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Notes

Pathogenic Groups Identified Among Isolates of *Rhynchosporium secalis*

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Scald, caused by *Rhynchosporium secalis* has been the major yield-reducing factor for barley production during the last decade. In this study, pathogenic groups of *R. secalis* were identified to obtain a global picture of the assembly of isolates involved in Syrian populations which is essential for the development of scald-resistant barley cultivars. To identify a number of pathogenic groups, 49 isolates collected over ten years from major barley growing areas in Syria were evaluated on five differential barley genotypes. Genotypes presented a continuous range of response from highly susceptible to moderately resistant, but none were immune to the disease. A cluster analysis placed isolates in six distinct differential pathogenic groups. Mean disease rating of 39.24% was the separation point between avirulent and virulent reactions. Isolate Rs46 exhibited distinct differential virulence patterns associated with high frequency across all genotypes. Hence, the data presented here provides crucial information for future selection of isolates to develop durable barley scald resistance.

Keywords **:** barley resistance, frequency of virulence, scald, pathogen population

Rhynchosporium secalis (Oudem.) J. J. Davis, the causal agent of scald, is an important pathogen of barley (*Hordeum vulgare* L.) found worldwide. This pathogen is considered economically important because it can cause marked reduction in yield and quality of the crop (Brown et al., 1996; Yahyaoui 2003; Meles et al., 2005).

Although fungicides can be effective in reducing scald severity (Stedman, 1980), the most effective and environmentally sound means of control is through the use of resistant cultivars. Development of stable forms of resistance to foliar diseases depends upon identification of resistances effective against the prevalent isolates in barley growing areas (Xi et al., 2000). Variations in barley resistance to scald were reported (Jørgensen and Smedegaard-Peterson, 1995; Xi et al., 2002; Zaffarano et al., 2006). The value of resistance to this disease depends mainly on its level and its durability in relation to the life span of the genotypes.

However, before resources are committed to control scald disease, the identification of pathogenic groups of isolates expressing differential virulence on barley genotypes should be evaluated. Field assessment is time-consuming and several environmental interactions such as inoculum and moisture make it impossible to obtain error-free estimates (Mert and Karakaya, 2003).

To effectively breed scald resistance barley, it is essential to have information on the pathogenicity groups in *R. secalis*. Therefore, the aim of the present research was mainly to investigate, the pathogenic groups of *R. secalis* collected from different regions of Syria isolates under controlled conditions, which can be used in future breeding programs to facilitate the deployment of effective resistance to *R. secalis* in barley.

Fungal isolates and inoculum preparation. Over ten years, more than 115 isolates of *R. secalis* were obtained from barley leaves showing scald symptoms in different regions of Syria. Leaves were placed in paper envelopes and allowed to air-dry at room temperature for at least 48 h. Dried leaves were soaked for 10 s in 70% ethanol, surface sterilized for 60 s in a 5% sodium hypochlorite solution, and rinsed in distilled water for 10 s like wise for the preceding lines. The sterilized leaves were cut into small pieces (5 mm in length) and transferred to Petri dishes containing potato-dextrose agar (PDA, Difco, MI, USA). After incubation for 48 h, at 15°C in the dark, fine glass needles were used to remove small tufts of mycelia that emerged around lesion borders. Mycelial tufts were transferred to Petri dishes containing lima bean agar (LBA). After incubation at 15°C for 1 to 2 weeks, colonies were spread across fresh LBA plates and incubated for an additional 2 to 3 weeks at 15°C. Spores and mycelial fragments were collected by adding 1 ml of sterile distilled water and scraping gently across the mycelium with a sterile glass microscope slide. In preliminary studies, different barley genotypes had been inoculated with 115 fungal isolates, evaluating host reactions and lesion forms. Emphasis was placed on selecting isolates that induced differential reactions on specific genotypes, leading to selection of the 49 monosporic isolates used in this study (Table 1). A suspension of conidia from 12- day-old cultures

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Table 1. Mean disease rating of 49 isolates of Rhynchosporium secalis on 5 barley genotypes used as differentials

Isolates (No.)	Year of collection	Mean rating					
		Tadmor	Igri	WI2291	Golf	Arabi Abiad	Mean
Rs1	1997	5.4	10.4	58.9	20.2	36.5	26.3h
Rs2	2004	40.7	49.8	77.1	43.7	79	58.6ef
Rs3	2004	32.3	29.8	85.5	37.4	70.6	51.1e
Rs4	2004	3.7	9.1	35.2	30.5	53.3	26.4h
Rs5	2004	43.6	59.6	89.2	79.7	87.6	71.9b
Rs6	2006	50.8	39.8	89.3	87	84.2	70.2bc
Rs7	2003	1.9	14.2	80.8	17	13	25.4h
Rs8	2006	20.6	56.6	39.4	55.9	40.5	42.6c
Rs9	2003	2.5	5.7	31.5	52	57.2	29.8gh
Rs10	2003	4.7	32.4	88.6	18.5	50.6	38.9ef
Rs11	2003	0.7	7.2	37	27.6	34	21.3i
Rs12	2001	1.3	4.8	20	33.6	26.6	17.3ij
Rs13	2004	12.6	10	90.2	81.6	81.6	55.2ef
Rs14	2001	15	53.5	40.3	54.1	44.5	41.4fg
Rs15	2003	7.6	19.2	78.8	28	52.1	37.1ef
Rs16	1997	8.5	12.3	58.4	27	62.6	33.8g
Rs17	2003	41	30	93.2	78.8	88.6	66.3cd
Rs18	2003	7.3	9.1	77.5	52.9	57.4	40.8fg
Rs19	2003	43.8	49.6	63.5	72.3	72.5	60.3bc
Rs20	2004	1.2	12.1	66.2	87	84.2	50.1e
Rs21	2004	6.1	4.7	58.2	17	13	19.8i
Rs22	2001	19.2	36.1	80.8	76.6	77.7	58.1e
Rs23	2001	2	7.9	30.4	14.3	21.1	15.1j
Rs24	2001	6.4	23.1	77.5	52.9	57.4	43.5fg
Rs25	2001	15.6	29.1	60.7	79.7	86.1	54.2gh
Rs26	2003	15	11.3	53.4	86.5	88.1	50.9e
Rs27	2003	7.2	8.7	53.8	17	13	19.9ij
Rs28	2003	43.8	34.4	70.5	74.6	77.8	60.2d
Rs29	2003	2.8	16.5	52.4	10	12.3	18.8ij
Rs30	2004	3.7	25.1	60	45.6	63	39.5de
Rs31	2006	7.1	9.6	68.8	27.1	64.6	35.5ef
Rs32	2006	7.2	7.6	70.4	28.3	56.6	34.1fg
Rs33	2006	3.1	12.9	51.1	12.1	26.6	21.2i
Rs34	2003	11.1	8.8	85	46.6	52	40.7fg
Rs35	2003	5.7	7.8	77	39.3	16.5	29.3h
Rs36	2003	3.9	7.2	77.5	46	53.8	37.7ef
Rs37	2006	48.6	29.3	93.3	88.5	89	69.7bc
Rs38	2006	22.1	23.5	89.1	73.6	82.3	58.1ef
Rs39	2006	1.1	8.6	24.8	66.3	69	33.9fg
Rs40	2006	9.7	19.5	61.5	86.5	88	53.0gh
Rs41	2006	0.3	11.9	48.9	17	22.6	20.1i
Rs42	2006	2.4	46.5	48.2	43.6	26.8	33.5de
Rs43	2006	4.2	7.3	76.3	24	27.8	27.9h
Rs44	2006	5	19.4	80.8	44.2	62.3	42.3fg
Rs45	2006	5.1	13.6	64.8	74.3	48.7	41.3fg
Rs46	2006	55.6	43.3	97	86.3	93.7	75.2a
Rs47	2006	4.6	5.3	41.2	33.8	58.9	28.8fg
Rs48	2006	3.3	5.9	40.9	50.5	40.2	28.1fg
Rs49	2006	2	7	43.8	29.6	17.6	20.0i
mean		13.65	20.55	64.05	48.5	54.75	

Values followed by different letters are significantly different at ($P < 0.001$) according to Newman-Keuls test.

was adjusted to 0.5×10^6 spores/ml using a hemacytometer. Tween 20 (polyoxyethylene-sorbitan monolaurate) was added as a surfactant (100 µl per liter) to the conidial suspension to facilitate dispersion of the inoculum over the leaf surface.

Pathogenicity test. Pathogenicity of the 49 isolates was conducted "under growth room conditions" using two different barley genotypes from Syria (Tadmor and Arabi Abiad), Germany (Igri), UK (Golf) and Australia (WI2291). They were chosen for their differential reactions to scald (Arabi et al. 2008). Seeds were surface-sterilized with 5% sodium hypochlorite solution for 5 min, washed three times in sterile distilled water, then planted in plastic flats ($60 \times 40 \times 8$ cm) filled with sterilized peatmoss, and arranged in a randomized complete block design with three replicates. Each experimental unit consisted of two rows of 18 seedlings per genotype. A full replicate consisted of 5 genotypes inoculated with each of the 49 isolates. Flats were placed in a growth chamber at 22 ± 1 °C (day) and 17 ± 1 °C (night) with a day length of 12 h and a relative humidity of 80~90%. Seedlings were irrigated with Knop's nutrient solution (1 g $NaNO_3$; 0.25 g KNO_3; 0.25 g $MgSO_4$ $7H_2O$; 0.25 g KH_2PO_4; and 10 mg $FeCl_3$ per 1000 ml of water). Plants were inoculated at growth stage 13 (Zadoks et al., 1974) by uniformly spraying each flat with 25 ml of conidial suspension with a hand-held spray bottle. Disease severity was assessed on the lamina of the second leaf from the base of each plant 17 days after inoculation, using the rating scale described by Salamati and Tronsmo (1997). Briefly, a 0-to-5 scale was used, where 0 = symptom free, 1 = traces or small necrotic flecks, 2 = some chlorosis or necrosis along margins, 3 = necroses but less than 40% affected tissue, 4 = necroses on 40 to 80% of the lamina, and 5 = more than 80% and up to a fully wilted leaf.

Statistical analysis. The experiment was repeated twice. The mean of disease severity was first calculated for each isolate separately, and the mean values for each group and correlations were calculated using STAT-ITCF program and Student-Newman-Keuls test (Anonymous, 1988). A separation point for resistant and susceptible classes was calculated by taking the average disease ratings of the two middle clusters and adding the standard error (SE) of the means (Eyal et al., 1985). The average disease rating of the two middle clusters was 38.6%. A standard error of the mean was calculated by taking the square root of the error mean square and dividing it by the square root of replicates (SE = error MS/r = 1.92/3 = 0.64). The final separation point was 38.6 + 0.64 = 39.24%. Genotypes rated higher than 39.24% were consider-zed susceptible and those lower than 39.24% were considered resistant. The frequency of virulence for each isolate was calculated by dividing the number of genotypes with a susceptible reaction by the total number.

Analysis of variance showed that there were highly significant differences ($P < 0.001$) among genotypes, isolates and their interaction (Table 1). WI2291 was susceptible; Arabi Abiad and Golf were moderately susceptible. Whereas, the genotypes Tadmor and Igri, were moderately resistant despite the fact that Igri possesses the BRR4 resistance gene to *R. secalis* (Bouajila et al., 2006).

The results demonstrated that the isolates varied greatly in pathogenicity. However, isolate, Rs46 (with an average of 75.20%) should be singled out as being extremely sever since unlike all other isolates, no single genotype could show any degree of tolerance against it.

Using cluster analysis, the isolates were split into six groups based on the infection response of each genotype (Table 2). Group 1 which contained 10 isolates (Rs1, 21, 27, 29, 33, 41, 11, 49, 23, 12) had the lowest virulence level (20%) associated with the lowest frequency of virulence (14%), whereas, group 6 which contained seven isolates (Rs5, 19, 6, 37, 46, 17, 28) exhibited a high virulence level (67.7%) and a low frequency of virulence (19.40%), the remaining groups were moderately susceptible to moderately resistant towards *R. secalis* (Table 2).

This study conducted under controlled conditions and at a specific seedling development stage demonstrated that none of the tested genotypes were immune to infection. Our data demonstrated that *R. secalis* is comprised of six

Table 2. Cluster analysis of mean disease rating of 49 isolates of *Rhynchosporium secalis* on 5 barley genotypes

Group	Isolates in group	Frequency of virulence (%)	Mean disease rating[a]
1	Rs1, 21, 27, 29, 33, 41, 11, 49, 23, 12	14.0%	20.0e[b]
2	Rs7, 43, 35	26.6%	31.7de
3	Rs10, 42, 15, 16, 31, 32, 30, 4, 9, 39, 36, 34, 24, 44, 47, 48, 45	47.0%	35.6d
4	Rs18, 8, 14	70.0%	41.6c
5	Rs13, 22, 38, 20, 26, 40, 25, 2, 3	62.2%	53.4b
6	Rs5, 19, 6, 37, 46, 17, 28	91.4%	67.7a

[a] Values are the average of the disease rating of five barley genotypes replicated three times, calculated on the basis of 0-5 scale, (see the text).
[b] Values followed by different letters are significantly different at ($P < 0.001$) according to the Newman-Keuls test.

distinct pathogenicity levels based on the infection response of barley genotypes. These results are in close agreement with those obtained by Tekauz (1991); Bouajila et al. (2006). The genotypes used in our study were effective for characterizing *R. secalis* pathogenicity since all gave clear response (high to low) to the pathogen isolates studied (Table 1). In our experiment, the amount of inoculum was controlled, and consistently produced sufficient numbers of well-separated lesions for assessing infection responses on barley plants. Presence of highly susceptible genotypes to this pathogen might be an indication that breeders can not rely on natural selection pressure since it is not practiced in a sufficiently uniform manner.

Our data demonstrated that there was significant variation in pathogenicity of *R. secalis* among Syrian isolates. This can be attributed to the genotype interactions, and that several genes for virulence are operating in the pathosystem (Brown et al. 1996; Goodwin et al., 1994). The observed variation in the virulence of *R. secalis* in this study is in line with the results of Forgan et al. (2007).

The results show that isolate Rs46 has the highest mean disease rating (75.20%) and a low frequency of virulence (19.40%). Therefore this isolate may be considered as a useful virulent isolate in the future plant-breeding program. Whereas, Rs12 and Rs23 isolates would be of no use in screening breeder's material due to its lack of virulence.

Characterization of different pathogenicity groups of *R. secalis* can facilitate the molecular mapping of scald and the selection of resistant lines in the field (Jørgensen and Smedegaard-Peterson, 1995; Zaffarano et al., 2006). The classification of plants into resistant and susceptible categories is a primary consideration in the selection of resistant breeding lines and in studies on host-pathogen genetics. However, if virulent pathotypes already exist, their increase will depend on their initial frequency and relative fitness. In addition, the results indicate a need to monitor the virulence situation in *R. secalis* which will facilitate studies on the inheritance of resistance to this pathogen. Moreover, knowledge of the *R. secalis* virulence spectrum may help in designing proper resistance breeding strategies.

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