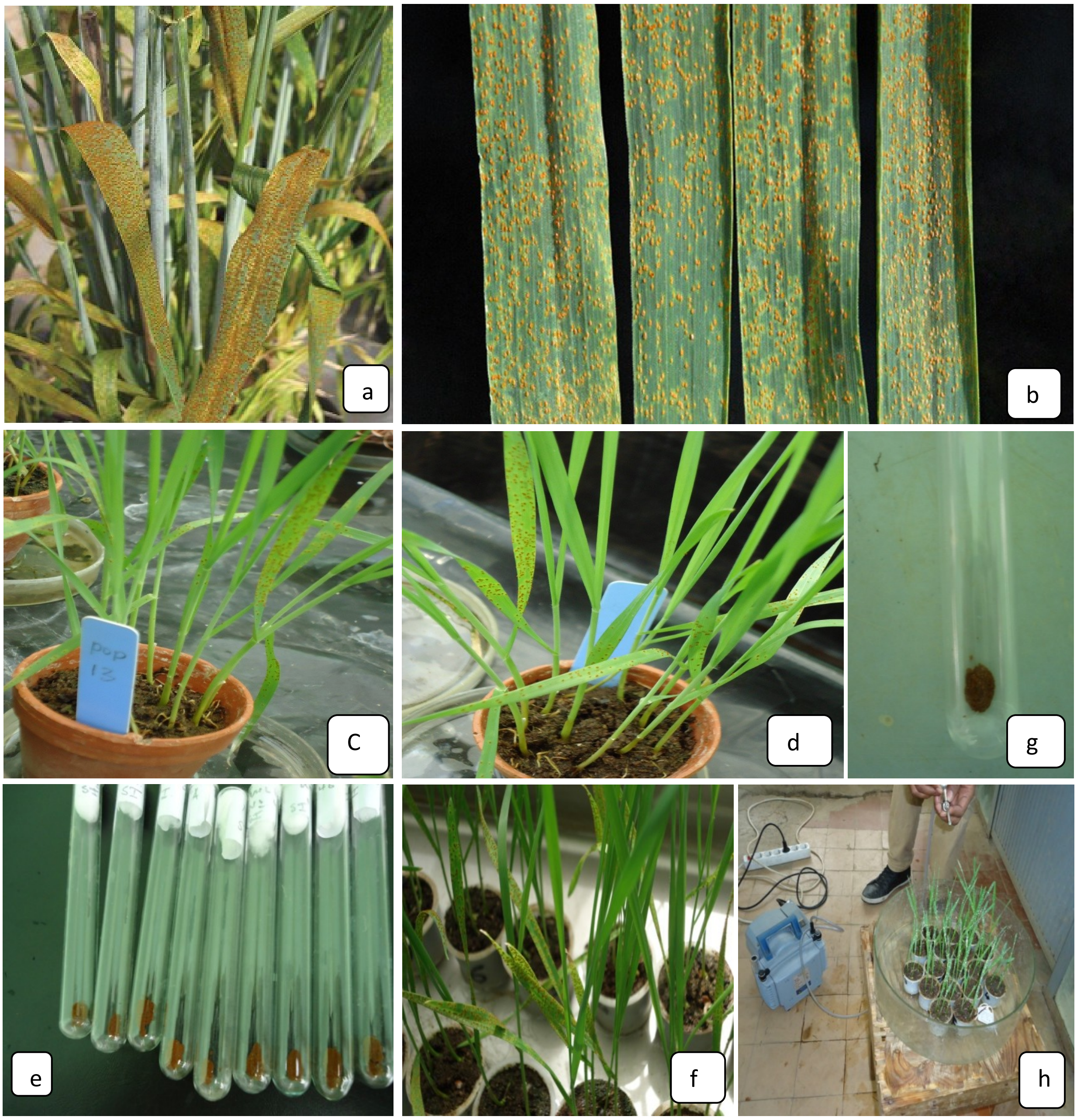

Fig. 1. a : i. Schematic overview of the protocols from field samples up to variety evaluation in greenhouse at APPRC, Ethiopia.

a. wheat field infected with leaf rust

b. Samples of infected wheat leaf rust

c. Inoculation of spores to Morocco for multiplication

d. Multiplication of Mono pustule on Morocco cultivar

e. Harvesting of mono pustules separately on test tubes.

f. Scoring of ITs on differentials using 0-4 scale (Long and Kolmer, 1989)

g. Spore collection from the virulent races separately on test tube

h. Inoculation of virulent race to cultivars for resistance evaluation.

i. Disease development of cultivars in glass house.

Table 1: List of sixteen wheat leaf rust differential hosts with their corresponding *Lr genes* and pedigree

Differential lines	*Lr* genes	Pedigree	Differential lines	*Lr* genes	Pedigree
RL6003	Lr1	TC*6/Centenario	RL6007	Lr3ka	TC*6/Klein Aniversario
RL6016	Lr2a	TC*6/Webster	RL6053	Lr11	TC*2 Hussar
RL6047	Lr2c	TC*6/Loros	RL6008	Lr17	Klein Lucero/ 6*TC
RL6002	Lr3	TC*6/Democrat	RL60 49	Lr30	TC*6/Terenzio
RL6010	Lr9	Transfer /6*TC	RL6051	LrB	TC*6/Carina
RL6005	Lr16	TC*6/Exchange	RL6004	Lr10	TC*6/Exchange
RL6064	Lr24	TC*6/Agent	RL6013	Lr14a	Selkirk /6*TC
RL60 78	Lr26	TC*6/St-1-25	RL6009	Lr18	TC*7/Africa43

Sources: Long and Kolmer, 1989

Table 2: Description of infection types to classifying the reactions of leaf rust on wheat seedlings in greenhouse

Infection types	Host response	Symptoms
0	Immune	No visible uredia
;	Highly resistant	Hypersensitive fleck
1	Resistant	Small uredia with necrosis
2	Resistant to moderately resistant	Small to medium size of uredia surrounded by necrosis or chlorosis
X	Mesothetic or Heterogeneous	A range of infection type from resistant to susceptible scattered randomly on a single leaf caused by single isolate
3	Moderately resistant to Moderately Susceptible	Medium size uredia with or without chlorosis
4	Highly susceptible	Large uredia without chlorosis
	Modified characters	
=	Low uredia	Uredia much smaller than the typical and at the lower limit of the infection type
-	Smaller uredia	Uredia smaller than normal
+	Large uredia	Uredia larger than normal
++	Largest uredia	Uredia much larger than typical and at the upper limit for the infection type

Source: Long and Kolmer, 1989

and with RH of 90%. Then after, seedlings were transferred to growth chamber where conditions were regulated at 12 hours photoperiod, 18-25°C and RH of 60-70% following the procedures mentioned earlier. After 12-14 days of inoculation, the spores from each mono pustule/ isolate were collected in separate test tubes (Fig 3 e) and stored at 4°C until they were inoculated on the differential hosts. This procedure was repeated until sufficient amount of spores are produced to inoculate the set of wheat leaf rust differential host (Table 1). By doing this a total of 40 isolates/mono pustules were developed from 40 wheat leaf rust samples. A pictorial schematic overview of the general protocol used for race analysis in APPRC green house, Ethiopia (Fig. 3 a - i).

Inoculation of P. triticina isolates to wheat leaf rust differential hosts

Six seeds each from 16 differential hosts and "Morocco" were grown in 3cm diameter pots containing soil, sand and compost at a ratio of 2:1:1 respectively. The susceptible variety Morocco was used as a check. 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 at 18-22°C and RH of 90%. Up on removal from the dew chamber, plants were placed separately in the growth chamber to avoid contamination. Greenhouse temperatures were maintained between 18-25°C.

Leaf rust assessment on differential hosts

Twelve days after inoculation, the infection types were scored for each isolate using the 0-4 scoring scale of Long and Kolmer (1989) (Table 2). Infection types were grouped in to two, where, Low (Resistance) = incompatibility (infection types: 0 to 2) and High (Susceptible) = compatibility (infection type: 3 to 4). Avirulence and virulence of cultures were determined by low (L) and high (H) infection types, respectively.

Designation of races of Puccinia triticina

Race designations were assigned as described by Long and Kolmer (1989). 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 (Table 3).

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 3). If an isolate produces a low infection type on Lr9 but a high infection type on the remaining 15 differential hosts, the race will be designated as TKTT.

Response of wheat cultivars to leaf rust races at seedling stage in green house.

The isolates of prevalent and virulent leaf rust races identified from the southeastern zone of Tigray were multiplied on the universally susceptible variety Morocco and collected in separate test tubes to inoculate wheat cultivars (Fig 3 f - i). Ten wheat varieties (Table 4) mainly cultivated in Tigray region were evaluated against the virulent race TKTT and dominant leaf rust races THTT and PHTT. Six seeds of each cultivar and Morocco were sown in 3 cm diameter plastic pots separately. Seven day old seedlings (when the first leaf is fully expanded and the second leaf is just emerged) were inoculated with spores of the mentioned races and incubated (Fig. 3 h). Varieties were arranged in complete randomized design (CRD) and replicated three times (Fig. 3 i). Data on infection types was recorded 12 days after inoculation using standard disease scoring scale 0- 4 (Long and Kolmer, 1989) (Table 2) and analyzed by using descriptive statistical analysis (means).

Table 3: Nomenclature of *Puccinia 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

Table 4: List of wheat varieties for evaluation of resistance against leaf rust races at their seedling stage in the greenhouse

S.N	Variety	Wheat type	Pedigree
1	Mekelle 1	Bread wheat	PARULA[2800]
2	Mekelle 2	Bread wheat	HINDI62/BOBWHITE/CPAN2099
3	Mekelle 3	Bread wheat	NA
4	Mekelle 4	Bread wheat	NA
5	Picaflor	Bread wheat	CHIL/PRL
6	Digalu	Bread wheat	SHA7/KAUZ
7	Dashin	Bread wheat	VEE 17/KVZ/BUHO"S"//KAL/BB
8	Ude	Durum wheat	CHEN / ALTAR- 84// JO69 CD 95294-9M- 030Y-040 PAP-2Y-OB
9	Dembi	Durum wheat	VZ466/61-130XLD SX GII"S" CM9605
10	Local cultivar	Bread wheat	NA
11	Morocco (Sus. check)	Bread wheat	NA

NA= not available

RESULTS AND DISCUSSION

Survey of wheat leaf rust in south eastern zone of Tigray

Plant disease assessment is to measure the amount of disease present in terms of incidence and severity of the individual plants. Keeping this in mind, survey of leaf rust was carried out from early to mid of September in 2013 in five districts of Southeastern Zone of Tigray. During the survey, 108 wheat fields were assessed for leaf rust distribution and intensity. Whenever disease assessments are made, growth stage of the plants is essential for meaningful comparisons between varieties, locations and years (Stubbs *et al*., 1986). In view of this, 2.8% of the wheat crop was at tillering to booting, 50% at heading to flowering and 47.2% at milk to dough growth stages. Leaf rust was observed in 2(66.7%), 45(83.3%) and 47(92.2%) of the 3, 54 and 51 wheat fields inspected during tillering to booting, heading to flowering and milk to dough growth stages of the crop respectively.

As far as disease intensity was concerned, leaf rust was found more important at heading to flowering and followed by milk to dough growth stages of the crop (Table 5). During heading to flowering growth stages, mean incidence of 67.2% and severity of 26.9% were recorded. The disease also caused high infection at milk to dough growth stages, with mean incidence and severity of 44.2% and 12.7%, respectively. Earlier study also indicated that, highest intensity of wheat leaf rust was recorded at heading to flowering growth stages of the crop (Mideksa, 2011).

Conversely, the lowest incidence and severity of leaf rust was recorded at tillering to booting growth stages of the crop with mean values 21.2% and 6.7%, respectively. This might be resulted from the fact that, the flag leaf which is the critical growth stage of the crop during which leaf rust reached its maximum severity level (Seck *et al.,* 1985), was not fully develop at tillering to booting growth stages as compared to heading to flowering and milk to dough growth stages of the crop . On top of this, damage is minimal during tillering due to the onset of colder temperatures that are likely to eliminate or reduce reproduction and spread of rusts.

Distribution and intensity of wheat leaf rust across districts

The disease was more prevalent at H/wejirat and Wukro districts with prevalence of 100% and 96.7% respectively, while districts of Enderta and S/samre showed similar distribution of wheat leaf rust, 85% each. In contrast, the lowest prevalence (45.5%) of leaf rust was registered at D/ Temben district. As a whole, wheat leaf rust was observed on 88% of the 108 wheat fields inspected in southeastern zone of Tigray (Table 6). This indicated that, the pathogen was widely distributed across the districts of the study area. As far as leaf rust intensity was concerned, different mean incidences and severities were recorded in all districts. 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 S/samre, with range of 0-100% and mean incidence of 60%. Districts of Enderta and H/wejirat also showed a considerable level of leaf rust incidence with mean values of 57.9% and 53.3%, respectively (Table 6). However, the lowest incidence of the disease (7.4%) was registered in D/Temben district. The overall mean incidence for the surveyed districts of southeastern zone of Tigray reached 48.4%.

Table 6: Prevalence and intensity of wheat leaf rust across districts in 2013 main cropping season

Districts	Altitude range (masl)	Number of fields inspected	Number of fields infected	Prevalence (%)	Incidence (%)		Severity (%)	
					Range	Mean	Range	Mean
H/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
S/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
D/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

Likewise, leaf rust severity showed similar trend as that of incidence in Wukro, S/samre and D/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 S/samre district, with range of 0-85% and mean severity of 26.1%. 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 D/Temben district. Unlike the three districts mentioned above, leaf rust severity did not show similar trend as that of incidence in Enderta and H/wejirat districts. Enderta and H/wejirat were ranked third and fourth in terms of incidence with mean values of 57.9 and 53.3%, respectively. Conversely, these districts were ranked as fourth and third in terms of severity with mean values of 9.5 and 14.1% following the same order. This indicated that, incidence did not show direct relationship to the severity of leaf rust in these districts. This might be associated with fact that, plants were counted as diseased whether they were exhibiting a single pustule or hundreds of pustules during recording incidence. But, in case of severity, plants with hundreds of pustules were considered as more diseased than plants with single pustule. Hence, incidence may have little relationship to the severity of the disease under such circumstance. The overall mean severity for the five districts of southeastern zone of Tigray reached 18.2% (Table 6).

Generally, leaf rust was found more important at Wukro, S/samre, H/wejirat, and Enderta districts. The high level of leaf rust intensity in these districts might be the cultivation of local cultivar or “Shahan” which is susceptible to wheat rusts in general and leaf rust in particular. This cultivar covered about 53.3, 55.6, 57.9 and 65% of the wheat area in Wukro, H/wejirat, Enderta and S/samre districts respectively (BoARD, 2005). Moreover, the cultivation of wheat in some areas of these districts during offseason could have played a significant role in rehabilitating the population of wheat leaf rust. Hence, these varieties 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 for growing wheat (meher and belg seasons) 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). However, the low prevalence and intensity of leaf rust in D/Temben district might be resulted from low temperature occurred during the season. In this district, the temperature was less than 10^{o}C (ENMA, 2013) which is below the optimum level of temperature. This low temperature likely eliminates or reduces the reproduction and spread of the rust. Earlier studies also confirmed that, at 10^{o}C, infection developed very slowly and restricted in size (Dyck and Johnson, 1983).

Distribution and intensity of wheat leaf rust across localities

The ‘within district’ comparison indicated that, highest prevalence (100%) of the diseases was recorded in all localities of H/wejirat. This implied that, the population of leaf rust was uniformly distributed across wheat fields of this district. However, these localities were infected with different levels of incidences and severities of leaf rust. The disease was more important in Adigudom, Hiwane and Freweyni localities with mean incidences of 83.8%, 65.3% and 10.9% in that order. The range of incidences in these localities also showed the same order as that of mean incidence. Similarly, the severity of this disease in these localities showed similar trend as that of incidences. The highest mean severity of 19% was recorded in Adigudom, followed by Hiwane with mean value of 17.6%. Contrary to this, the lowest severity of

Table 7. Prevalence and intensity of wheat leaf rust across localities of Southeastern Zone of Tigray, in 2013 cropping season

District	Kebele (locality)	Altitude range (masl)	No. fields inspected	No. fields infected	Prevalence (%)	Incidence (%)		Severity (%)	
						Range	Mean	Range	Mean
H/wejirat	Hiwane	1966-2066	10	10	100	19-95.5	65.3	1-40	17.6
	Adigudom	2048-2198	10	10	100	42.5-100	83.8	5-75	19
Wukro	Freweyni	2012-2195	7	7	100	5-30	10.9	5-10	5.7
	Genfel	1935-2399	16	15	93.8	0-100	63.6	0-95	28.4
	Adimekel	1927-2091	14	14	100	5-100	62.7	1-95	46.1
S/Samre	Degen	2001-2339	11	8	72.7	0-82	36.8	0-30	10
	Mytekli	1961-2123	9	9	100	20-100	83.2	5-85	42.2
Enderta	Quiha	2022-2292	10	8	80	0-100	65.1	0-20	8.8
	Illala	1912-2225	10	9	90	0-92.9	50.7	0-25	10.2
D/Temben	D/Temben	2431-2654	11	5	45.5	0-24	7.4	0-10	4.1
Total/mean	10	1912-2654	108	95	88	0-100	53	0-95	19.2

Table 8. Prevalence and intensity of leaf rust in different agro ecologies of Southeastern zone of Tigray in 2013 cropping season.

Altitude range (masl)	Number of Inspected field	Number of Infected fields	Prevalence (%)	Incidence (%)		Severity (%)	
				Range	Mean	Range	Mean
1801-2300	91	84	92.3	5-100	59.9	Trace- 95	21.4
2300 -2654	17	11	64.7	1-100	29	Trace-15	5.6
Total/Mean	108	95	78.5	1-100	44.5	Trcea-95	13.5

Source: IFPRI, 2006

wheat leaf rust was recorded in Freweyni with mean value of 5.7%. In Wukro district, the disease was also highly distributed with prevalence of 93.8% in Genfel and 100% in Adimeskel. These localities showed similar mean incidences of leaf rust with 63.6% in Genfel and 62.7% in Adimeskel wheat fields. However, they showed differences in severities of the disease, in which the highest mean severity of 46.1% recorded in Adimeskel and 28.4% in Genfel wheat fields. The low incidences and severities in these localities resulted from low or free infection scored from durum wheat varieties, Ude and Dembi grown in both localities for seed multiplication. The prevalence and intensity of the disease were different across localities of Degen and Mytekli in S/samre district. Leaf rust was uniformly distributed in Mytekli with prevalence of 100%, while 72.7% was recorded in Degen wheat fields. The highest mean incidence (83.2%) and severity (42.2%) were recorded where the highest prevalence (100%) was noted in Mytekli. In the same way, the lowest mean incidence (36.8%) and severity (10%) were recorded where the lowest prevalence (72.7%) was noted in Degen. This indicated that, the distribution of the disease in these localities sowed the same trend as that of incidence and severity.

In contrast, less similarity trend among prevalence, incidence and severity of leaf rust was observed in Enderta localities. The highest prevalence of 90% and mean severity of 10.2% were recorded in Illala, while prevalence of 80% and mean severity of 8.8% were recorded in Quiha. But, a higher mean incidence of 65.1% was scored in Quiha, followed by Illala with 50.7%. Comparing with the listed localities, the lowest prevalence and intensity of leaf rust was recorded in D/Temben wheat fields. In this study area, the disease was distributed intermediately with prevalence of 45.5% and mean vales of 7.4% and 4.1% incidence and severity respectively (Table 7).

Distribution and intensity of wheat leaf rust across altitude ranges

Of 108 wheat fields inspected, approximately 84% of the fields were found with altitude range of 1801-2300 m. a. s. l, while the remaining 16% were found between 2300-2654 m. a. s. l. The highest prevalence of the disease was recorded at altitude range between 1801-2300 m. a. s. l. Out of 91 wheat fields inspected in this altitude range, the disease was found in 84 wheat fields. Similarly, the highest incidence and severity of wheat leaf rust were recorded at this altitude range of 1801-2300 m. a. s. l with range and mean values of 5-

100% and 59.9% and Trace-95% and 21.4% respectively. However, the distribution and intensity of wheat leaf rust reduced at higher altitudes. Relatively low prevalence (64.7%) of leaf rust was recorded at altitude range of 2300-2652 m. a. s. l. In the same way, the range and mean values of incidence and severity were reduced to 1-100% and 29% and Trace-15% and 5.6%, respectively. The overall mean incidence and severity of wheat leaf rust for the midland (1801-2300 m. a. s. l) and highland (2300-2654 m. a. s. l) of the study area reached 44.5 and 13.5%, respectively (Table 8). In general, though, the disease was more important in midland areas, it was also distributed in the highland of the study area with considerable amount of intensity and prevalence. This might be associated with the fact that, wheat leaf rust occurs wherever wheat is grown and it is the most widely distributed of all cereal rusts (Knott, 1989). Moreover, the adaptability of leaf rust to different climates play a significant role for the widely distribution of the pathogen (Roelfs and Singh, 1992). This result is also similar with the findings of Dagnachew (1967). He reported that, leaf rust is endemic at different altitudes of wheat growing regions of Ethiopia.

Prevalence and intensity of wheat leaf rust by wheat type and variety

Tesfaye and Getachew (1991) reported that tetraploid wheat (durum) species cultivated in Showa, Gojam, Gonder, Wello and Tigray occupied 60% of the total wheat area in the country. However, recent studies that were conducted in the above mentioned regions and in the country as whole (Belaynesh, 2010) as well as this survey showed hexaploid wheat (bread) was dominating over the tetraploid wheat species. In view of this, about 95% of wheat fields in the study area were covered by bread wheat varieties. The local cultivar (locally named as Shahan) dominated the bread wheat varieties and covered 56.5% of wheat field in the study area. This was followed by Picaflor, Dashen and Mekelle-1 which comprised 16.7%, 6.5% and 5.5%, respectively. On the other hand, durum wheat varieties, Ude and Dembi covered only 4.5% of wheat fields in southeastern zone of Tigray.

During the survey, the prevalence and intensity of leaf rust varied between durum and bread wheat varieties (Table 9). Though, durum wheat varieties, Ude and Dembi showed prevalence of 100%, the intensity of the disease was lower on these varieties compared to Dashen, Digalu, Picaflor, Mekelle-3, Mekell-1 and local cultivar (Shahan). The range incidence and severity of leaf rust in durum varieties varied between 0-10 percent each respectively. The lowest mean incidence (2.5%) and severity (2.5%) of leaf rust was recorded on variety Dembi and followed by Ude, with mean incidence of 5% and severity of 3.8% across the three fields. The low intensity in these varieties was resulted from their resistant response recorded in all wheat fields of the study area. This finding is in agreement with previous report 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, the highest intensity of leaf rust was recorded in bread wheat varieties at different levels of incidences and severities. The prevalence and intensities of leaf rust in Mekelle varieties were lower as compared to Dashen, Digalu, Picaflor and Local cultivar. The prevalence of leaf rust in these varieties ranged between 50 -100% of the fields cultivated. The leaf rust incidences and severities in these varieties varied between 0-19.1%

Table 9. Prevalence and intensity of leaf rust on varieties grown in south eastern zone 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 *	2000-2626	3	3	100	0-10	5	0-10	3.8R
Dembi*	1973-2614	2	2	100	0-5	2.5	0-5	2.5R

and 0-10%, respectively.The lowest incidence and severity of the disease was recorded in Mekelle-4 with mean values of 2.5% each, respectively (Table 9). Moreover, these varieties demonstrated moderately resistant to resistant reaction to leaf rust populations in their inspected fields.

High intensity of wheat leaf rust was found on commercial bread wheat varieties of Dashen, Digalu, Picaflor and Local cultivar as compared with the other varieties. The intensity of the disease in these varieties varied between 0-100% in incidence and 12-27% in severity (Table 9). Varieties, Dashen, Digalu and Picaflor demonstrated moderately susceptible to moderately resistant response to leaf rust across locations. However, the local cultivar consistently showed susceptible response to the disease in all the study areas. As a result, the highest mean incidence of 72.9% and severity of 27% were recorded on this cultivar. The disease was prevalent on 90.2% of the fields cultivated with local cultivar. The long period cultivation and increase in susceptibility from time to time by leaf rust population probably makes the local cultivar highly infected. In addition, the wide cultivation of this cultivar in the study area 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 landrace cultivars that are susceptible to the disease; even though a large number of improved cultivars of wheat have been released.

In Ethiopia, leaf rust is one of the most important diseases of wheat and its recurrent outbreaks have threatened wheat production in the country (Bedabo, 2002). 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). Generally, most of the varieties demonstrated different response across localities, among varieties and even within the same variety. This variation might be the result of differences in host growth stage. Susceptibility and resistance are often highly correlated with host growth stage even with races specific resistance. A host may be subjected to a heavy inoculum density with favorable infection period at critical growth stage, while other host may not be confronted with similar circumstances when it is at the critical stage (Roelfs, 1992). Environmental difference across districts and variation in aggressiveness among population of wheat leaf rust can also result different responses even with the same varieties.

Identification of P. triticina races in southeastern zone of Tigray

Race analysis provides essential information in determining the range of pathogenic variation in a specific region, screening resistance in cultivars, confirming that host responses are due to race changes, understanding the mechanism of variation as well as in determining the direction of research and breeding programs before the pathogen becomes a threat to wheat crop (Abebe et al., 2013). Using the international system of nomenclature for *P.triticina* (Long and Kolmer, 1989), 22 races were identified from 40 mono pustules or isolates based on their reactions on 16 differential hosts.

Distribution and diversity of P. triticina races across districts

As far as race distribution was concerned, 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 isolated from three or four districts of the study area (Table 10), which indicated that they were widespread throughout southeastern zone of Tigray. PHTT was detected eight times in the population of wheat leaf rust collected from Wukro, H/wejirat and Enderta districts while, PHRT was isolated six times from S/samre, D/ Temben, Wukro and Enderta populations 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, S/samre and H/wejirat and Wukro, Enderta and H/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 10). 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, H/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. This genetic similarity between *P. triticina* isolates of these districts is in line with the findings of McVey *et al*. (2004), who reported similar level genetic similarity between *P. triticina* populations collected from Egypt in 1998 to 2000 and from southern and central plains of United States in the same period. The within districts comparison also indicated that, some genetic similarities among isolates of Wukro, H/wejirat, S/samre and D/Temben were observed. Out of the 11 isolates collected in wukro district, 36.4% of the isolates showed genetic similarity and resulted in race PHTT. In H/wejirat district, 20 and 30% of the isolates were resulted in FHRT and PHTT races respectively. This indicated that, only 50% of the leaf rust isolates showed genetic diversity in this district. Isolates of S/samre also showed genetic similarity as a result; races THTT and PHRT were detected two times each from 10 isolates of leaf rust. In the same way, PHRT was identified from two isolates collected from D/ Temben district.

Their geographic proximity, absence of barriers and cultivation of similar bread wheat cultivars among Wukro, H/wejirat and S/samre districts might have played significant role for race similarity. On top of this, these races might be more fit or easily adapted with the environment of these districts. D/Temben district on the other hand is geographically isolated by mountains from other places. Thus, the possibility of migration of urediospores of wheat leaf rust to and from this district is much 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 10). 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. 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.

Table 10: Distribution of *P. triticina* races across districts of Southeastern Zone of Tigray

Races	Districts					Isolates	Frequency (%)
	Wukro	Enderta	S/samre	D/Temben	H/wejirat		
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

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 11). 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 what production in the region in general and Southeastern zone 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 (Table 11). The "L" group races, LBBM and LBDC were the least virulent, producing compatible reaction only on three *Lr* genes (*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 (Table 11).

Approximately 55% of the races identified in Southeastern zone 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 11). 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 depend on the type of wheat cultivars grown (Singh, 1991), and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*., 1982). 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 12). 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 11. Virulence /avirulence spectrum of *P. triticina* races collected from Southeastern Zone of Tigray in 2013

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 12: 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

The *Lr18* displayed consistently high infection type to all isolates of *P. triticina* collected from southeastern zone 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". This showed that absolute compatibility of *Lr18* to all races of *P. triticina* was demonstrated. This information may provide a clue either *Lr18* is effective and expressed at adult plant or completely lost from its differential line, RL6009. However, further study on the effectiveness of *Lr18* against wheat leaf rust population is required to consolidate this conclusion. 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 S/samre district. McIntosh *et al*. (1995) also reported that, *LrB* was ineffective to leaf rust isolates in most geographic areas of Australia. 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. 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). On the other hand, *Lr9, Lr24,* and *Lr2a* were found to be effective to most of wheat leaf rust populations (Table12). 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). This finding is also in a good agreement with the previous studies which stated that, no virulence to *Lr9* was found (Hussain *et al*., 1980). Similarly, *Lr24* was found to confer resistance to 95% of the tested leaf rust isolates. This gene was ineffective only by two virulent races, TKTT and PJTT identified from Wukro and S/samre isolates respectively. Mesterhazy *et al.* (2000) and Kolmer (2003) also stated that, *Lr24* is generally effective in many wheat producing areas of the world. Virulence on *Lr*2a was also rare and found to be effective to 82.5% of leaf rust isolates. Anteneh (2011) also reported that, *Lr2a* was exhibited effectiveness to wheat leaf rust races. Hence, these genes can be used as potential sources during wheat breeding programs for resistance to wheat leaf rust.

Response of wheat varieties to leaf rust races at seedling stage in greenhouse

In this study, seedlings of 10 commonly grown wheat varieties and the universally susceptible check, Morocco were screened against the most virulent and dominant races of leaf rust (TKTT, THTT and PHTT) identified in APPRC greenhouse. The reaction of wheat varieties to leaf rust 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) (Table 13).

Table13. Response of wheat cultivars to dominant and virulent races of wheat leaf rust at seedling stage in greenhouse in 2013 growing season

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

Among the tested wheat varieties, eight (Mekelle-1, Mekelle-2, Mekell-3, Mekelle-4, Picaflor, Digalu, Dashen and local cultivar), seven (Mekelle-1, Mekelle-2, Mekelle-3, Mekelle-4, Picaflor, Dashen and local cultivar) and five (Mekelle-3, Mekelle-4, Picaflor, Dashen and local cultivar) of them produced susceptible reaction to TKTT, THTT and PHTT races, respectively. Five bread wheat varieties namely, Mekelle-3, Mekelle-4, Picaflor, Dashin and local cultivar were susceptible to the three races. Varieties, Mekelle-1 and Mekelle-2 were susceptible to TKTT and THTT races, but resistant to PHTT. Digalu was only susceptible to TKTT but showed resistance to THTT and PHTT races. The data showed that, the more the virulent race, the more susceptibility on many bread wheat varieties was recorded and vice versa.

Most varieties showed more resistance under the natural infection in the field than at seedling stage in the green house. All the Mekelle varieties, Mekelle-1, Mekelle-2, Mekelle-3 and Mekell-4 showed moderately resistance to resistance under the natural infection in the field, while they showed susceptibility to at least two of the virulent races of leaf rust at seedling stage in the greenhouse. Similarly, Picaflor, Dashin and Digalu showed moderately susceptible to moderately resistance reaction under the natural infection, while they showed susceptible reaction to at least one of the races of leaf rust at their seedling stage.

This variation might be resulted due to, these varieties may have genes responsible for adult plant resistance at field condition, but poorly expressed at their seedlings in the greenhouse. The presence of conducive environment in the greenhouse provided optimum development to leaf rust as compared to the field where environmental conditions might not regulated based on the requirement of the pathogen. Hence, this circumstance also contributed for the susceptible response of these varieties at their seedling stage in the green house. Moreover, varieties in the greenhouse were evaluated through the inoculation of the most virulent and dominant races of leaf rust and resulted susceptible reaction on the seedlings of the above mentioned varieties. However, varieties in the field have a chance to be infected with weak population of leaf rust. In effect, low infection type or resistance to leaf rust could be observed in these varieties under field condition. The local cultivar however, showed susceptible reaction under natural infection and greenhouse inoculations for leaf rust population. This cultivar had high infection types similar to that of "morocco". Therefore, this cultivar can be assumed as having no effective gene (s) at its seedling and adult plant growth stages. Both durum wheat varieties, Ude and Dembi have demonstrated resistance to leaf rust at adult plant in the field and seedling stages in the greenhouse.

In general, 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 race (s) after short period of releasing.

CONCLUSIONS

Leaf rust is one of the most important foliar diseases of wheat in Tigray region in general and south eastern zone in particular. This study indicated that, all the identified races of *P. triticina* have shown genetic uniqueness as compared to the previously identified races in Ethiopia. The variation over location, wheat varieties grown and environmental conditions, especially temperature might be contributed for the uniqueness of the races. Approximately, 81% of the *Lr* genes were ineffective to more than 55% of *P. triticina* isolates. High virulence frequencies (≥72.5%) have been found on the resistance genes *Lr2c, Lr26, Lr1, Lr3, Lr16, Lr3ka, Lr11, Lr30, LrB, Lr10, Lr14a* and *Lr18*. However, *Lr* genes *9, 24* and *2a* were found effective to 100%, 95% and 82.5% of the tested isolates. Hence, these genes are among the most important genes which could be used as sources of resistance to wheat leaf rust. Evaluation for wheat varieties for their resistances is very important in integrated leaf rust management. In this study, a total of 10 commonly grown wheat varieties were evaluated against three virulent and dominant races of leaf rust at seedling stage in greenhouse and these varieties showed broad infection types from 1 (small uredia surrounded by necrotic area) to 4 (large uredia without chlorosis). Generally, the study confirmed the presence of wider range of virulence among the population of wheat leaf rust races, indicating the presence of genetic diversity among the races in the study area. 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 into 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. Leaf rust is highly variable even within a single cropping season, and breakdown the previously resistant varieties. Hence, it has to be surveyed regularly to determine its current status and to take action before the pathogen becomes a risk to wheat production. Virulence has been observed on all of the *Lr* genes except *Lr9*. Thus, searching for new source of leaf rust resistant genes is necessary to maintain leaf rust resistance. The leaf rust resistant gene *Lr9* was identified as effective gene to all leaf rust isolates. Hence, it should be utilized in breeding program with other effective genes through gene pyramiding as the additive effects of these genes offer the cultivar a wider base of leaf rust resistance. The results from both seedling test and field survey revealed that local cultivar exhibited susceptibility to leaf rust populations. Hence, Breeders and/ Plant pathologists should replace this cultivar by developing resistant varieties that does not follow gene-for-gene specificity. The Ethiopian durum wheat landraces are potential sources of leaf rust resistance. Hence, their resistant genes should be exploited in wheat breeding programs. Finally, plant pathologists and /or breeders should use this data as a base line during resistant variety development program in the study area.

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