Pathogenic Variation of *Rhynchosporium secalis* Isolates from Ethiopia*

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Abstract

Rhynchosporium secalis, the causal agent of barley leaf scald is a highly variable pathogen in areas where the disease is reported. To design an effective breeding program and exercise other disease control practices, information on the pathogenic variability of this pathogen is critical. In this study, the variability of 24 single spore isolates was studied on seedlings and adult plants of 14 barley differentials. Seedlings test showed wide variability of the pathogen in that the 24 isolates were grouped into 17 distinct pathotypes. Pathotypes Et16 and Et17 were the most virulent and overcame the resistance in 11 differential genotypes whereas pathotypes Et1, Et2 and Et3 were virulent on only one genotype each. Environmental conditions, farming practices and the cultivation of barley largely as landrace populations are assumed to generate variability of the pathogen in Ethiopia. In general, there was a strong correlation between the seedling and adult plant tests. Since scald is most destructive at early crop growth stages, seedling resistance is vital to reduce the disease epiphytotics, to differentiate isolates and to predict adult plant resistance. The shifts in population composition and the greater variability of *R. secalis* detected in this study suggest regular sampling of the pathogen within a given region is warranted.

Introduction

Barley leaf scald (leaf blotch) caused by *Rhynchosporium secalis* (Oud.) J.J. Davis is an important disease of barley in many parts of the world where the crop is cultivated (Ali et al. 1976; Jackson & Webster 1976; Salamati & Tronsmo 1997; Shipton et al. 1974). In the highlands of Ethiopia, scald is one of the principal diseases of the crop that inflicts heavy epidemics and consequently leads to yield losses as high as 67% (Eshetu 1985; Yitbarek et al. 1996). The economic importance of the disease varies depending on the crop genotypes, farming practices and environmental conditions.

Evidence for sexual reproduction in *R. secalis* is currently lacking. The fungus is known to produce new recombinants asexually and this means of reproduction appears to be an effective mechanism for the generation of diverse pathogenic groups that leads to the identification of a number of classes referred to as pathotypes or physiological races.

The population of *R. secalis* is reported to exhibit greater variability in many parts of the world (Ali et al. 1976; Jørgensen & Smedegaard-petersen 1995; Robinson et al. 1996; Salamati & Tronsmo 1997). In Ethiopia, Kiros & Mengistu (1994) characterized 24 single spore isolates from different barley genotypes and localities on 10 standard barley differentials and classified these into 19 pathotypes. As populations of this fungus

1995; McDonald et al. 1999), it is necessary to check the current composition of a *R. secalis* population and monitor changes in the frequency of the most virulent pathotypes.

The designation of resistance genes in barley genotypes that used to differentiate isolates of *R. secalis* is complex and inconsistent (Baker & Larter 1963; Dyck & Schaller 1961; Habgood & Hayes 1971; Starling et al. 1971). The varied number and type of the differentials used, and the lack of standardized experimental protocols have further created difficulties in comparing the findings of different studies. Therefore, reference was at large made to the work by Goodwin et al. (1990) in selecting barley differential lines for this study.

Growing resistant cultivars is one way of controlling leaf scald. However, the type of resistance (qualitative or quantitative) may play a significant role in the durability of resistance. The wide diversity in the pathogen is one of the main causes for the breakdown of a newly deployed resistance within a short period in cultivars with only one or two major resistance genes (Jackson & Webster 1976). Thus, before initiating an effective breeding program for resistance to leaf scald, knowledge on the pathogenic variability of the fungus is pivotal.

This study was undertaken to examine the pathogenic variability of single spore isolates of the fungus collected from different barley genotypes and locations in Ethiopia using both seedlings and adult plants of the standard barley differentials. Moreover, a comparison between the present and the earlier study in Ethiopia was attempted to understand shifts in the pathogenic variability of the fungus.

Materials and Methods

Isolation of the fungus

Barley leaves with scald symptoms collected from various genotypes at 33 locations during the main seasons of 1997 and 1998 were used to isolate the fungus. The regions, locations and barley genotypes from which leaf scald infected leaves were collected are shown in Table 1. Collected samples were first air dried and subsequently stored in a refrigerator at about 5^{0}C. Leaves with scald lesions were cut into 1 - 1.5 cm long pieces and then surface disinfected in 70% ethanol for 15-20 seconds. In case of very dry leaves, segments were first soaked in distilled water for 20- 30 min prior to disinfection in 70% ethanol. The leaf pieces were further disinfected in 0.5% sodium hypochlorite solution for 90 seconds, transferred onto 9cm plates that contained a Difco Lima Bean Agar (LBA) and placed at 20^{0}C in the dark.

Spores from a pure colony of each isolate were scraped off with a wire loop and suspended in 1 ml of distilled water. The suspension was first adjusted to 10^3 spores/ml, streaked onto fresh plates of LBA and incubated as described above. After 10 to 12 hrs, plates were examined microscopically and a germinating single spore was transferred onto a fresh plate of LBA and incubated at 20^{0}C in the dark for 2 weeks. Then each single spore derived colony was transferred into test tubes of LBA and stored in the dark at 5^{0}C until required. Each isolate was then inoculated on the susceptible line, Ardu-12-8c, and re-isolated on LBA in order to revive the aggressiveness of the isolate prior to the seedling and adult plant tests.

Table 1. Origin of *Rhynchosporium secalis* isolates used in pathogenecity tests.

Isolate No. [a]	Place of sampling	Region	Barley genotype
1	HRC[b]	Shewa	Variety?
6	Near HRC	Shewa	HB42
7	Near HRC	Shewa	Local landrace
8	Ambagiogis	Gonder	Local landrace
9	Absala Guagsa Shikudat	Gonder	Local landrace
10	Ilala	Gonder	Local landrace
11	HRC	Shewa	Ardu 12-8c
14	Robgebaya	Shewa	Local landrace
15	SRC	Shewa	BLR/sc # 12 Acc. No 1172
16	SRC	Shewa	ICB-92 0724
17	SRC	Shewa	Ardu 12-8C
18	Alidoro, Degem	Shewa	Local landrace
19	Abote, Degem	Shewa	Local landrace
20	Tarmaber zuria	Shewa	Local landrace
21	Gudoberet/ Ansase	Shewa	Local landrace
22	D.Birhan Zuria Layemush/Keyet Asagert	Shewa	Local landrace
24	Mezezo	Shewa	Local landrace
25	D.Birhan zuria Bakelo	Shewa	Local landrace
26	D.Birhan zuria Chacha	Shewa	Local landrace
27	Ankore Degem	Shewa	Local landrace
30	Bekoji Expt. Station.	Arsi	Ardu-12-8c
31	Tikurinchini	Shewa	Local landrace
32	Tikurinchini	Shewa	Local landrace
33	Altufa	Shewa	Local landrace

[a] *Isolate number corresponds to the field sample number.*
[b] *HRC = Holeta Research Center.*
[c] *SRC = Sheno Research Center.*

Seedling test

Seeds of 14 barley genotypes, provided by Prof. M. Johnston, Montana State University, Bozeman, were used to differentiate *R. secalis* isolates. The differential genotypes used and their corresponding designated resistance genes are listed in Table 2. The 14 differentials and a susceptible genotype, Ardu-12-8c, were first planted in a 9cm plastic pot that contained a mixture of soil, sand and compost at a ratio of 1:1:1 v/v/v. In each pot, 5 seedlings of a genotype were grown and 4 pots were used for each isolate/genotype combination. One pot of each genotype was placed in a plastic tray and watered from the bottom to avoid seedling damage during watering. Trays were placed in the glasshouse at 16hr daylight and temperature of around 20^{0}C until required. The experiments were arranged in a split plot design where the isolate was used as the main plot and the differentials as sub-plot treatments. The sub-plot treatment was arranged in a completely randomized design with 4 replicated trays. The whole experiment was repeated once.

Trays were transferred into a climate chamber a day before inoculation when the second leaf was completely unfolded and the third leaf just visible. Throughout the experiment, the climate chamber was programmed with a day/night temperature of 18/14^{0}C, 12hr light of 75m E m^{-2} s^{-1} and 95% relative humidity (RH).

Table 2. Barley genotypes used as differentials to identify pathotypes of *Rhynchosporium secalis* isolates from Ethiopia.

Cultivar	CI number[a]	R gene	References
Abyssinian	668	Rh9	Baker & Larter 1963
Atlas	4118	Rh2	Dyck & Schaller 1961
Atlas 46	7323	Rh2, Rh3 Rh Rh-Rh3-Rh4	Dyck & Schaller 1961 Habgood & Hayes 1971 Starling et al. 1971
Brier	7157	Rh Rh, rh6 Rh-Rh3-Rh4	Dyck & Schaller 1961 Habgood & Hayes 1971 Starling et al. 1971
Forrajera	8158	Rh4?	Harrabi, Pers. communication
Jet	967	rh6, rh7 rh^5, rh 6	Baker & Larter 1963 Habgood & Hayes 1971
Kitchin	1296	Rh 9	Baker & Larter 1963
La Mesita	7565	Rh4 Rh^4, Rh 10 Rh-Rh3-Rh4	Dyck & Schaller 1961 Habgood & Hayes 1971 Starling et al. 1971
Modoc	7566	$Rh4^2$ Rh^2, rh6 Rh-Rh3-Rh4	Dyck & Schaller 1961 Habgood & Hayes 1971 Starling et al. 1971
Nigrinudum	2222	rh8	Wells & Skoropad 1963
Osiris	1622	Rh4 Rh^4, rh6, Rh10	Dyck & Schaller 1961 Habgood & Hayes 1971
Steudelli	2266	rh6, rh7	Baker & Larter 1963
Trebi	936	Rh4 Rh-Rh3-Rh4	Dyck & Schaller 1961 Starling et al. 1971
Turk	5611-2	Rh3, Rh5 Rh, rh6 Rh-Rh3-Rh4	Dyck & Schaller 1961 Habgood & Hayes 1971 Starling et al. 1971

[a] *Accession number of Cereal Crop Research Branch, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland.*

Preparation of inoculum and inoculation

To produce inoculum of the various isolates, each isolate was first transferred onto LBA plates and incubated in the dark at 20^0C for 21 days. Colonies of an isolate were scraped off and suspended into distilled water. The spore suspension was then filtered through a 0.1mm mesh gauge to remove mycelial fragments and concentration of the spores was adjusted to 1.5 x 10^6 spores/ml. The whole set of differential seedlings were then inoculated by spraying 75 ml of spore suspension that contained 3 drops of the surfactant Tween 20 (polyoxyethylen sorbitanmonolaurat). After inoculation, seedlings were kept in the dark at about 100% RH for 48 hr to enhance infection. Fifteen days after inoculation (15 dpi), the seedlings were rated for scald infection using a 0-4 disease rating scale (Jackson & Webster 1976), where 0 = no visible disease symptoms, 1 = small discrete lesions confined to the leaf margins and tips, 2 = small lesions often with dark margins scattered over the leaf, 3 = large coalescing on more than 50% of the leaf often with irregular wilted grayish-green areas without dark margin, and 4 = up to 100% of the leaf covered with grayish-green wilted areas without dark margins and finally with total collapse of the leaf. Seedlings with disease ratings of 0-2 were classified as resistant and 3 - 4 as susceptible. The mean disease value was calculated from 5 seedlings per pot and used for the analysis of variance.

Moreover, the disease index (DI), a quantitative measurement of disease severity useful to summarize the information from disease ratings of 0, 1, 2, 3 and 4 was used to characterize barley differentials. For each isolate and differential combination, DI was calculated using the following formula:

$$DI = \frac{\sum_{i=0}^{4} jfi}{4\sum_{i=0}^{4} fi},$$

Where, fi = number of individuals in the j^{th} category and 4 is the maximum disease score in the 0-4 disease rating scale. DI values range between 0 if every individual in an entry scores 0 and 1 if all individuals score 4. The DI values were subjected to a two-way analysis of variance using the MSTATC (1989) statistical package. Means were separated by the Students-Newman-Keuls' (S-N-K) test at the 5% significance level.

Adult plant test

Those *R. secalis* isolates that were virulent on a greater number of the genotypes used in the seedling tests were further tested on adult plants of the 14 barley differentials. Two seeds of each genotype were planted in a 9cm plastic pot and 2 pots were used for each isolate/genotype combination. Three successive sowing dates at two weeks' interval were carried out in order to obtain plants of a similar growth stage at the time of inoculation. Plants were kept in the glass house until required. When plants reached the flag leaf stage, i.e. flag leaf completely unfolded, they were transferred into the climate chamber with the same conditions as described for the seedling test. One pot of each differential was placed in a plastic tray and two trays were used for the inoculation by a single isolate. Plants were inoculated by spraying 85 ml of spore suspension/2 trays that contained 1.5 x 10^6 spores/ml. The experiment was arranged in a similar way as the seedling test, but the sub-plot treatments were completely randomized with two replicated trays. The disease severity was assessed on each plant at 15 dpi and 30 dpi using the 0-4 disease rating scale and the average disease data calculated from the two plants in a pot were used for the analysis of variance. The whole experiment was repeated once.

Results

R. secalis was successfully isolated from 24 of the 33 leaf samples collected. Attempts were made repeatedly to isolate the fungus from the remaining samples but with no success. Single spore cultures from each of the 24 isolates were prepared and used for the subsequent experiments.

All 24 isolates were able to induce a susceptible reaction on the susceptible line, Ardu-12-8c (Table 3). Isolates Et 8, 14, 15, 17, 21 and 22 were virulent only on the differential Brier. Isolate Et 11 and Et 6 were virulent only on Modoc and Atlas, respectively. On the other hand, Et 27 and Et 33 were the most virulent, each inducing a susceptible reaction on 11 differential genotypes. These two isolates originated from locations distant from each other. Isolate Et 31 from a location close to Et 33 was also virulent on 9 differential genotypes. Other isolates were virulent only on 3 to 6 differentials, and no isolate was virulent on the whole set of differentials.

The differential genotypes used varied in their reactions depending on the type of isolate. The differential Brier was susceptible to 21 of the 24 isolates, followed by Steudelli and Jet, each being susceptible to 12 and 11 isolates, respectively. In contrast, the differential Osiris was resistant to all isolates and this was reflected by the significantly lower DI value. Atlas, Atlas 46 and La Mesita were each susceptible to only 2 isolates and exhibited significantly lower DI values. The remaining differential genotypes were susceptible to between 4 and 9 isolates, whereas Ardu-12-8c was susceptible to all and had the highest DI values.

Table 3. Mean disease rating and disease index values on barley differentials inoculated with 24 isolates of *Rhynchosporium secalis*

Differential	*R. secalis* isolates																								Mean disease
	1	6	7	8	9	10	11	14	15	16	17	18	19	20	21	22	24	25	26	27	30	31	32	33	index[a]
Abyssinian	1.0[b]	1.0	1.0	0.0	1.0	1.0	0.5	1.0	1.0	1.3	1.0	3.5	2.0	2.0	1.3	2.0	1.5	2.0	3.5	4.0	0.9	3.8	1.8	3.9	0.36 f
Atlas	1.0	3.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	0.3	1.0	1.5	1.3	2.0	1.0	0.3	3.0	1.0	1.0	1.0	0.0	0.1	0.0	1.3	0.22 j
Atlas 46	0.0	0.0	1.0	1.0	0.5	1.0	0.8	0.0	0.5	1.8	0.0	1.0	1.0	2.0	1.0	0.0	3.0	1.0	1.0	1.0	0.0	0.9	0.8	3.4	0.21 j
Brier	2.0	2.0	4.0	3.0	4.0	4.0	1.0	4.0	3.5	4.0	3.0	3.5	3.5	4.0	3.0	3.5	4.0	4.0	4.0	4.0	2.8	3.8	3.0	4.0	0.73 b
Forrajera	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	3.8	0.5	1.0	0.0	1.0	1.0	3.0	2.0	4.0	0.0	3.3	0.5	4.0	0.22 j
Jet	3.0	1.0	2.0	1.8	2.5	3.0	1.0	1.0	1.0	1.3	1.0	2.0	3.0	2.0	1.3	2.0	3.5	3.0	4.0	4.0	1.6	2.6	3.1	3.8	0.48 c
Kitchin	0.0	1.0	1.0	0.0	3.5	0.0	0.0	1.3	1.0	1.0	1.0	2.0	0.0	1.0	1.0	0.5	1.0	0.5	3.3	4.0	1.0	3.8	3.1	4.0	0.31 g
Modoc	3.0	1.0	1.0	1.5	0.0	1.3	1.0	3.0	1.0	1.0	3.0	2.0	3.8	2.0	0.5	1.0	0.0	2.0	2.0	2.0	4.0	2.5	3.8	2.4	4.00.42 d
La Mesita	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	4.0	1.0	1.0	0.5	2.3	0.11k
Nigrinudum	4.0	1.0	2.0	1.0	3.5	2.0	1.0	0.5	1.0	1.5	1.0	1.0	1.8	1.5	2.0	2.0	1.3	0.5	4.0	3.0	0.6	2.4	1.5	3.9	0.39 e
Osiris	0.0	1.0	0.3	0.0	1.0	0.0	0.3	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0	1.0	1.0	2.0	1.0	0.5	0.0	0.3	0.0	0.08 l
Steudelli	2.0	1.0	3.0	1.0	1.5	3.0	1.0	1.5	0.0	1.0	0.8	1.3	3.0	3.0	1.8	1.5	4.0	3.0	4.0	4.0	2.3	3.3	3.6	3.8	0.48 c
Trebi	1.0	0.0	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	0.0	3.0	0.5	1.0	0.5	0.0	1.0	1.8	1.0	4.0	1.8	3.8	0.8	3.8	0.28 h
Turk	0.0	0.0	3.0	1.0	1.0	2.0	0.3	1.0	0.0	3.3	0.0	1.0	0.8	4.0	0.3	0.5	3.0	1.0	1.0	4.0	0.8	0.3	0.5	1.0	0.26 i
Ardu-12-8c	3.5	4.0	4.0	3.0	4.0	4.0	3.5	4.0	3.0	4.0	3.0	3.0	4.0	4.0	3.0	4.0	4.0	4.0	3.5	4.0	4.0	4.0	3.8	4.0	0.76 a

Means in a column followed by the same letter are not significantly different at p £ 0.05 as determined by S-N-K test.

[b]*Disease rating 0 -2 = resistant, 3 and 4 = susceptible.*

The virulence analysis of *R. secalis* isolates led to the identification of 17 pathotypes labeled 1 to 17 (Table 4). Pathotypes were designated based on the number of differentials (resistance genes) they attacked. Pathotype 1 was found to consist six of the 24 isolates and pathotypes 4 and 7 were each represented by two isolates. Each of the remaining pathotypes was represented by only one isolate. Pathotypes 11 to 17 were virulent on at least 5genotypes. Pathotypes 16 and 17 were most virulent to overcome the resistance in 11 differential genotypes.

Table 4. Pathotypes of *Rhynchosporium secalis* identified and number of susceptible differential genotypes

Pathotype	*R. secalis* isolate	Genotypes susceptible
1	Et 8, 14, 15, 17, 21, 22	Brier
2	Et 6	Atlas
3	Et 11	Modoc
4	Et 10, 19	Brier, Jet, Steudelli
5	Et 30	Brier, Modoc, Steudelli
6	Et 1	Jet, Modoc, Nigrinudum
7	Et 7, 20	Brier, Steudelli, Turk
8	Et 16	Brier, Modoc, Turk
9	Et 9	Brier, Jet, Kitchin, Nigrinudum
10	Et 25	Brier, Forrajera, Jet, Steudelli
11	Et 32	Brier, Jet, Kitchin, Modoc, Steudelli
12	Et 18	Abyssinian, Brier, Forrajera, Modoc, Trebi
13	Et 26	Abyssinian, Brier, Jet, Kitchin, Nigrinudum, Steudelli
14	Et 24	Atlas, Atlas 46, Brier, Jet, Steudelli, Turk
15	Et 31	Abyssinian, Brier, Forrajera, Jet, Kitchin, Modoc, Nigrinudum, Steudelli, Trebi
16	Et 33	Abyssinian, Atlas 46, Brier, Forrajera, Jet, Kitchin, La Mesita, Modoc, Nigrinudum, Steudelli, Trebi
17	Et 27	Abyssinian, Brier, Forrajera, Jet, Kitchin, La Mesita, Modoc, Nigrinudum, Steudelli, Trebi, Turk

The adult plants response of the differential genotypes to the 6 most virulent isolates of *R. secalis* varied depending on the isolate/genotype combinations (Table 5). Among the genotypes, Osiris was found to be resistant to all the isolates and consistently had very low DI values. Atlas, Atlas 46, La Mesita and Turk were each susceptible to two isolates and also had lower DI values than the other genotypes, while Nigrinudum was susceptible to 3 isolates. Differentials Brier, Modoc and Trebi, on the other hand, were susceptible to all isolates comparable to Ardu-12-8c.

In general, disease assessments made at the seedling stage significantly correlated ($r = 0.81$, $p < 0.001$) to disease data taken at the adult plant stages of the barley differentials inoculated with the most virulent isolates. However, the interaction between certain differentials and isolates revealed a differential response between the seedling and the adult plant stages. For instance, isolates Et 18 and Et 31 resulted in a susceptible reaction on greater number of differentials at the adult plant than at the seedling stage. The remaining 4 isolates induced a susceptible reaction on the same number of differentials at both growth stages. Some differentials that were resistant at the seedling stage were susceptible at the adult plant stage.

differentials that were resistant at the seedling stage were susceptible at the adult plant stage. For instance, La Mesita, which exhibited seedling resistance to isolate Et 18 was susceptible at the adult plant stage. Likewise, Trebi and Atlas 46 were susceptible at the adult plant stage to isolates Et 24 and Et 31, respectively. In general, the barley differentials exhibited a lower disease severity at the adult plant than the seedling stage.

Table 5. Mean disease rating and disease index values on adult plants of barley differentials inoculated with selected isolates of *Rhynchosporium secalis* from Ethiopia.

Differential	Isolate						Mean disease index
	Et 18	Et 24	Et 26	Et 27	Et 31	Et 33	
Abyssinian	2.5 bc[β]	1.25 c	2.25 a	2.75 ab	2.25 abc	3.25 ab	0.51 e
Atlas	1.0 ef	2.75 b	0.5 b	0.5 c	0 f	2.75 b	0.26 h
Atlas 46	0.5 fg	0.75 c	0.75 b	1.0 c	2.75 ab	2.5 b	0.31 g
Brier	2.5 bc	2.75 b	2.75 a	3.25 ab	2.25 abc	2.75 b	0.57 d
Forrajera	3.25 a	0.5 c	2.75 a	3.25 ab	2.75 ab	3.25 ab	0.56 d
Jet	1.25 ef	2.75 b	3.25 a	2.75 ab	1.75 bc	3.25 ab	0.53 de
Kitchin	1.25 ef	0.75 c	2.75 a	2.75 ab	3.25 a	2.5 b	0.47 f
La Mesita	2.25 cd	0.75 c	1.0 b	2.25 b	1.25 cde	1.75 c	0.33 g
Modoc	3.25 a	2.75 b	3.0 a	3.5 a	2.5 abc	3.75 a	0.67 b
Nigrinudum	1.75 de	0.5 c	3.25 a	2.75 ab	1.5 bcd	3.25 ab	0.46 f
Osiris	0 g	0.5 c	0.5 b	0.5 c	0.25 ef	0.5 d	0.08 i
Steudelli	1.25 ef	2.75 b	3.25 a	2.75 ab	2.25 abc	3.25 ab	0.55 de
Trebi	3.0 ab	2.75 b	2.25 a	3.25 ab	2.75 ab	3.5 ab	0.62 c
Turk	0.5 fg	2.25 b	0.5 b	2.75 ab	0.5 def	1.0 d	0.29 gh
Ardu-12-8c	3.5 a	3.5 a	3.25 a	3.5 a	2.75 ab	4.0 a	0.73 a

[β] Mean disease rating in a column followed by the same letter are not different at p £ 0.05 as determined by S-N-K test. The underlined values indicate higher mean disease ratings on the adult plants than on seedlings.

A comparison between the results of the present study and those from a similar work by Kiros and Mengistu (1994), based on the type of differentials used in both studies, indicated that except for Nigrinudum and Turk, that were matched by a similar number of pathotypes, the other genotypes were susceptible to a lesser number of pathotypes in 1999 than 1994. On the other hand, Jet was susceptible to more pathotypes in 1999 than 1994. Examination of the pathotypes that were virulent on Nigrinudum in both studies revealed dissimilarities in their geographical origins. Of the 5 pathotypes that were pathogenic on Turk in the present study, only pathotype 5 originated from a similar location as pathotype 18 of the earlier study.

Discussion

The reactions of the standard barley differentials to the isolates tested revealed a higher number of pathotypes in the 1997-98 field population of *R. secalis* from Ethiopia. The pathotypes were designated as 1 to 17 based on an increasing number of genes matched. This type of designation was used in several other studies and found useful when a few number of differential genotypes are used (Goodwin et al. 1990).

The present study, based on a fairly small number of *R. secalis* isolates demonstrated the presence of a greater variability in the pathogen similar to the earlier study in Ethiopia (Kiros &

Mengistu 1994). It was similar to the results from California, (Jackson & Webster 1976), Denmark (Jørgensen & Smedegaard-petersen 1995) and Norway (Salamati & Tronsmo 1997). As the resistance gene designation of the standard differentials is not yet clarified, it is preferred to use the term pathotypes instead of races as suggested by Ali et al. (1976). Among the pathotypes, the pathotypes 16 and 17 were the most complex, each overcoming the resistance of 11 barley differential genotypes. Pathotype 1, which was represented by six isolates, and the pathotypes 2 and 3 each represented by one isolate were the least complex pathotypes, each able to match the resistance in only one genotype. The present study also showed that except pathotypes 1, 4 and 7, all the remaining were represented only by a single isolate and this may suggest sampling and testing of more isolates from a location.

Resistance controlled by a major gene is usually expected to be effective at all growth stages of the host plant and is less affected by changes in growing environments. In most studies, isolates had been characterized mainly on seedlings, and there is a possibility that any adult plant resistance could have passed unnoticed. This adult plant resistance may depend on other more complex characters such as the morphology and physiology of the host, reduced spore germination, reduced penetration and slower growth rate of the pathogen. In our study, there was a significant correlation between the seedling and adult plant stage for resistance to leaf scald. Nevertheless, there were differential genotypes that were resistant at the seedling stage but susceptible at the adult plant stage depending on *R.* secalis isolate. In a different study, the genotype La Mesita, that was resistant at the seedling stage exhibited susceptibility to the same isolates when inoculated at the adult plant stage in the field (Robinson et al. 1996), and in controlled environment tests (Jones et al. 1995). Conversely, the response of ten genotypes was reported to remain unchanged between seedling and 6-week old plants after inoculation with 12 different isolates (Jackson & Webster 1976). Hence, the seedling test was suggested to be valid for the differentiation of pathotypes, and was considered predictable for the adult plant response except in a few of the genotype/isolate combinations. In Ethiopia, barley leaf scald is a destructive disease of barley primarily during the seedling stage probably due to the prevailing cool temperature and high moisture conditions at this stage compared to the adult plant stage. Therefore, resistance at the seedling stage is considered to play a vital role in reducing disease epiphytotics and plant yield losses.

In Western Australia, infected wild grasses, imported barley grain and other plant parts are assumed as the source of the genetic diversity in *R. secalis* (Ali et al. 1976). Recently, McDonald et al. (1999) studied the genetic structure of *R. secalis* in Australia using RFLP markers and were able to classify 214 distinct pathotypes from 265 isolates. This high level of diversity had not been expected for an asexually reproducing pathogen and they proposed that the sexual stage of *R. secalis* may occur on stubble between growing seasons and play a role in producing new genetic recombination followed by exclusively asexual propagation. The variability reported in populations of *R. secalis* worldwide further exemplifies a long-unresolved question in pathogenic fungi if there are some undetected forms of genetic recombination responsible for the high variability exhibited in a presumably asexual population. On the other hand, there was no obvious explanation for the high variability of the fungus in Denmark (Jørgensen & Smedegaard-Petersen 1995), which geographically is a fairly uniform and isolated country, nevertheless, each part of the country was reported to have its own separate population of the pathogen. Infected seed and a

favorable environment are considered to enhance the wide pathogenic variability in Norway (Salamati & Tronsmo 1997). In addition, isolates originating from other regions and the cultivation of a wider range of cultivars are assumed to increase the diversity of the fungus. Breeding for resistance to *R. secalis* and cultivation of different barley genotypes short distances from each other are assumed to generate variability in the pathogen population. These assumptions may not be the key factors promoting pathogenic variability in Ethiopia. First, none or only a very few of the differential genotypes are cultivated in Ethiopia. Secondly, breeding of barley following Mendelian principles in this country is only 30 years old. Hence, newly developed cultivars occupy only a small portion of the barley area in the country and this in turn would be expected to have little influence to exert changes in the pathogen population.

The generation of a wider variability in the population of *R. secalis* is assumed to be related not only to the environmental conditions, but also to different cultural practices. Most barley growing regions in Ethiopia has two rainfall periods allowing cultivation of the crop twice in a year. This lack of a long crop free period between the two seasons and the favorable environment can be assumed to promote disease epidemics. Moreover, barley is genetically a most diversified crop in Ethiopia and is grown mainly as landrace populations. These landraces often may have adapted to specific microclimatic conditions even in locations fairly close to each other. This in turn may have promoted the establishment of very different germplasms of the host plant and of different resistance gene patterns in the landraces as well. Hence, the high genetic diversity of *R. secalis* in Ethiopia is assumed to originate in the high variability of the host in the country. Genetically, each landrace population consists of individuals with a varying degree of resistance to the disease (Yitbarek et al. 1998). The evaluation of barley landrace populations for resistance to barley leaf scald revealed the presence of considerable differences even among individual single lines from the same population and among populations collected from fields over short distances. Thus, it is not surprising that this broad genetic variation in the host has generated considerable pathogenic variability in the pathogen population within a given locality or region. This is likely facilitated by the generally short horizontal distance over which the spores of *R. secalis* are disseminated (McDonald et al. 1999, Shipton et al. 1974).

Attempts were not made to compare the distribution of *R. secalis* pathotypes among geographical origins since some regions like Arsi were only represented by a single sample. Nevertheless, differences in the distribution of the pathotypes were found in which pathotype 1, most widely distributed, was observed in samples collected from fields in the Gonder region and in the Eastern and Western parts of the Shewa region. Pathotype 2 was found in the Northern and Western parts of Shewa region. Interestingly, pathotype 4 was obtained from the field samples of the North Shewa and Gonder regions where both are geographically far apart from each other. Hence, these findings support the previous suggestions that the pathogen has a wider range of variability even within a single geographical zone (Goodwin et al. 1992). It is, therefore, desirable to sample local populations of *R. secalis* to determine the population structure within a region.

The broad resistance of the Cv. Osiris is in agreement with findings in Norway (Salamati & Tronsmo 1997) and in Denmark (Jørgensen & Smedegaard-Petersen 1995). The *R. secalis* population was reported to be highly variable in Norway where 42 isolates were classified into 32

was found to be pathogenic on this genotype. Similarly, among 38 Danish isolates that were classified into 28 pathotypes none of them was pathogenic on Osiris.

The information obtained from this study is valuable for the development of new barley genotypes with broad resistance to this economically important disease in Ethiopia. However, breeding based on race specific resistance may not be efficient since the pathogen might overcome such resistance due to its wide range of variability. In this regard, multiple race specific resistance in combination with race nonspecific resistance will be ideal to combat the disease. For this purpose the cv. Osiris appears to be promising source of wide resistance. However, whether the nature of resistance in Osiris is more of a nonspecific or due to an accumulation of a higher number of what we traditionally call "major genes" is not yet clear. In case of barley scald, a distinct differentiation between "major genes" and "minor genes" on one hand and between "specific" and "nonspecific" becomes quite difficult.

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