The Wheat Leaf Rust Pathogen in Certain Areas of Ethiopia and its Effects on Durum Wheat Cultivars

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Abstract

In brief, the study conducted in Ethiopia aimed to assess the impact of leaf rust, a significant threat to wheat cultivation, caused by the Puccinia triticina pathogen. The primary objectives were to identify the physiological races of this pathogen and evaluate the seedling resistance of Ethiopian durum wheat cultivars against them. The research collected twenty-four mono pustule isolates of P. triticina from various regions in Ethiopia and identified three distinct races: EEEE, BBBN, and BBBQ. Of these, BBBN was newly discovered in Ethiopia and displayed lower virulence compared to BBBQ and EEEE. EEEE showed high virulence, infecting a significant portion of tested durum wheat cultivars but interestingly not infecting Thatcher and its isoline, commonly used as leaf rust differential cultivars. The resistance of thirty-six commercial durum wheat cultivars and three landrace cultivars against the identified leaf rust races was assessed under controlled conditions.Several cultivars demonstrated resistance against all three leaf rust races, while the three landrace cultivars were found to be susceptible to all pathogen races. This study provides valuable insights into the diversity of leaf rust races affecting wheat in Ethiopia and identifies cultivars with promising resistance traits, offering potential strategies to mitigate the adverse effects of this disease on wheat production.

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

Introduction

Wheat is one of the staple food crops that provides 20% of protein and calories for human consumption worldwide (FAO, 2015). The demand for wheat grows over the coming decades due to increasing population and wheat food preference (Wageningen FSC, 2016). In developing countries, studies predicted 34-60% demand growth by 2050 (Hugo, 2014). In Sub-Saharan Africa, wheat consumption is expected to grow by 38% by 2023 due to population growth and urbanization (Rosegrant *et al*., 2010). The world wheat productivity is growing at rate of 1%; however, it must grow by about 40% over the current rate to meet the demand (Wheat Agri-food system proposal, 2016).

Wheat has been cultivated in Ethiopia since antiquity. In his seminal work on crop domestication in the 1920s, Vavilov identified six species of wheat and proposed Ethiopia to be the center of diversity for durum wheat.

Currently, bread wheat (*Triticum aestivum)* and durum wheat (*T. durum)* are the most important species cultivated in Ethiopia. currently, wheat is among the most important crops grown in Ethiopia, both as a source of food for consumers and as a source of income for farmers. Wheat and wheat products represent 14% of the total caloric intake in Ethiopia, making wheat the second-most important food, next maize (19%) and ahead of tef, sorghum, and enset (10-12% for each) (FAO, 2015). Regarding the area of cultivation, wheat is the fourth most widely grown crop after tef, maize, and sorghum. In the gross production value of cereal crops, wheat ranked 3rd after tef, maize and approximately tied with sorghum (REAP, 2015).

Ethiopian farmers have been growing durum wheat (*Triticum turgidum* var. *durum*) for centuries. Durum wheat covered 60-70% of the wheat cultivation area in Ethiopia in the 1980s (Negassa, 2012). However, the introduction of widely adapted, high yielding semi-dwarf bread wheat varieties from international breeding programs has replaced the durum wheat landraces. currently the proportion to durum wheat is estimated to be 20% of the total wheat area Negassa (2012). Tessema and Bechere (1998) reported that improved durum varieties covered less than 20% of the total durum wheat area because of a lack of a modern seed market and farmers' low purchasing power. Most of the durum wheat grown in Ethiopia is thus landraces consisting of large numbers of different genetic lines (Arega, 2007).

Ethiopian durum wheat landraces are conspicuously diverse and unexploited. They possess high variation, which is important for durum wheat improvement (Teklu and Hammer, 2008). Ethiopian durum wheat landraces are unique sources of useful traits (Amri, 1990) although collections have not been fully exploited in breeding programs. The use of crop diversity is one of several approaches to improving agricultural productivity and is a key to achieving global food security (Tilman, 2001). Knowledge of existing genetic diversity and its distribution in crop species is useful for germplasm conservation and selection of parents with diverse genetic background, thereby delivering crop improvement more efficiently.

Crop landraces described as geographically or ecologically distinct populations that show conspicuous diversity in their genetic composition both among populations (landraces) and within them (Brown, 1978) and display genetic variation for useful quantitative and qualitative characters (Harlan, 1975). These landraces not exploited by the Ethiopian wheat improvement program, despite the country's large genetic diversity of durum wheat. These unused genetic resources, however, have contributed to world wheat improvement. For instance, Klindworth *et al.* (2007) found that the Ethiopian durum wheat landrace *ST464* was one of the major sources of stem rust resistance, *Sr13*, the only known gene for resistance to *Ug99* or race *TTKSK*, a new stem rust race currently threatening wheat production worldwide. Virulence to *Sr13* and *Sr9e* detected at Debre Zeit, Ethiopia, and the Durable Rust Resistance in Wheat Project (DRRW) have selected the location as an international screening site for durum wheat since 2005.

In Ethiopia, more than 100 bread and 40 durum wheat varieties released for production since 1950s. However, the national average yield of wheat is still 2.6 quintal per hectare (CSA, 2017), which is far less than potential yields of 6-8 quintal per hectare, and the average yield of durum wheat is even less than 1.3 tons per hectare. Conversely, the demand for durum wheat has steadily increased in Ethiopia in the last decades, particularly due to the emergence of many food processing industries (REAP, 2015). The demand has also been projected to grow over the coming decades due to population growth and change of food habit (Valin *et al.*, 2014)

The low productivity is mainly attributed to lack of durable resistant varieties to the prevalent wheat rusts. Leaf rust, caused by *Puccinia triticina* Eriks. is one of the most important diseases in Ethiopia (Ayele *et al*., 2008).

In Ethiopia, yield loss due to leaf rust reached 70-75% on 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, an incidence of 30.2% was recorded for leaf rust in Oromia, Amhara, SNNPR, and Tigray regions. The average prevalence of leaf rust for the above-mentioned locations was 53.3 %. (Tesfay, 2016)

In Tigray region of Ethiopia wheat disease 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 Southeastern zone in particular (MARC, 1998). The four-year surveys in Tigray region showed that reaction in wheat varieties to leaf rust varied, depending on the resistance or tolerance levels of the varieties and specific environmental growing conditions (Teklay, 2015). Generally, about 73% of wheat cultivars were affected by the disease at varied levels of intensity except for three varieties (Mekelle 4, Shorima, and Danda'a). The highest prevalence (80%), the peak severity level (100%) was noted for Kubsa (HAR 1685) and local mixtures, indicating their susceptibility to the disease. The reaction of the disease for Kubsa (HAR 1685), local mixture, and unspecified bread wheat mixtures exhibited up to susceptible (Teklay, 2016).

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, it requires regular continuous search for new sources of resistant genes in the cultivated and wild forms of wheat. 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

The objective of this paper was not to conduct a comprehensive leaf rust survey, but rather to focus on race analysis and seedling evaluations of Ethiopian durum wheat varieties for the identified races. During the research period, we were unable to collect a sufficient number of leaf rust samples from the major wheat-growing areas of Ethiopia. As a result, we obtained only 24 samples from certain zones across the country.

The procedure involved in this study comprised the collection of wheat leaf rust samples from three distinct zones within Ethiopia: East Shewa, North Gondar, and Bale. A total of 24 infected samples were gathered, with 9 from East Shewa, 5 from North Gondar, and 10 from Bale. To conduct the collection, infected wheat fields were surveyed along main roads and accessible routes in each zone, with assessments conducted at intervals of 5-10 km. utilizing a systematic approach, each field was thoroughly examined in an X pattern. Freshly infected young leaves were carefully harvested using sterile scissors and placed in glassine bags (Teklay, 2015). These samples were then transported to the laboratory at the Debre Zeit Agricultural Research Center under controlled dry conditions. Upon arrival, the samples underwent a 24-hour drying period at ambient room temperature. Spores from each sample were collected using a motorized pump and stored in gelatin capsules. The capsules were maintained at 4°C in a dry environment for subsequent analysis. Throughout the process, meticulous documentation of pertinent info-rmation including location, variety name, and GPS data was diligently recorded. This methodological app-roach ensures the systematic and professional collection, preservation, and documentation of wheat leaf rust samples for comprehensive analysis and study.

The process of isolating and multiplying *P. triticina* inoculum: Bulked spores obtained from infected dried leaf rust samples were gathered in a gelatin capsule using a vacuum pump. These spores were then mixed with light mineral oil (specifically, soltrol 130) and applied onto seven-day-old seedlings of a susceptible durum wheat cultivar, RL6089, utilizing a motorized pump. Sub-sequently, the plants were misted with fine droplets of distilled water and placed in a plastic dew chamber for 24 hours at a temperature range of 18-22°C with nearly 100% relative humidity (RH). Following this, the seedlings were moved to a greenhouse bench with a temperature range of 18-25°C and RH of 70%. A portion of the spore samples was stored in a refrigerator at 4°C as a contingency plan.

Seven days post-inoculation, leaves containing a single fleck were chosen from the lower part of a leaf. The remaining leaves in each pot were removed using sterilized scissors. A single leaf witha fleck was encased in a cellophane bag and securely fastened at the base with a rubber band to prevent cross-contamination, as described by Fetch and Dunson (2004).

Approximately 12-14 days after inoculation, when the mono pustules were well developed, each mono pustule was gathered in a gelatin capsule using a motorized vacuum pump. Spores collected from a mono pustule were mixed with lightweight mineral oil (soltrol 130) and inoculated onto seven-day-old seedlings of susceptible durum cultivars, specifically RL6089. Similarly, about 12- 14 days after inoculation, spores from each mono pustule were collected in separate gelatin capsules as previously mentioned and stored at 4°C for later use in the inoculation of leaf rust differential lines. These procedures were repeated until a sufficient amount of single spores were produced for race analysis purposes, resulting in the development of 24 mono pustule isolates for race analysis.

Inoculating Puccinia triticina isolates onto wheat leaf rust differential lines: five seeds from each of the 16 distinct genetic lines, as well as a susceptible control line (RL 6089) i.e susceptible durum line obtained from USA small grain collection center, table 1. were cultivated in pots measuring 4 cm diameter. The pots contained a mixture of soil, sand, and compostin a 2:1:1 (volume/volume/volume) ratio i.e parts from total volume of the pot.

Table 1. List of wheat differential lines with their corresponding *Lr genes* used for race analysis.

S. No	Differential lines	Lr genes	Pedigree
1	RL6003	Lr1	TC*6/Centenario
2	RL6016	Lr2a	TC*6/Webster
3	RL6047	Lr2c	TC*6/Loros
4	RL6002	Lr3	TC*6/Democrat
5	RL6010	Lr9	Transfer /6*TC
6	RL6005	Lr16	TC*6/Exchange
7	RL6064	Lr24	TC*6/Agent
8	RL60 78	Lr26	TC*6/St-1-25
9	RL6007	Lr3ka	TC*6/Klein Aniversario
10	RL6053	Lr11	TC*2 Hussar
11	RL6008	Lr17	Klein Lucero/ 6*TC
12	RL60 49	Lr30	TC*6/Terenzio
13	RL6051	LrB	TC*6/Carina
14	RL6004	Lr10	TC*6/Exchange
15	RL6013	Lr14a	Selkirk /6*TC
16	RL6009	Lr18	TC*7/Africa43
17	RL6089	Susceptible check	

A suspension of spores, amounting to approximately 3- 5 mg per ml, was prepared by mixing a single pustule-derived spore in light mineral oil (soltrol 130). This suspension was then sprayed onto seedlings that were seven days old using a motorized pump. Subsequently, the treated seedlings were placed in a dew chamber with close to 100% relative humidity at a temperature range of 18-22°C for duration of 24 hours. Following this incubation period, the seedlings from each pot were transferred to a greenhouse, within individual plastic enclosures to prevent any potential contamination. The greenhouse conditions were regulated to maintain temperatures between 18-25°C and humidity levels between 60-70% (Kolmer and Marcelis, 2016).

Evaluate leaf rust on different lines to determine the involved race: couple of weeks post- inoculation, disease severity was assessed using a scale ranging from 0 - 4, with scores grouped into low and high infection types based on resistance or susceptibility. Virulence was determined accordingly: low infection types (L) denoting resistance, and high infection types (H) indicating susceptibility. Physiological race designations followed Long and Kolmer's method (1989), with sixteen differential lines grouped into four sets. Each isolate received a four-letter race code based on its reaction with these lines. For example, a code like TKTT signifies low infection on one line and high infection on others. This process was applied to all 24 isolates to establish their race nomenclature.

Three distinct races of *P. triticina* were employed to infect 36 durum wheat cultivars, along with three landrace lines and a susceptible control. The seedling growth, inoculation, incubation, and disease assessment followed standardized procedures. Infection types were assessed using a 0-4 scale twelve days post-inoculation, following the methodology outlined by Long and Kolmer (1989). Cultivars and landraces were categorized as resistant or susceptible based on their reaction to each isolate's infection, characterized by low or high infection types.

Result and Discussion

Race identification: Three distinct virulent pathotypes of *Puccinia triticina*, identified as BBBQ, BBBN, and EEEE, have been found in various regions of Ethiopia Table 2 and figure 1. Among these, the EEEE pathotype is the most prevalent, constituting 91.6% of occurrences, while BBBQ and BBBN were each detected only once, making up 4.1% each. This EEEE pathotype, also known as the Eth-race, exhibits avirulence towards the Thatcher line of bread wheat but virulence towards Ethiopian durum wheats. Despite its prevalence in Ethiopia, this pathotype has not been identified elsewhere. It appears to be a remnant of an earlier *P. triticina* population adapted to durum wheat varieties before the widespread cultivation of bread wheat. This observation aligns with previous findings of *P. triticina* isolates in Ethiopia that were virulent on durum wheat yet avirulent on Thatcher wheat, as documented by Roelfs *et al.* (1992) and Ordonez and Kolmer (2007).

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

If the EEEE race isolates have long been established in Ethiopia on tetraploid landrace cultivars, it would be anticipated that these isolates would exhibit a higher level of genetic diversity compared to other more recently introduced types, as suggested by Kolmer and Acevedo (2016). *Puccinia triticina* isolates with significant virulence to durum wheat have recently been discovered and characterized in various regions including Mexico (Singh *et al.*, 2004), France (Goyeau *et al.*, 2011), Spain (Martinez *et al.*, 2005), Italy (Mantovani *et al*., 2010), Israel, Turkey (Kolmer *et al*., 2009), Argentina (Ordonez and Kolmer, 2007), and Ethiopia (Mengistu *et al.*, 1991; Kolmer and Acevedo, 2016). The BBBQ phenotype found in Ethiopia shows similarity to isolates identified from durum wheat in various other countries, as per unpublished data from Kolmer. Originating from emmer wheat, this phenotype exhibits virulence solely towards lines carrying genes LrB and Lr10, while being avirulent to many bread wheat cultivars. Its survival and spread rely on the presence of durum wheat, as highlighted by Kolmer and Acevedo (2016).

In 2001, the 'Altar C84' durum wheat in Mexico became susceptible to durum leaf rust (BBBQ), later determined to carry Lr72 (Herrera-Foessel *et al*., 2014). This led to susceptibility in many other CIMMYT cultivars and durum germplasm globally. Further virulence variations to durum wheat cultivars may exist in the BBBQ isolate from Ethiopia and elsewhere, necessitating the development of a differential set of durum wheat genotypes for proper assessment, as the Thatcher differentials are inadequate in detecting such virulence variation in these isolates.
Isolates displaying the BBBN phenotype were found to be virulent on LrB and Lr14a. These isolates, collected from the bread wheat cultivar Sofumer in Bale zone, may exhibit some virulence towards bread wheat cultivars. While BBBN has been previously detected in Mexico and other regions worldwide, where it was reported as virulent to Lr72 (Singh *et al*., 2004), its identification in Ethiopia is a novel finding, although requiring further verification.

In this study, only twenty-four isolates were collected from the Oromia and Amhara regions due to the low prevalence of wheat leaf rust. Consequently, the number of characterized isolates was limited, resulting in the identification of only three phenotypes. Kolmer and Acevedo (2016) also noted a small number of phenotypes from a wide geographical area and large collections. Conversely, other populations of *P. triticina* collected from relatively small geographical areas in Tigray, Ethiopia, reported many phenotypes (Tesfaye *et al*., 2016).

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Table **2**. Distribution of *Puccinia triticina* races and their virulence characteristics.

Zone	Woreda	Altitude (m)	Latitude	Longitude	Wheat type	Identified races	Virulent to
N/Gondar	Wogera	2800	12.75	37.70	Durum	EEEE	AV
N/Gondar	Wogera	2771	12.75	37.73	Durum	EEEE	AV
N/Gondar	Ambagiorgis	2947	12.76	37.63	Durum	EEEE	AV
N/Gondar	Dabat	2599	13.02	37.87	Durum	EEEE	AV
N/Gondar	L/Armachiho	2625	12.70	37.50	Emmer	EEEE	AV
E/Shewa	Gimbichu	2392	8.58	39.09	Emmer	EEEE	AV
E/Shewa	Gimbichu	2351	8.58	39.13	Emmer	EEEE	AV
E/Shewa	Gimbichu	2470	9.01	39.07	Emmer	EEEE	AV
Bale	Sinana	2443	7.05	40.06	Emmer	EEEE	AV
Bale	sinana	2424	7.05	40.09	Emmer	EEEE	AV
Bale	Sinana	2435	7.06	40.01	Emmer	EEEE	AV
Bale	Sinana	2450	7.06	40.13	Emmer	BBBQ	LrB & Lr10
Bale	Sinana	2430	7.05	40.12	Emmer	EEEE	AV
Bale	Sinana	2405	7.04	40.15	Emmer	EEEE	AV
Bale	Sinana	2382	7.19	39.97	Bread	BBBN	LrB & Lr14a
Bale	Sinana	2426	7.12	40.23	Emmer	EEEE	AV
Bale	Sinana	2388	7.20	39.97	Emmer	EEEE	AV
Bale	Sinana	2446	7.15	39.95	Emmer	EEEE	AV
E/Shewa	Ada	1900	8.44	38.57	Durum	EEEE	AV
E/Shewa	Ada	1900	8.44	38.57	Durum	EEEE	AV
E/Shewa	Ada	1900	8.44	38.57	Durum	EEEE	AV
E/Shewa	Ada	1900	8.44	38.57	Durum	EEEE	AV
E/Shewa	Ada	1900	8.44	38.57	Durum	EEEE	AV
E/Shewa	Ada	1900	8.44	38.57	Durum	EEEE	AV

AV= Avirulent to all thatcher differential lines.

Durum wheat cultivars and landraces underwent seedling tests to assess their response to the identified race: Seedlings of 36 widely cultivated Ethiopian durum wheat varieties, along with some landrace cultivars, were assessed for their resistance to leaf rust phenotypes (EEEE, BBBQ, and BBBN) identified in the study as shown in table 2. The cultivars were grouped based on their reactions to these leaf rust races.

In Group 1, cultivars like Utuba, Lelisa, and others displayed resistance to all three leaf rust races, while in Group 2, Mcd4-32 showed resistance to EEEE and BBBQ only. Group 3 contained cultivars resistant to EEEE, such as Mcd7-42. Similarly, Group 4 included cultivars like Mossobo resistant to BBBQ only. Group 5 cultivars, like Tesfaye and Ejersa, were resistant to BBBQ and BBBN but susceptible to EEEE. Group 6 cultivars, including Arendeto and Tob-66, showed resistance to BBBN only. Finally, Group 7 contained cultivars susceptible to all three leaf rust races.

The BBBN phenotype was less virulent on durum wheat cultivars compared to BBBQ and EEEE. EEEE was highly virulent, infecting 42.5% of tested cultivars, while BBBQ showed intermediate virulence, infecting about 27.5%. Some cultivars showed resistance under field conditions despite susceptibility at the seedling stage, possibly due to adult plant resistance genes or different environmental factors.

Varieties like Megenagna and Selam showed resistance under field conditions despite susceptibility at the seedling stage, suggesting potential for durable resistance. Others like Bekelcha and Utuba demonstrated high resistance at both seedling and adult stages, while cultivars like Boohai and Tate showed moderate resistance across both stages.

Table 3. Seedling response of Durum wheat cultivars to Leaf rust races.

S. NO	Cultivars	Leaf rust races		
		EEEE	BBBQ	BBBN
1	Utuba	1	; ,1	0
2	Leliso	1C	1	0
3	Ejersa	3	1	0
4	Worer	1C	0	1^{+}
5	Bekelcha	2	1	0, ;
6	Tate	2^{+}	1	0
7	Bichena	3	2	0
8	Flakit	1^{+}	0	0
9	Foka	1	1C	1
10	Oda	3	2	2
11	Arendato	3	3	0
12	Hitosa	1	1	0
13	Yerer	1	3	1^{+}
14	Ilani	3	2^{+}	0
15	Kilinto	1	1C	;
16	Denbi	1	2	2
17	Megenagna	3	3	3^{+}
18	Tob-66	3	3	2
19	Ginchi	1	3	0
20	Cocorit-71	3	1^{+}	1^{-}
21	Quamy	1	3	;
22	Obsa	3	3	2
23	Toltu	3	3	3
24	Ude	1	2^{+}	0
25	Kokate	3	1^{+}	1
26	Selam	3C	3	3
27	LD-357	2	2	0
28	Boohai	1	1	0
29	Mukuye	1	1	0
30	Robe	1	1	0
31	Mangudo	1	1	0
32	Assasa	1	1	1
33	Malefia	3	0	0
34	Mossobo	3	0	3
35	Alem tena	1^{+}	;	0
36	Tesfaye	3	2	1^{+}
37	Mcd4-12	3	0	3
38	Mcd4-32	0	0	4
39	Mcd7-42	0	3	4
40	Morocco	4	3	3

Conclusions

The study presents a detailed analysis of the virulence patterns of *Puccinia triticina*, the causative agent of wheat leaf rust, in Ethiopia. Three distinct pathotypes—EEEE, BBBQ, and BBBN—were identified, with the EEEE pathotype being the most prevalent, representing 91.6% of the collected isolates. This pathotype exhibits avirulence towards the Thatcher line of bread wheat but is virulent towards Ethiopian durum wheats, suggesting it may be a remainder of an older *P. triticina* population adapted to durum wheat prior to the widespread cultivation of bread wheat in the region.

The BBBQ and BBBN pathotypes, each constituting 4.1% of the isolates, show virulence towards specific leaf rust resistance genes, LrB and Lr10, and LrB and Lr14a, respectively. The BBBQ pathotype, similar to isolates identified in various other countries, appears to be associated with durum wheat, while BBBN was found in the bread wheat cultivar Sofumer in the Bale zone, a novel finding for Ethiopia that warrants further verification.

The study also conducted seedling tests on 36 widely cultivated Ethiopian durum wheat varieties, along with some landrace cultivars, to assess their resistance to these identified leaf rust races. The results revealed a range of resistance levels across different cultivars, with some showing resistance to all three pathotypes and others susceptible to one or more. Notably, the EEEE pathotype was found to be highly virulent, infecting 42.5% of the tested cultivars, while the BBBQ pathotype exhibited intermediate virulence.

The findings underscore the complexity of leaf rust virulence in Ethiopia and highlight the need for continuous monitoring and the development of resistant wheat cultivars. The identification of specific pathotypes and their virulence profiles provides critical information for breeding programs aimed at enhancing wheat resistance to leaf rust. Additionally, the potential for durable resistance in some cultivars, despite susceptibility at the seedling stage, emphasizes the importance of field-based evaluations alongside seedling tests. This study contributes to a deeper understanding of the dynamics of *P. triticina* populations in Ethiopia and offers valuable insights for the management of wheat leaf rust in the region.

Conflict of Interests

The authors have not declared any conflict of interests.

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