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Characterization of wheat leaf rust pathogen (*Puccinia triticina*) in some parts of Ethiopia and seedling evaluation of durum wheat (*Triticum turgidum*) cultivars to the pathogen

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Leaf rust caused by the pathogen *Puccinia triticina* is one of the important diseases of wheat in Ethiopia; and yield loss due to this disease has reached up to 75%. A study was carried out to detect physiologic races of the pathogen from some parts of Bale, Gondar and Shewa and to identify seedling resistance of Ethiopian durum cultivars. Twenty-four mono pustule isolates were made, and out of those, only three *P. triticina* races namely; BBBB, BBBN and BBBQ were identified. Race BBBN was detected for the first time in Ethiopia and was less virulent to commercial durum wheat cultivars than BBBQ and BBBB, while phenotype BBBB was relatively highly virulent to Ethiopian durum wheat and it infect 42.5% of the tested cultivars and is not virulent to Thatcher and Thatcher isoline leaf rust differential cultivars. Thirty six commercial durum wheat cultivars and three land race cultivars were evaluated against those three leaf rust pathogen races at seedling stage in the green house at Debre-Zeit Agricultural Research Center. Varieties Utuba, Lelisa, Worer, Bekelcha, Tate, Filakit, Foka, Alem tena, Hitosa, Kilinto, Denbi, Ude, LD-357, Boohai, Mukuye, Robe, Mangudo and Assasa exhibited resistance reaction (; to 2) to all the three leaf rust races, while the rest showed susceptible at least to one of the race. All the three land race cultivars (Mcd4-12, Mcd4-32 and Mcd7-42) were susceptible to all pathogen races.

Key words: Durum wheat, leaf rust, *Puccinia triticina*, race, seedling resistance.

INTRODUCTION

Wheat is a worldwide staple food crop that provides 20% of protein and calories for human consumption (FAO, 2015), and its demands are progressively growing with the elevations of human population (Wageningen FSC, 2016); however, world wheat productivity is growing at 1% rate.

In Ethiopia, wheat is an important crop, where it represents 14% of caloric intake and considered as

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second most important food crop (FAO, 2015) in areas of cultivation of crops in Ethiopia (REAP, 2015). Currently, the rapid urbanizing sub-Saharan Africa, subjected to increase wheat consumption is about 38% at 2023 (Geraled et al., 2010) and this elevation in wheat demand in Ethiopia may be due to the emergence of many food processing industries (REAP, 2015).

The use of crop diversity is a key approach to improve productivity and achieve food security (David et al, 2001). Where, Ethiopian durum wheat landraces are diverse and possess high variation that has not been exploited (Teklu and Hammer, 2008). Ethiopian landraces contributed to world wheat improvement for instance and the Ethiopian durum wheat landrace *ST464* was one of the major sources of *Sr13 that is, Ug99* resistance gene (Klindworth et al., 2007). On the other hand, more than 90 bread and 36 durum wheat varieties had been used for production since 1950s. In Ethiopia however, the national average yield is still 2.78 t/ha, which is lower than potential yields of 8 to 10 t/ha (CSA, 2017).

In general, the low productivity is mainly attributed to lack of durable resistant varieties to the prevalent wheat rusts. Hence, leaf rust caused by *Puccinia triticina* Eriks, is one of the most important diseases in Ethiopia (Ayele et al., 2008) where the production loss due to leaf rust reached 70-75% in susceptible wheat varieties at hot spot areas (Ayele et al., 2008; Mengistu et al., 1991; Draz et al., 2015; Ordonez et al., 2010; Shimelis and Pretorius, 2005). During 2007-2009 cropping seasons, incidence of 30.2% was recorded for leaf rust in Oromia, Amhara South, and Tigray regions. The average prevalence of leaf rust for the above mentioned locations was 53.3% (Tesfay et al., 2016).

Recently in Tigray region, 22 races of *P. tiriticina* were identified, among them PHTT and PHRT were dominant, but the broadest virulence spectrum was recorded from TKTT race, making all Lr genes ineffective except Lr 9. While the evaluation of these varieties, *Mekelle-3, Mekelle-4, Picaflor,* and *Dashin* were susceptible to TKTT, THTT, and PHTT races, *Mekelle-1 and Mekelle-2* were susceptible to races TKTT and THTT, but resistant to PHTT. But the *Digalu* was only susceptible to TKTT race. Unlike bread wheat varieties, the durum varieties, *Ude* and *Dembi* were resistant to these races (Tesfay et al., 2016). In general, most information on identification of *P. triticina* races were obsolete, and even the recent studies of race identification and identification of resistance wheat cultivars were limited to some parts of Tigray region and some bread wheat cultivars only.

In Ethiopia, wheat production used to be characterized by high biodiversity in crops and low input systems, and the management of wheat rusts largely relied on genetic host resistance (Sewalem et al., 2008); however, host resistance might not always be readily available in commercial cultivars. In addition, this requires regular and continuous search for finding a new source of resistant genes in the cultivated and wild forms of wheat.

Till date, most of the research works on wheat diseases focused on yellow rust and stem rust in Ethiopia, while, research on wheat leaf rust is limited. Therefore, characterization of *P. triticina* isolates and identification of resistance in durum wheat cultivars and landraces to the prevailing races would be essential for breeding and variety deployment strategies in the country.

MATERIALS AND METHODS

Collection of wheat leaf rust samples

A total of 24 leaf rust infected samples were collected from east Shewa (9), North Gondar (5) and Bale (10) zones in Ethiopia, respectively. The infected wheat fields were assessed along the main roads and accessible routes in each survey districts at 5-10 km interval based on vehicle odometers and each field assessed in X patterns, and all necessary information like location, variety name and GPS data were taken. Four to five freshly infected young leaves were taken using sterile scissors and placed in glassine bags; the infected leaf samples were transported to a laboratory at Debre Zeit Agricultural Research Center under dry conditions and then allowed to dry at room temperature for 24 h. Spores from each sample collected using motorized pump in a gelatin capsule and then stored at 4°C under dry conditions for a while.

Isolation and multiplication of *P. triticina* inoculum

The bulked spores from infected dried leaf rust samples were collected in a gelatin capsule using a vacuum pump; then, the spores were suspended in light mineral oil (that is, soltrol 130) and sprayed on seven days old seedlings of susceptible durum wheat cultivar *RL6089* using motorized pump. The plants were then misted with fine droplet of distilled water and incubated in a plastic dew chamber for 24 h at 18-22°C with close to 100% RH. Then, the seedlings were transferred to a greenhouse bench of temperature 18-25°C and RH of 70%. A portion of spore samples was kept in a refrigerator at 4°C as a backup.

Seven days after inoculation leaves containing a single fleck were selected from the base of a leaf. The remaining leaves within a pot removed using sterilized scissors. A single leaf with fleck was covered with cellophane bag and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunson, 2004). About 12-14 days after inoculation when the mono pustule was well developed, each mono pustule were collected in gelatin capsule using motorized vacuum pump. Spores harvested from a mono pustule were suspended with light weight mineral oil (soltrol 130) and inoculated on to seven days old seedlings of susceptible durum cultivars that is, RL6089. About 12-14 days after inoculation, the spores from each mono pustule were collected in separate gelatin capsule as indicated above and stored at 4°C to be used for inoculation of leaf rust differential lines. These procedures were repeated until sufficient amount of single spores produced for race analysis purpose. In this way, 24 mono pustule isolates were developed for race analysis.

Inoculation of *P. triticina* isolates to wheat leaf rust differential lines

Five seeds from each 16 differential lines and susceptible check, *RL 6089* were grown in 4 cm x 4 cm diameter pots containing soil, sand and compost in 2:1:1 (v/v/v) ratio, respectively. A single

pustule derived spores(approximately 3-5 mg of spores per ml) was suspended in light weight mineral oil (soltrol 130) and sprayed on to seven days old seedlings using motorized pump and then placed in dew chamber close to 100% RH at 18-22°C for 24 h. Then, seedlings in each pot were transferred to the greenhouse within a plastic cubicle to avoid contamination. The greenhouse temperature and humidity were maintained between 18-25°C, 60-70%, respectively.

Leaf rust assessment on differential lines and race designation

Twelve days after inoculation, disease scores (infection types) were taken using 0-4 scoring scale employed by Long and Kolmer (1989). Infection types were grouped into low and high reaction type; where low refers to resistance or incompatibility (infection type 0-2) and high refers to susceptible or compatibility (infection type 3-4). Accordingly, a virulence and virulence of isolates were determined by low (L) and high (H) infection types, respectively.

Physiological race designations were made as described by Long and Kolmer (1989); while the sixteen differential lines were grouped into four sets in the following order: Group1; Lr1, Lr2a, Lr2c, Lr3; group 2; Lr9, Lr16, Lr24, Lr26; group 3; Lr3ka, Lr11, Lr17, Lr30; and group 4; LrB, Lr10, Lr14a and Lr18. Each isolate assigned a four letter race code based on the reaction of differential lines (Long and Kolmer, 1989). For instance, low infection type (L) on the four hosts on a set was assigned with a letter B, while high infection type (H) in the four hosts was assigned with a letter T. Hence, if an isolate produces low infection type on the 16 differential hosts, the races were assigned with a four letter race code BBBB. In the same way, an isolate which produces a high infection type on the 16 wheat leaf rust differential hosts have a race code TTTT. If an isolate produces a low infection type on Lr9 but a high infection type on the remaining 15 differential hosts, the race is designated as TKTT. In such a way, the race nomenclatures were completed for all 24 isolates.

Seedling tests of durum cultivars and landraces

Three different *P. triticina* races were used to inoculate 36-durum wheat cultivars, three landraces lines and susceptible check. Similar procedures were applied as indicated above for seedling growth, inoculation, incubation and disease scoring. Data on infection types were recorded 12 days after inoculation using standard disease score scale 0-4 (Long and Kolmer, 1989). Resistance and susceptible cultivars and landraces were determined by low and high infection types for each isolates.

RESULTS AND DISCUSSION

Identification of *P. triticina* races in Bale, Gondar and Shewa

Three different virulent phenotypes (races) of *P. triticina* were found in three zones in Ethiopia (East Shewa, Bale and North Gondar) as described in Table 1. Races BBBQ, BBBN and BBBB were detected from Bale zone, while BBBB was identified from East Shewa and North Gondar zones as shown in Figure 1. Leaf rust race BBBQ was virulent only to the Thatcher lines with genes LrB and Lr10, whereas, leaf rust race BBBN was virulent only to the Thatcher (bread wheat) lines with LrB and Lr14a genes. Out of the three identified races, BBBB is the most frequent and predominant with a frequency of 91.6%, whereas the remaining two (BBBQ and BBBN) were detected once each with a frequency of 4.1%. Race BBBB is virulent to Thatcher line (bread wheat); thus, virulence to leaf rust resistance genes in the Thatcher iso-line series could not be determined for this phenotype. Phenotype BBBB is virulent on durum wheat, but virulent on Thatcher and other susceptible bread wheat cultivars and has not been detected elsewhere except Ethiopia, and temporarily named as ETH-BBBB race.

P. triticina isolates from Ethiopia that was virulent to durum wheat but not virulent to Thatcher wheat was previously noted (Roelfs et al., 1992; Ordonez and Kolmer, 2007b). Before a large cultivation of bread wheat in Ethiopia, ETH-BBBB isolate may be left over from *P. triticina* population that was adopted to Ethiopian durum wheat cultivars. If this isolate had been present in Ethiopian durum land race cultivars, it may have higher genetic variability than other recently introduced *P. triticina* isolates (Kolmer and Acevedo, 2016). Isolates of *P. triticina* with high virulence to durum wheat have recently been found and characterized in Mexico (Singh et al., 2004), France (Goyeau et al., 2011), Spain (Martinez et al., 2005), Italy (Mantovani et al., 2010), Israel and Turkey (Kolmer, 2009), Argentina (Ordonez and Kolmer, 2007b) and Ethiopia (Mengistu et al., 1991; Kolmer and Acevedo, 2016). On the other hand, the *BBBQ* was identified in Ethiopia as other isolates recovered from durum wheat in other countries. This phenotype was collected from emmer wheat and had virulence only to lines with genes *LrB* and *Lr10,* while*, BBBQ* was a virulent to many bread wheat cultivars; thus, availability of this isolate may be influenced by the presence of durum wheat (Kolmer and Acevedo, 2016).

Similar findings were reported as Altar C84 durum wheat which may become susceptible to durum leaf rust (BBBQ) in Mexico (Singh et al., 2004). Other study determined that Altar C84 carried Lr72 (Herrera-Foessel et al., 2014) and many other CIMMYT cultivars and durum germplasm from around the world were susceptible to this new virulence phenotype of *P*. *triticina.* Additional virulence variation to durum wheat cultivars may be present in the *BBBQ* isolate from Ethiopia and other countries Kolmer and Acevedo (2016); however, a differential set of durum wheat genotype will need to be developed to properly assess such isolates, because the Thatcher differentials do not detect much virulence variation in these isolates.

The isolates with phenotype *BBBN* was virulent on *LrB* and *Lr14a*. These isolates may have some virulence to bread wheat cultivars, because *BBBN* was isolated from bread wheat cultivar (Sofumer) collected in Bale zone. Phenotype *BBBN* previously detected in Mexico and other parts of the world are reported as virulent to *Lr72* (Singh et al., 2004), but detected for the first time in Ethiopia, however, further works and conformations

Table 1. Identified *P.triticina races* in some parts of Ethiopia during 2107.

Race	Virulent	No. of isolate	Location
BBBB	-	22	Bale East-Shewa and North Gondar zones
BBBQ	LrB and Lr10	1	Bale zone
BBBN	LrB and Lr14a	1	Bale zone

Figure 1. The distributions of the *P. triticina* races across the assessed areas.

required.

In this study, only twenty-four isolates were collected from Oromia and Amhara regions of Ethiopia because of very low prevalence of wheat leaf rust, hence, number of characterized isolates was limited, and only three phenotypes identified. Also, Kolmer and Acevedo (2016) identified small amount of phenotypes from wide geographical area and large amount of collections. Conversely, in other populations of *P. triticina* collection even from relatively small geographical area large number of phenotypes was reported in Tigray, Ethiopia (Tesfay et al., 2016).

Seedling tests of durum wheat cultivars and landraces to identified races

Seedlings of 36 commonly grown Ethiopian durum wheat varieties and some land race cultivars including susceptible check morocco were evaluated for the seedling resistance against phenotypes (*BBBB, BBBQ,* and *BBBN*) of leaf rust identified in the current study. Based on the reaction to the leaf rust phenotypes, cultivars were categorized into seven groups.

Group 1: Cultivars with seedling resistance to all leaf rust races; Utuba, Lelisa, worer, Bekelcha, Tate, Filakit, Foka, Alem tena, Hitosa, Kilinto, Denbi, Ude, LD-357, Boohai, Mukuye, Robe, Mangudo and Assasa exhibited resistance reaction (; to 2) to all the three leaf rust races while the rest 18 were susceptible at least to one race.
Group 2: Cultivars with seedling resistance to BBBB and BBBQ only; the landrace Mcd4-32 was resistance to BBBB and BBBQ.
Group 3: Cultivars with resistance to BBBB only; the land race Mcd7-42 was resistance to BBBB race only.
Group 4: Cultivars with resistance to BBBQ only; Mossobo and landrace line Mcd4-12 were resistance to BBBQ phenotype.
Group 5: Cultivars with seedling resistance to BBBQ and BBBN races; Tesfaye, Ejersa, Bichena, Oda, Ilani, Cocorit-71, Malefia and Kokate showed resistance

reaction to BBBQ and BBBN phenotypes, but susceptible to BBBB.
Group 6: Cultivars with seedling resistance to only BBBN phenotype; Arendeto, Tob-66 and Obsa were resistance to BBBN only.
Group 7: Cultivars with no seedling resistance to all the three leaf rust races; Megenagna, Toltu and Selam showed susceptible reaction to all of the three races. However, Megenagna and Selam showed high level of field resistance under field condition (Habtamu, 2019).

Phenotype BBBN was less virulent on commercial durum wheat cultivars than BBBQ and BBBB. Only 20% of tested cultivars were infected by this race; however, this less virulent to durum cultivars might be because of its detection for the first time in Ethiopia and the isolate collected from bread wheat, thus, not well adapted the environment and not withstand the durum wheat cultivars. While phenotype BBBB was relatively highly virulent to Ethiopian durum wheat and it infect 42.5% of the tested cultivars, it is not virulent to Thatcher and Thatcher isoline leaf rust differential cultivars, and this findings is in agreement with the previous report by Kolmer and Acevedo (2016). In addition so far, this phenotype was not reported elsewhere except Ethiopia. Phenotype BBBQ have intermediate virulence, and it infect about 27.5% of the tested durum cultivars.

Megenagna and Selam showed resistance reaction under natural infections while susceptible reaction to all the three races at seedling stage in the greenhouse. Similarly, Mossobo and three landrace lines (Mcd4-12, Mcd4-32 and Mcd7-42) showed resistance reaction under field condition (Habtamu, 2019), but, susceptible reaction to at least one of the races at their seedling stage. This variation might be due to the varieties that may have genes responsible for adult plant resistance at field condition, but poorly expressed at their seedling stage in the green house. In addition, varieties in the green house were evaluated through inoculation of virulence races of leaf rust. However, varieties in the field have a chance to be infected with weak population of leaf rust. Hence, this circumstance might be the cause for the resistance variation of varieties in their adult and seedling stages. Such adult plant and seedling resistance variations within single variety were also previously reported (Tesfaye et al., 2016). According to Parlevliet (1988), wheat cultivars that exhibited intermediate (2+) and/or susceptible (>3$^-$) infection types at seedling stage may possess race non -specific or adult plant resistance, thus, cultivars Megenagna, Selam, Mossobo, Mcd 4-12, Mcd4-32 and Mcd7-42 may provide durable resistance because field assessment results confirmed their slow rusting characters (Habtamu, 2019). Bekelcha and Utuba showed high level of resistance in both seedling and adult plant stages, whereas, Boohai, Tate, Denbi, Worer, Mukuye and Mangudo demonstrated moderate level of resistance in both seedling and adult plant stages.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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