

## VIRULENCE SPECTRUM TO BARLEY (*HORDEUM VULGARE*L.) IN SOME ISOLATES OF *COCHLIOBOLUS SATIVUS* FROM SYRIA

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### SUMMARY

Isolates of *Cochliobolus sativus* (Ito and Kurib) Drechsl. ex Dast.[anamorph, *Bipolaris sorokiniana* (Sacc. in Sorok.)] that cause common root rot (CRR) symptoms on barley (*Hordeum vulgare* L.) were collected in 1998 and 1999 from fields in different regions of Syria. Their virulence spectra were determined using 11 barley cultivars. Cultivars exhibited a continuous range of response from very susceptible to moderately resistant but none was immune from the disease. A cluster analysis indicated that the isolates exhibited distinct differential virulence patterns with three groups. Mean disease rating of 4.37 was the separation point between avirulent and virulent reactions. Isolate CRR16 had the highest mean virulence with lowest variance across all cultivars; and thus it was proposed to be considered as a physiological race. To incorporate adequate levels of resistance into future barley cultivars, disease evaluations should be made with *Cochliobolus sativus* isolates that express the full spectrum of virulence found in Syria.

*Key words:* barley, (*Hordeum vulgare* L.), *Cochliobolus sativus*, common root rot, virulence spectrum.

### INTRODUCTION

The main causal agent of common root rot (CRR) of barley (*Hordeum vulgare* L.) and other small grains in the semi-arid climates is *Cochliobolus sativus* (Ito and Kurib) Drechsl. ex Dast.[anamorph, *Bipolaris sorokiniana* (Sacc. in Sorok.)]. This pathogen causes small but consistent losses in yield over large areas which in total represent significant losses to the grains industry. Piening *et al.* (1976) estimated barley yield losses from CRR to be 10%, while Ledingham *et al.* (1973) reported that, CRR was responsible for an annual wheat yield reduction of 5.7% in Canada.

In Syria, Van Leur (1991) reported a 40% yield loss in barley due to infection by *C. sativus*. The importance of soil borne pathogens is expected to increase among regions as traditional cereal- fallow rotations are replaced by continuous cereals (Van Leur and Ceccarelli, 1990). The development of resistant or tolerant barley cultivars to CRR were considered to be the most economic way for controlling this disease (Sallans, 1965). Barley resistance to CRR is genotype dependent (Duczek, 1984) and also affected by soil inoculum (Duczek *et al.*, 1985). The Syrian barley landraces populations are often referred to as a source of CRR resistant genes (Van Leur *et al.*, 1989). However, before resources are committed to control CRR disease, the identification of *C. sativus* isolates expressing differential virulence on barley cultivars should be evaluated. The pathogen is a highly variable organism and there are several virulence types (Fetch and Steffenson, 1994).

Under field conditions, it is impossible to obtain error-free estimates for resistance to CRR, due to several factors such as time and intensity of infection, level of inoculum, genotype and environmental interactions (Verma *et al.*, 1976).

The purpose of this study was to investigate, under controlled conditions, the virulence spectrum of *C. sativus* isolates collected from different regions of Syria in 11 barley cultivars originated from widely dispersed areas.

### MATERIALS AND METHODS

**Isolation and maintenance of isolates.** During 1998 and 1999, more than 102 isolates of *C. sativus* were produced from barley subcrown internodes (SCI) showing CRR symptoms in different regions of Syria. The surface of SCIs were sterilized in 10% sodium hypochlorite solution (NaOCl) for 3 min, soaked three times (5 min) in sterile distilled water, and dried between filter papers. SCIs prepared in this way were transferred to Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI) and incubated for 10 days, at 22 ± 1°C in the dark to allow mycelial growth.

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During a preliminary study, 9 isolates were selected on the basis of morphological and physiological criteria (virulence) for evaluation on 11 barley cultivars. A suspension made with conidia from 12-day old *C. sativus* cultures was adjusted to about  $5 \times 10^7$  conidia per milliliter and 40 ml of spore suspension was mixed in a plastic Petri dish with 50 g sterile neutralized peat.

**Host plants.** Eleven barley cultivars used in the study (Table 1) were chosen for their differential reactions, agricultural characteristics and diverse origins. Universal susceptible controls (cv. 'Arizona' and 'WI2291', from USA) were included in the experiment.

**Table 1.** Barley cultivars used to determine differences in pathogenicity of isolates of *C. sativus* collected from different regions of Syria.

Cultivar	Row type	Origin
Thibaut	6	France
Selina	2	France
Golf	2	England
Smash	6	Belgium
Arizona	6	USA
WI 2291	2	USA
CI-5791	2	Ethiopia
Furat-2	6	Syria
<sup>a</sup> AECS 83	2	Syria
AECS 76	6	Syria
AECS 71	6	Syria

<sup>a</sup> Atomic Energy Commission of Syria.

The seeds were surface-sterilized with 5% sodium hypochlorite solution for 5 min and then washed three times in sterile distilled water. They were inoculated by mixing thoroughly with peat-gum-conidia inoculum and planted in plastic flats (60 x 40 x 8 cm) filled with sterilized peatmoss. To minimize varietal differences in length of subcrown internodes (SCIs) and to increase the severity of disease, the seeds were planted on 6 cm depth (Kokko *et al.*, 1993). The cultivars were arranged in a randomized complete block design with three replicates. Each experimental unit consisted of a row of 18 seedling per cultivar. A full replicate consisted of the plots of 11 cultivars inoculated with each of the 9 isolates; this full test was repeated three times. Flats were placed in a growth chamber at temperatures 22-1°C (day) and 17-1°C (night) with a daylength of 12 h and a relative humidity of 70-80%. Seedlings were irrigated by Knop nutrient solution (1 g NaNO<sub>3</sub>; 0.25 g KNO<sub>3</sub>; 0.25 g MgSO<sub>4</sub> 7H<sub>2</sub>O; 0.25 g KH<sub>2</sub>PO<sub>4</sub>; and 10 mg FeCl<sub>3</sub> per 1000 ml water).

**Disease rating.** Disease ratings were made 7 weeks after inoculation using a 1-5 scale (Arabi and Jawhar, 1999) according to the percentage of infected area of the subcrown internodes, where 0 (immune, no lesions); 1 = HR (highly resistant), small light brown lesions covering 1-10% of the SCI; 2 = R (resistant), light brown lesions covering 11-25% of the SCI; 3 = MS (moderately susceptible), light brown/black lesions covering 26-40% of the SCI; 4 = S (susceptible) black lesions covering 41-75% of the SCI; 5 = HS (highly susceptible) black lesions covering 76-100% of the SCI.

Results were analyzed by means of cluster analysis program from Unweighted Pair Group Means Analysis (UPGMA) (Fitch and Margoliash, 1967), using mean disease rating for each cultivar across three replicates. 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 4.33. 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 ( $SE = \text{error MS}/r = 0.11/3 = 0.037$ ). The final separation point was then  $4.33 + 0.037 = 4.37$  (Eyal *et al.*, 1985). Cultivars rated higher than 4.37 were considered susceptible and those lower than 4.37 were considered resistant. The frequency of virulence for each isolate was calculated by dividing the number of cultivars with a susceptible reaction by the total number of 11 cultivars.

The Newman-Keuls test (1988) was employed to determine significant differences among means of disease ratings.

## RESULTS

Lesions appeared on the SCIs three weeks after sowing. Therefore, the number of plants exhibiting lesions in each treatment after 7 weeks was considered as being the maximum possible response to the treatment. The disease symptoms (discoloration and necrosis of the SCIs) were always more severe in the very susceptible cultivars that were infected with virulent isolates. The isolates were clustered into three groups (Table 2). Cluster 1 was composed of one isolate CRR16. This cluster had the highest virulence ratings, with a mean disease rating 5.72 and a frequency of 100%, the highest among all clusters. Cluster 2 was composed of three virulent isolates (17, 15 and 10) with a mean disease rating of 4.75. Whereas, cluster 3 was made up of five isolates (8, 9, 11, 13, and 20) of intermediate virulence, with a mean disease rating 3.79.

**Table 2.** Cluster analysis of mean disease rating of 9 isolates of *C. sativus* on 11 barley cultivars.

Cluster	Isolates in cluster	Frequency of isolates (%)	Mean disease rating <sup>a</sup>
1	CRR16	100	5.72a <sup>b</sup>
2	CRR15, CRR17, CRR10	58	4.75b
3	CRR8, CRR9, CRR11, CRR13, CRR20	32	3.79c

<sup>a</sup> Values are the average of the disease rating of 11 barley cultivars replicated three times, calculated on the basis of 1-6 scale, (see the text).

<sup>b</sup> Means followed by the same letter are not significantly different ( $P > 0.001$ ) according to Newman-Keuls test.

Table 3 shows the mean disease rating and variance of each isolate when tested on the 11 barley cultivars, with the variance ranging between 0.08 and 2.34.

**Table 3.** Mean disease rating and variance of 9 isolates of *C. sativus* tested on 11 barley cultivars.

Isolate	Variance <sup>b</sup>	Mean disease rating <sup>a</sup>
CRR16	0.08	5.72a
CRR15	1.08	4.94b
CRR17	2.27	4.76c
CRR10	1.78	4.55d
CRR9	2.34	4.12e
CRR13	1.88	4.00e
CRR20	1.76	3.94e
CRR11	1.69	3.67f
CRR8	2.03	3.30g

<sup>a</sup> Values are the averages of the disease rating of 11 barley cultivars replicated three times, calculated on the basis of 1-6 scale (see the text).

<sup>b</sup> Infection type of each cultivar replicated three times ( $n = 33$ ).

Analysis of variance showed that there were highly significant differences ( $P < 0.001$ ) among cultivars, isolates and their interaction. Genotypes infected with *C. sativus* isolates were classified according to the resistance groups defined above (Table 4). 'WI2291', 'Arizona', 'AECS 83' were highly susceptible; 'Selina' and 'CI-5791' were susceptible; 'Smash', 'Thibaut' and 'AECS 76' were moderately susceptible. Whereas, 'AECS 71', 'Golf' and 'Furat-2' were resistant. These cultivars had a significantly lower percentage of SCIs infected with the disease than did the other cultivars. However, within group, analysis of variance showed that there were significant differences among certain

cultivars. The mean disease ratings for cultivars ranged between 5.85 and 2.63 (Table 4). The isolates are listed decreasing pathogenicity order according to the means for all cultivars.

The results demonstrated that the isolates varied greatly in virulence. However, isolate CRR16 should be singled out of this group as being extremely severe since unlike all other isolates, no single variety could show any degree of tolerance against it. With an average of 5.7 and no significant differences among the cultivars in their response, CRR16 showed highest virulence level.

The degree of virulence for isolates CRR15, 17, 10, 9, 20 and 13 ranged between 4.94 and 3.94 and thus are classified as virulent causing severe discoloration (Table 4). However, significant differences were found among them and also among the varieties response to each of them. Isolates CRR11 and 8 expressed moderate virulence but expressed high virulence on the very susceptible cultivars (Table 4).

The virulence frequency of the isolates studied is shown in Table 2. The isolate CRR16 had the highest percentage of virulence frequency (100%) and a mean disease rating of 5.72, whereas, the isolates CRR8, 9, 11, 13 and 20 had the lowest percentage of virulence frequency (32%) and a mean disease rating of 3.79.

## DISCUSSION

This study conducted under controlled conditions demonstrated that none of the tested cultivars was immune from disease. However, certain cultivars (*i.e.* 'AECS 71', 'Golf' and 'Furat-2') showed a level of resistance higher than the reference cultivar 'AECS 76' which is generally considered to be moderately resistant (Arabi and Jawhar, 1999). This confirms the importance of the Middle-East as a source of resistant genotypes to CRR. On the other hand, American cultivars ('Arizona' and 'WI2291') were found to display the highest susceptibility ratings to the pathogen with a mean of 5.67 and 5.85 respectively. These results are in agreement with those of Van Leur *et al.*, 1991.

The variation in the genotypes reaction to *C. sativus* suggests that partial resistance to the disease is most likely controlled by several minor genes, making resistance more difficult to breakdown when compared with single genes of major effect. However, the presence of highly susceptible cultivars to this pathogen might be an indication that breeders can not rely on natural selection pressure since it is not practiced in sufficiently uniform manner (Bailey *et al.*, 1988). Duczek *et al.* (1985) demonstrated that relatively low inoculum densities can maximize disease symptoms. In our experi-

**Table 4.** Mean disease rating<sup>a</sup> of 9 isolates of *Cochliobolus sativus* on 11 barley cultivars used as differentials.

Cultivar	Isolate									Mean
	CRR16	CRR15	CRR17	CRR10	CRR9	CRR13	CRR20	CRR11	CRR8	
WI2291	A	A	A	A	A	A	B	A	A	A
	6.00a <sup>b</sup>	6.00a	6.00a	6.00a	6.00a	6.00a	5.33a	6.00a	5.33a	5.85
Arizona	A	A	A	A	A	BC	A	B	A	B
	6.00a	6.00a	6.00a	6.00a	6.00a	4.67c	6.00a	5.00bc	5.33ab	5.67
AECS 83	A	A	A	A	A	AB	B	BC	A	B
	6.00a	6.00a	6.0a	5.67a	6.00a	5.33a	5.00a	4.67a	5.00a	5.52
Selina	A	A	AB	A	B	AB	D	E	D	C
	6.00a	5.67a	5.33a	5.67a	5.0a	5.33a	3.67b	3.33b	2.00c	4.67
CI-5791	A	B	B	A	B	BC	C	D	B	C
	6.00a	5.00b	5.00b	5.00b	4.67b	5.00b	4.33bc	4.00c	4.00c	4.78
Smash	A	A	A	A	D	D	B	E	D	D
	5.67a	5.00ab	5.67a	4.00bc	3.67c	4.00bc	4.00bc	4.33bc	3.67c	4.44
Thibaut	A	A	A	A	D	D	B	E	D	D
	6.00a	5.67a	6.00a	5.33ab	3.00c	3.00c	5.00b	3.00c	2.00d	4.33
AECS 76	A	C	C	B	C	C	E	F	CD	E
	5.67a	4.00b	4.00b	4.00b	4.00b	4.00b	3.00c	2.00d	3.00c	3.74
Furat 2	A	C	C	D	D	2E	E	E	D	F
	5.33a	4.00b	4.00b	2.33d	3.00c	2.00d	3.00c	3.00c	2.00d	3.19
Golf	A	C	D	BC	E	2E	F	E	D	G
	5.00a	4.00b	2.33d	3.33c	2.00d	2.00d	2.00d	3.00c	2.00d	2.85
AECS 71	A	D	D	CD	E	DE	F	F	D	H
	5.33a	3.00b	2.00c	2.67bc	2.00c	2.67bc	2.00c	2.00c	2.00c	2.63
Mean	5.73A	4.94B	4.76C	4.55D	4.12E	4.00E	3.94E	3.67F	3.30G	

<sup>a</sup> Values are the means of 9 isolates based on a 1-6 scale; see the text.

<sup>b</sup> Means followed by the same small letter (line) and preceded by the same capital letter (column) are not significantly different ( $P > 0.001$ ) according Newman-Keuls test.

ments the level of inoculum was controlled, and so precise assessment could be made on barley cultivars reaction to CRR. Moreover, Huang and Tinline (1976) observed no differences in the infection processes between resistant and susceptible cultivars.

Race non-specific or partial resistance is frequently considered to be characterized by 'slower development of fewer, smaller less prolific lesion' compared with a susceptible cultivar, and can be divided into two components: (i) size of lesions and (ii) duration of lesion development. These components may be present throughout the plant life, or only at particular growth stages. The finding that size of lesion was a component of resistance, at least at the seedling stage, supports the results of other workers (Hetzler *et al.*, 1991).

Our data demonstrated that there was great variation in the pathogenicity of *C. sativus*. This can be attributed to the cultivars interactions and it is assumed that several genes for virulence are operating in the pathosystem (Hetzler *et al.*, 1991). The specificity of pathosystems and the different types of resistance have been defined by Van derPlank (1984).

Considering the genetic variability of the *C. sativus*, different levels of virulence were observed in our study, as has been reported by Fetch and Steffenson (1994) and by Hetzler *et al.* (1991). The presence of moderate or weak pathogenic isolates could conceivably interfere

with identification of lines that are highly resistant or immune to *C. sativus* and even slightly susceptible to other organisms that produce similar symptoms (Sallans and Tinline, 1965). The results show that the isolate CRR16 has the highest mean disease rating 5.7 and a frequency of 100% with a low variance 0.08. Therefore this isolate may be considered as a physiological race.

Plating subcrown internodes of cultivars used in this study revealed that 'Golf', 'AECS 71' and 'Furat-2' had significantly lower percentage of SCIs that were infected by *C. sativus* than the other cultivars. The amount of infection of the SCIs by *C. sativus* might serve as a useful criterion for final selection of resistant lines from crosses involving 'Golf', 'AECS 71' and 'Furat-2'. This could be extremely useful in cases where resistance level from these cultivars is being combined with other sources of resistance. In addition, the results indicate a need to monitor the virulence situation in *C. sativus*, which will facilitate studies on the inheritance of resistance to CRR. Moreover, knowledge of *C. sativus* virulence spectrum may aid in designing proper breeding strategies.

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