Importance and Pathogenic Variation of Wheat Tan Spot in Ethiopia

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Abstract

Tan spot or yellow leaf spot is one of the major diseases of wheat worldwide; however, its significance has not been well explored so far in Ethiopia. This study was initiated to examine its distribution, importance and pathogenic variability in major wheat producing regions of Ethiopia. A survey was conducted mainly in Arsi and some wheat fields at Debre-Zeit and Holetta in 1998. Fifty-seven leaf samples were collected and analyzed in the laboratory at Kulumsa. Out of these, 70% were infected by the tan spot fungus. The disease severity varied from low to high levels according to locations. Based on the greenhouse preliminary pathogenicity tests, five isolates were selected for further characterization in comparison with four isolates of different origin. Nine tan spot isolates were used to inoculate 24 bread wheat cultivars under controlled environments. There were significant differences among isolates, cultivars and isolate x cultivar interactions. The Ethiopian isolate (ET57/98) was the most aggressive followed by the Indian isolate (Ind-119). Applying a hierarchical agglomerative cluster analysis and centroid method, the isolates were grouped into two large clusters while the cultivars formed more than five clusters. Despite the presence of significant isolate cultivar interactions, their differences were not distinct enough to categorize them into physiologic races. Twenty-one commercial bread wheat cultivars from Ethiopia were inoculated with a mixture of three aggressive isolates at two-leaf stage under controlled conditions and only three cultivars had shown adequate level of resistance. Therefore, the wheat improvement program in Ethiopia should focus on developing genotypes with resistance to tan spot.

Introduction

Tan spot caused by *Pyrenophora triticirepentis* (Died.) Drechs., anamorph: *Drechslera tritici-repentis* (Died.) Shoem., is an important disease of wheat in many parts of the world (Dubin 1983, Rees & Platz 1990). The fungus has been reported to infect many grass species (Krupinsky 1992).

The main source of primary inoculum of the fungus is ascospores released from pseudothecia matured on wheat stubble after harvest on the soil surface. Under moist weather conditions, the disease spreads from the bottom to the upper leaves, causing necrotic and/ or chlorotic lesions. Moderate to severe infection of the tan spot fungus could inflict 30-50% vield loss (Raymond et al. 1985, Shabeer & Bockus 1988). Crop rotation, burning or incorporating crop residues, spraying fungicides and the use of resistant cultivars has been recommended for the control of tan spot (Bockus & Claassen 1992). However, the use of resistant cultivars remains to be the most reliable in controlling this and many other diseases of wheat. Several host-pathogen interaction studies on wheat and P. triticirepentis have been conducted both in the field and in the greenhouse (Evans et al. 1999). Moreover, variations in the pathogenicity among isolates of the tan spot fungus were reported (Schilder & Bergstrom 1990).

In Ethiopia, *P. tritici-repentis* is a relatively unexplored disease of wheat. Its occurrence was first reported by Waller (1974) during his visit to examine the *Triticale* diseases in Ethiopia. The importance of tan spot has been underestimated in Ethiopia. Most likely, the disease symptoms have been confused with those of septoria tritici blotch caused by *Mycosphaerella graminicola* and *Stagonospoa nodorum* blotch caused by *Phaeospheria nodorum* (E.Müller) Hedjaroude.

This study was initiated to observe the distribution and importance of tan spot in major wheat producing regions of Ethiopia and to examine its pathogenicity in comparison with isolates of different origin and finally to evaluate the commercial bread wheat cultivars from Ethiopia for their resistance to this pathogen.

Materials and Methods

Disease survey

A survey was conducted in major wheat producing regions of Ethiopia in 1998. Wheat fields, which were suspected to be infected by the tan spot, were visited around the Holetta and Debre-Zeit Research Centers while a formal survey was carried out in the Arsi region in two separate routes. The first route was from Kulumsa – Digelu – Bekoji – Asasa – Ardita – Goffer and the second route was from Kulumsa – Gonde – Etheya – Hurutta – Diksis – Arsi-Robe. The survey was conducted at different growth stages of the crop. During the survey, wheat fields were randomly selected at 10 Km distance along the main roads. Wheat fields showing which appeared to be tan spot or yellow spot lesions were noted as high, medium and low according to disease severity. A total of 57 plots or fields were visited and the same number of leaf samples was collected. The samples were dried at room temperature and temporarily stored at 4°C.

Isolation of the pathogen

isolation of the fungus was accomplished following the procedure of Sah & Fehrmann (1992) in the laboratory at Kulumsa. Leaf segments (ca. 0.5 cm⁻) were disinfected in 70% ethanol for 20 sec and further surface sterilized in 0.5% sodium hypochlorite solution for 1 minute, and rinsed twice for 30 sec in sterile distilled water. The leaf segments were placed on potato-dextrose-agar (PDA) and incubated in a 12/12 hr light and dark regime at 20°C. After 3 to 4 day of incubation, the culture was checked for the development of brown conidiophores of pale brown 3-5 septated cylindrical conidia under stereomicroscope and compound a microscope, respectively. Twenty-one representative samples were randomly selected and a monospore isolate was made for each selected sample. The isolates were multiplied according to the methods of Sah & Fehrmann (1992) on PDA/V8 medium (150 ml V8 juice, 10 g Difco potato-dextrose agar (PDA), 3 g CaCO₃, 10g Bacto agar and 850 ml of distilled water). After inoculating five different bread wheat cultivars at Kulumsa, five isolates were selected based on the disease severity for further study in comparison with four isolates of different origin (Table 1).

Multiplication of the isolates

Small plugs (0.5 cm²) from each fungal culture were transferred onto PDA/V8 medium in plastic petri-plates and incubated in the dark at 20°C for 6 days. Then the mycelium was scraped with a sterilized bent metal rod. The culture then was incubated at 20°C under fluorescent light for 24 hr followed by a 24 hr dark period at 16°C. An inoculum suspension from each culture was prepared by flooding the plates with distilled water. The suspension was sieved through double cheesecloth twice to remove mycelia and hyphal fragments. A drop of Tween 20 (Polyoxyethylene sorbitan monolaurate) was added per 100 ml suspension and a concentration of 3 x 10³ conidia/ml was used for inoculation.

Table 1. List and the origin of isolates of the tan spot fungus which were used to study variations in their pathogenicity on 24 bread wheat cultivars

No	Isolate no.	Location	Year	Country ¹	
1	Kny2	Njoro	-	Kenya	
2	Ind-119	-	-	India	
3	Bay2a	Bayern	-	Germany	
4	Rh26	Reinshof	1999	Germany	
5	Et-5/98	Sagure	1998	Ethiopia	
6	Et-57/98	Kulumsa	1998	Ethiopia	
7	Et-45/98	Holetta	1998	Ethiopia	
8	Et-15/98	Hurutta	1998	Ethiopia	
9	Et-01/98	Goffer	1998	Ethiopia	

Tan spot isolates from Germany, Kenya and India were obtained from the Institute of Plant Pathology and Plant Protection, Goettingen.

Wheat cultivars

Twenty-four bread wheat cultivars of different origin (Ethiopia, Latin America and Europe) were selected after a preliminary test against tan spot isolates in the greenhouse at Kulumsa. From each genotype, seven seeds were sown into 3 (5 x 5 cm) Jiffy pots containing soil, sand and compost (1:1:1 v/v/v). The seedlings were grown in the greenhouse until the full emergence of the second leaf (ca. two weeks old). The seedlings in each pot were thinned out to five per pot in order to have healthy and vigorous plants. About 2g/100 ml fertilizer (N:P₂O₅:K₂O₇) 15:11:15) was applied twice to each tray containing 24 pots.

Experimental procedures

The experiment was arranged in complete randomized design in a split-plot layout. The isolates were assigned to the main plots (trays) and each cultivar was randomized in a tray. Both the main plots and cultivars in the sub-plots were randomized. The treatments were replicated three times. The first and the second leaves were inoculated uniformly until a run-off point using an atomizer driven by compressed air attached to a glass tube sprayer. Seedlings in two trays were sprayed with distilled sterilized water for control. Inoculated plants were left in the open air for ca. 30 minutes to dry. Then the plants were transferred to a climate chamber with a temperature of 20°C and a photoperiod of 12/12

dark/light (17,000 lux). The cabin was misted intermittently, five seconds per 30 minutes for 48 hr, and the relative humidity in the cabin was 90-100%. Thereafter, the RH in the cabin was adjusted to 80% until the time of disease assessment.

Disease assessment

Disease notes were taken both from the first and second leaves 8-9 days of post inoculation (dpi). The disease assessment was accomplished using a lesion type (LT) that was based on a 0-5 scale (Lamari & Bernier 1989, Francl & Jordahl 1994). The 0-5 scale used was as follows: 1 = small dark brown to black spots without any surrounding chlorosis or tan necrosis (resistant), 2 = small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant), 3 = small dark brown to black spots surrounded by a distinct chlorotic or necrotic lesion generally coalescing (moderately susceptible), 4 = dark brown or black spots surrounded by chlorotic or tan necrotic zones and some lesions coalescing (susceptible), 5 = dark brown or black centers that may not be distinguishable and most lesions consisting of coalescing chlorotic or tan necrotic zones (highly susceptible) and 0 = indicates an apparently immune response.

Data analysis

The data were analyzed using MSTAT C (1989) statistical package. The mean values were separated by the Students-Newman-Keuls' test (S-N-K) at the 5% probability level. Moreover. agglomerative hierarchical cluster analysis was applied to determine the relationship of the isolates of tan spot based on their pathogenicity on 24 bread wheat cultivars and of the wheat cultivars based on their patterns of resistance to the 9 isolates. The cluster analysis was executed using SYSTAT, version 8.0 statistical package (SYSTAT 1998) and the data were clustered based on a centroid method (Schilder & Bergstrom 1990, Sah & Fehrmann, 1992).

Resistance in commercial wheat cultivars

Finally, 21 commercial bread wheat cultivars from Ethiopia were tested against a mixture of three selected tan spot isolates (Et-57/98, Ind-119 and Bay2a) at two-leaf stage. The experiment was arranged in a complete randomized design and replicated three times. The experimental procedures and disease assessment methods were similar to the above experiment.

Results

The survey has indicated that about 70% of wheat leaf samples under investigation were infected by the tan spot fungus. We were not able to isolate the tan spot fungus from 30% of the leaf samples therefore the data are omitted in Table 2. The highest tan spot disease incidences were noted at Goffer, Kulumsa, Debre-Zeit and Arsi-Robe.

All the nine isolates that were tested under controlled environment on 24 bread wheat cultivars were pathogenic to all cultivars used in the experiment. There were highly significant differences between tan spot isolates, bread wheat cultivars and their isolate x cultivar interactions. The mean of each isolate on 24 bread wheat cultivars and the response of each cultivar to nine isolates,

respectively were compared (Table 3). There were clear differences among isolates in their pathogenicity on certain bread wheat cultivars

The Ethiopian isolate *Et*-57/98 was the most aggressive one, which inflicted moderate to susceptible lesion types (LT, 3.0-4.1) on 18 bread wheat cultivars, followed by the Indian isolate *Ind*-119. The isolates *Et*-57/98 and *Ind*-119 incited different lesion types on at least three bread wheat cultivars (Ralle, HAR1709 and ET13).

Two large groups of similar isolates (Et-57/98, Bay2a, Rh26, Et-5/98, Ind-119, Kny2 and isolates Et-45/98, Et-15/98, Et-01/98) were identified by cluster analysis using the centroid method (Fig.1). The two Ethiopian isolates (Et-57/98 and Et-5/98) and two German isolates (Bay2a and Rh26) were in the first group. Based on their reaction to nine tan spot isolates, the cultivars could be grouped into different clusters (Fig. 2). The six susceptible cultivars ET13. HAR845, HAR710, Thasos and Bluejay formed one cluster. There was similarity among the three resistant cultivars Munk, Trigo35 and HAR719. The moderately resistant cultivars HAR1685 and Trigo 38 fitted in one cluster. The remaining had intermediate to moderately susceptible reaction and the majority of them fitted into one large cluster.

Table 2. Occurrence of tan spot in leaf samples which were collected from different wheat fields in Ethiopia in 1998.

Accession number ⁵	Location	Altitude (ml)	Soil type	Cultivar	Growth stage	Tan spot
Ptr1 /98	Goffer	2400	black clav	HAR710	69	high
Ptr 3/98	Kulumsa	2200	clay	HAR1709	8	high G
Ptr 4/98	Digelu	2500	black clay	K6295-4A	8	low
Ptr 5/98	Digelu	2500	black clay	Payon 76	8	low
Ptr 6/98	Ardita	2300	clay loam	Explor#8	68	moderate
Ptr 8/98	Ardita	2300	clay loam	F8#2	<u>. ව</u>	moderate
Ptr 9/98		2300	clay loam	HAR1522	: <u>0</u> 3	moderate
Ptr 10/98	Temella S.F ®	2300	loam	HAR1522	61	moderate
Ptr 11/98	Asasa	2300	clay loam	FH6-1-7	65	moderate
	Tiyo	2400	clay	HAR1685	පු	WO
Ptr 15/98	Hurutta	2250	clay	Dashen	<u>6</u>	WO
Ptr 17/98	Hettosa	2250	clay	HAR710	66	WO
Ptr 19/98	Diksis	2600	black clay	HAR1709	3	low
Ptr 20/98	Diksis	2600	black clay	HAR1685	28	OW
Ptr 21/98	Diksis S.F *	2600	black olay	HAR604	3 5	moderate
Ptr 23/98	Robe/Jinja	2450	clay Sign	TAR/10	28	ngn
Ptr 25/98	Robe/Jinia	2450	clay	Explor #19	3	high
Ptr 26/98	Robe/Jinja	2450	clay	Local durum	57	nig S
Ptr 29/98	Robe	2420	black clay	MRVT #8	59	low
Ptr 32/98	Robe	2420	black clay	FH6-1-7	9	low
Ptr 33/98	Robe	2420	black clay	MRVT#4	59	low
Ptr 34/98	Rcbe	2420	black clay	MRVT#3	8	low
Ptr 36/98	Robe	2420	black clay	HAR1709	18	moderate
Ptr 3//98	XODE O	2420	black clay	MT V #10	35	WO
Ptr 39/98	Holetta	2400	red clav	liknown	<u> </u>	low
Ptr 40/98	Holetta	2400	red clay	HAR1522	<u>න</u>	low
Ptr 43/98	Holetta	2400	red clay	ET13	<u>ඇ</u>	moderate
Ptr 44/98	Holetta	2400	red clay	FH8 tall	<u>S</u>	moderate
Ptr 45/98	Holetta	2400	red clay	FH8 tall	9	moderate
Ptr 47/98	Etheya	2250	clay	HAR1522	9	moderate
Ptr 48/98	Debre-Zeit	1980	black clay	durum	う '	nigh
Dtr 52/08	7000	2450	Clay	CT13	2 4	high
Ptr 53/98	ROS C	2450	Clay	FT13	<u> </u>	
Ptr 54/98	Rcbe	2450	clay	HAR604	<u>ფ</u>	ng S
Ptr 55/98	Robe	2450	clay	unknown	49	moderate
Ptr 56/98	Robe	2450	clay	durum	<u>ල</u>	high
Ptr 57/98	Kulumsa	2200	clay	HAR1709	71	nigh

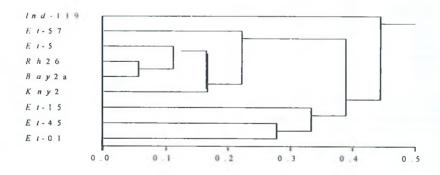
* Accession numbers both in the field and laboratory. * Samples collected from the state farms. "The two digit growth scales are according to Zadoks et al. (1974).

* Tan spot disease severity (High= \geq 30%, moderate =10 to 20 % and low \leq 5% severity).

Table 3. Mean lesion types incited by 9 isolates of P. tritici-repentis on 24 bread wheat cultivars.

					Infectio	n type	(0-5)			
Bread wheat cultivars	Rh26	Et-15	Et-45	<i>Ind</i> 119	Kny2	Et-01	Et-5	Et-57	Bay2a	Mean
Thasos	3.7aB	3.0a-c	3.0ab	3.9a-d	3.7a	2.8a-c	3.6a-c	3.8a-c	3.6a-c	3.5ª
Trigo 35	2.0cd	1.8 ^{fg}	1.81	1.8 ^{f-h}	2.2d-f	1.9ef	2.0ef	2.2e	1.7e-g	1.99
HAR1685 (Attila's')	2.0cd	2.3b-f	1.9ef	2.5e-g	2.5b-e	1.5 ^{fg}	2.4d-f	2.8с-е	2.5b-f	2.5ef
HAR719 (Lira's')	1.5 ^d	1.69	1.39	1.1h	1.4f	1.5 ^{fg}	1.8f	1.3f	1.29	1.4 h
Ralle	2.8a-c	2.8a d	2.3c-/	2.4e-g	3.3a-c	2.5b-e	2.8b-e	3.8a-c	3.1a-d	2.9bc
Palermo	2.9a-c	2.6 ^{b-e}	2.2d-f	2.4e-g	2.3c-f	2.0d-f	2.4d-f	2.8c-e	2.7а-е	2.6 de
Naxos	3.1a-c	3.1ab	3.0ab	3.1c-f	3.3a-c	2.7b-d	2.8b-e	3.5ad	2.9a-e	3.0 bc
K6290-Bulk	2.9a-c	2.2 ^{d-g}	2.5 ^{b-e}	2.8d-f	2.6b-e	2.4b-e	2.9 ^{b-e}	3.3a-e	2.3c-g	2.6 de
Milan	2.8a-c	2.3c-f	2.3c-f	2.8d-f	2.30-1	1.9ef	3.2b-d	3.2a-e	2.8а-е	2.6 de
Trigo 38	2.3b-d	2.0e-g	2.2d-f	2.5e-g	2.2d-f	2.3с-е	2.5c-f	3.0a-e	1.9d-g	2.3f
Munk	2.1cd	1.0h	1.8 ^f	1.3gh	2.0ef	1.29	2.4d-f	2.3 ^{de}	1.5 ^{fg}	1.79
Laketch	3.1a-c	2.5b-e	2.8a-d	2.8d-f	3.3a-c	2.5 ^{b-e}	3.1b-d	3.1a-e	3.3a-c	3.0 bc
HAR1709	3.5ª	2.3b-f	2.5 ^{b-e}	3.7a-e	2.8a-e	2.8a-c	3.2 ^{b-d}	2.8b-e	3.4a-c	3.0 bc
HAR604	3.3ab	3.0a-c	3.0ab	3.3b-e	2.8a-e	2.6b-e	3.0b-e	3.1a-e	3.2a-d	3.0 bc
Anza	3.5ª	3.0a-c	2.9a-c	4.4ab	3.2ª-d	2.8a-c	4.1a	4.0ab	3.8ab	3.6 a
Mango	2.7a-c	2.1d-g	2.4 ^{b-f}	3.0c-f	2.3c-f	2.3с-е	3.2 ^{b-d}	3.0a-e	2.7а-е	2.6de
Tonicci	2.8a-c	2.7a-e	2.3b-f	3.2b-f	3.4ab	3.1ab	3.2b-d	3.7a-c	3.0a-d	3.0 bc
HAR407 (Vee's)	2.6a-c	3.1ab	2.3b-f	2.7d-f	2.6b-e	2.8a-c	2.8ь-е	3.3а-е	2.8a-e	2.8cd
Gara (Bow's)	3.4ab	2.9a-c	2.8a-d	3.0c-f	3.3a-c	2.6b-e	2.8 ^{b-e}	3.2a-e	2.8a-e	3.0 bc
Triso	3.8a	2.6b-e	2.4b-f	3.6ª-e	2.8a-e	3.1ab	3.1b-d	3.6a-c	3.7ab	3.2b
HAR710 (MRL/BUC)	3.5ab	3.0ª-c	3.3a	4.3a-c	3.3a-c	3.4a	3.8ab	4.1a	3.8ab	3.6 a
Bluejay	3.3ab	2.4b-f	2.8a-d	4.0a-d	3.2ª-d	3.0a-c	3.6a-c	3.3a-e	3.3a-c	3.2b
HAR845	3.7a	3.3a	3.3a	4.6a	3.3a-c	3.2ab	4.2a	3.5a d	4.0a	3.7 a
ÉT13	3.3ab	3.1ab	3.0ab	4.4ab	3.3a-c	3.0a-c	3.8ab	3.4a-d	3.3≥€	3.4 a
Mean	2.9ab	2.5b	2.5b	3.1a	2.8b	2.5b	3.0a	3.2a	2.9ab	

According to S-N-K tests, the mean disease ratings with the same letters in the column are not significantly different at the 5% probability level.



Distance between cluster centroids

Fig. 1. Dendrogram showing virulence similarity and successive clustering of 9 isolates of *P. tritici-repentis* on 24 bread wheat cultivars.

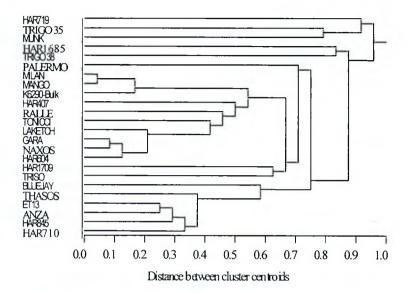


Fig.2. Dendrogram showing similarity and successive clustering of 24 bread wheat cultivars based on their resistance/susceptibility to 9 isolates of *P. tritici-repentis*.

Table 4. The reaction of Ethiopian bread wheat cultivars to a mixture of tan spot isolates.

No.	Cultivars	Lesion type 0-5) ^α	No.	Cultivars	Lesion type (0-5) ^α
1	HAR1899	3.0 ^{a-e}	12	BATU	2.0e-g
2	HAR1407	2.8a-f	13	DASHEN	1.29
3	HAR416	1.8 ^{fg}	14	GARA	2.8≄1
4	HAR1775	2.2d-f	15	HAR407	2.2d-f
5	HAR1522	3.2a-d	16	K6295-4A	3.2a-d
6	HAR1595	3.3a-c	17	ET13	3.8a
7	HAR1868	3.7ab	18	DERESELIGN	2.7b-f
8	HAR710	3.7ab	19	K6290-BULK	2.5c-f
9	HAR1685	2.5c-f	20	ENKOY	2.7b-f
10	HAR604	3.5a-c	21	PAVON 76	2.5c-f
11	HAR1709	3.8a			

** According to S-N-K tests, the mean disease ratings with the same letters in the column are not significantly different at the 5% probability level.

All the 21 bread wheat cultivars from Ethiopia were infected by the tan spot fungus. However, there were significant differences among cultivars in resistance to tan spot (Table 4). Thus, the cultivars could be grouped into three based on their disease reaction. The susceptible to moderately susceptible (HAR1709, ET13, HAR710, HAR1868, HAR 604, K6295-4A, HAR 1899, HAR1522, HAR 1595) the moderately resistant (Dashen, HAR 416 and Batu) and the other cultivars can be considered as intermediate types.

Discussion

Until now, detailed research work on tan spot of wheat is lacking in Ethiopia. This study has revealed that tan spot is widely distributed in major wheat producing regions in Ethiopia. About 70% of the leaf samples that were collected from the field had the tan spot fungus while the rest had other fungi such as *Septoria* spp., *Phoma* spp., and *Alternaria* spp. (data not presented). Thus, it is necessary to compliment field tan spot survey with

laboratory work because disease symptoms can be confounded with others. The tan spot severity in this study varied from low to high depending on locations. Tan spot has not been detected at high altitudes, Bekoji (2700 masl) and Meraro (2900 mals) indicating that the fungus is better adapted to the warmer climatic conditions.

The identification of a physiological specialization of a pathogen is an important step for the development of disease resistant cultivars in any hostpathogen system where major genes control resistance and this applies mainly for obligate parasites such as cereal rusts. However, isolate x cultivar specificity has been considered in host-pathogen system where physiological specialization is mainly based on quantitative differences in disease expression such as caused by Septoria spp. (Scharen et al. 1985). Tan spot has also been reported to exhibit isolate x cultivar specificity. Schilder & Bergstrom (1990) tested 17 isolates of P. tritici-repentis on 12 wheat cultivars and reported significant isolate x cultivar interactions; however, they could not group them into distinct physiological races. Sah & Fehrmann (1992) tested the virulence patterns of 19 isolates of different origin on eight wheat cultivars and they identified highly significant cultivar x isolate interactions. Similarly, in this study significant levels of isolate x cultivar interactions were detected. Nevertheless, the differences among isolates were not distinct enough to categorize them into physiological races. Still there is a consensus that the more aggressive types should be employed in germplasm screening.

There were genetic differences among the 21 commercial bread wheat cultivars from Ethiopia in their resistance to tan spot under controlled conditions. All the cultivars were infected and no immune reaction. The cultivars could be grouped into three (susceptible to moderately susceptible, intermediate and moderately resistant). Only three cultivars were

moderately resistant to the pathogen. Several host-pathogen interaction studies on wheat and P. tritici-repentis have been conducted both in the field and in the greenhouse (Rees & Platz Correlated results were obtained between resistance tests at the seedling and a dult plant growth stages (Evans et al. 1999). The study has indicated that tan spot could be a common disease of wheat in Ethiopia and most of the commercial bread wheat cultivars lack adequate level of resistance to the pathogen. In the past, wheat cultivars have been released without properly exposing them to the pathogen. Therefore, the wheat-breeding program in Ethiopia should aim at developing tan spot resistance genotypes.

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