Occurrence of Rot Rot in Barley in an Experimental site in Northwest Syria and Varietal Differences in Resistance of *Cochliobolus sativus*

J.A.G. van Leur[1], W.E. Grey[2], Liang Qu[3] and M.Z. Alamdar[1]

(1) International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

(2) Montana State University, Bozeman, USA.

(3) Institute for Application of Atomic Energy (CAAS), Beijing, P.R. China

Abstract

Van Leur, J.A.G; W.E. Grey; Liang Qu and M.Z. Alamdar. Occurence of root rot on barley in an experimental site in northwest Syria and varietal differences in resistance to *Cochliobolus sativus*. Arab. J. Pl. Prot. 9(2):129 - 133 .

A total of 45 barley lines were evaluated for root rot symptoms under natural infection conditions in Northwest Syria. Significant differences were found among varieties in the discoloration of subcrown internodes. The symptoms were found to be associated with *Cochliobolus sativus* and, to a lesser extent, with *Fusarium oxysporum* and *F. sambucinum*. Seedling tests confirmed the pathogenicity of the *C. sativus* isolates, but differences in disease rating among cultivars in the seedling test were not similar to those of adult plants in the field.

Key words: Root rot, Barley, *Fusarium* spp., *Cochliobolus sativus*.

Introduction

Root rot of barley is caused by one or more fungi, depending on the location *Cochliobolus sativus* and *Fusarium spp.* are commonly encountered pathogens. Early infection may cause seedling death, but the disease rarely cause death to the adult plant. Head sterility may be associated with *Fusarium culmorum,* but symptoms of root rot are often less obvious, and restricted to below-ground plant parts (14). Whether infection of the roots results in yield loss depends largely on environmental factors and the plant's ability to compensate for the damage.

Barley is a major crop in the Syrian Arab Republic with 1.5 - 2 million hectares seeded annually, almost exclusively under rainfed conditions. Inputs in barley cultivation in Syria are low and the long-term average yields do not exceed 1 t/ha (1). The growing demand for barley can only be met by intensifying production as further expansion of the cultivation into marginal lands may lead to environmental degradation. Changing farming systems, in particular the replacement of the traditional barley-fallow rotation and the replacement of traditional cultivars, may have a large influence on plant diseases (16). Conflicting reports exist on the effect of cereal monoculture on the development of root rot. Wildermuth (17) found that root rot was promoted by wheat monoculture. However, Peining *et al.* (7) (1969) found fallow to increase root rot, while fertilizer application restricted its development. Deep seeding, recommended for low rainfall conditions, increases root rot development (7, 3, 9).

Little work has been reported on root rot in Syria. *Fusarium spp.* were found to be associated with root rot symptoms on wheat (5), but there are no reports of root rot of barley. Varietal differences in resistance or crop losses due to common root rot have not been reported on wheat or barley in West Asia.

The most economic way of controlling root rot either in traditional or in improved farming systems is by disease resistance (10). It is therefore important to investigate the level of root rot resistance of barley cultivars presently used in West Asia, as well as that of breeders' material developed for improved dryland farming systems. This paper reports the first results on pathogen identification of common root rot on barley in Syria, as well as an investigation on differences in disease resistance among barley varieties.

Material and methods

Field survey. The field survey was carried out during the 1987 - 88 season in a yield testing site used by the Barley Improvement Project of the International Center for Agricultural Research in the Dry Areas (ICARDA). The site Breda is located in the drier zone (long-term annual rainfall 269 mm) of Northwest Syria and previous sampling had shown a relative high occurrence of root rot. Samples were taken out of two yield trials planted in a lattice design with two replications. Each trial consisted of 25 entries of which 20 were lines under test and five were check varieties, common to both trials. Out of the five checks, two were local cultivars and three were improved lines. Tested material was of diverse origins, partly advanced breeding lines, partly pure-line selections from Syrian and Jordanian landraces. None of the material had been previously tested for resistance to root rot and was solely chosen for agronomic performance in different environments. Each plot consisted of six rows of 5m long, seeded by an Oyjord experimental planter with a eow distance of 0.3m and a seed rate of

120 kg/ha. The land on which the experiments were grown was kept fallow during the 1986 - 87 season, and received 20kg N and 40 kg P_2O_5 before seeding in November 1987. Samples were taken in early May 1988 when plants were in the soft dough stage. The border rows in each plot were uprooted and a minimum of 50 plants with a subcrown internode (SCI) of at least 1 cm were evaluated. The SCIs were cut off, taken to the laboratory, and scored on the extent of their discoloration according to the scale used by Grey and Mathre (4), «Clean» = no discoloration; «Slight» = pinpoint lesions, less than 20% of the SCI discolored; «Moderate» = extended linear lesions, 20% - 50% discolored; «Severe» = more than 50% discolored. The plot mean of the percentage discolored area was calculated using the midpoint of each scoring class (10%, 35% and 75% for «Slight», «Moderate», and «Severe» respectively) and used in statistical analysis.

Isolation from subcrown internodes. Identification of pathogens from plant tissue was carried out in the Plant Pathology Department of Montana State University, Bozeman, USA. SCIs were surface sterilized in 10% Chlorox (equals 0.5% Sodium Hypochlorite) for 3 min. A minimum of 16 SCIs representing individual plants in each of the four disease reaction classes were plated on each of three media. The plates were incubated for 10 days at room temperature (22°C) and the fungal colonies were identified.

The three media included a semiselective medium for *Cochliobolus sativus* (12), a semiselective medium for *Fusarium* spp. (6), and a general growth medium. The semiselective media were composed of 17 g/l Czapek-Dox Agar, 1 g/l yeast extract, and 0.1 g/l of the antibiotic Kanamycin sulfate. After autoclaving and cooling, 0.002 g Benomyl (active ingredient in the fungicide Benlate) and 0.1 g of the antibiotic Streptomycin in 5 ml 70% ethanol were added for 1 l of the selective *Cochliobolus* agar (SCA). For the semi selective *Fusarium* agar (SFA), 0.003g 2,6-dichloro-4-nitroaniline (active ingredient in the fungicide «Botran») and 0.1 g Streptomycin in 5 ml 70% ethanol were added to the cooled medium. The general growth medium consisted of 39 g/l potato dextrose agar (PDA) (Difco) with Kanamycin sulfate, which was acidified with 25% lactic acid to a final pH of 5.8 and with Strptomycin added to the cooled medium.

Pathogenicity test of *C. sativus* isolates. A mixture of five cultures of *C. sativus,* originating from SCIs with a disease rating of «Severe», were used as inoculum for the pathogenicity tests. Cultures were grown for two weeks at room temperature on standard PDA media, to which 0.1 g/l of the antibiotic Kanamycin sulfate was added before autoclaving. Spores were harvested by adding water with 0.005% Tween 20 to the plates and scraping the cultures with a microscope slide.

Eight varieties were selected for seedling tests, six of which showed a contrasting performance in the field survey in Breda, while two lines, not present in the field tests, were selected from preliminary seedling tests. Seed was placed in a small vessel filled with a suspension of 10^5 spores/ml. The vessels were placed under vacuum for 2 hr to enhance the chance of spores entering beneath the seedhull, after which the seeds were germinated on filter paper moistened with the spore suspension for 42 hr at 20°C. Plastic cylinders (3 cm diameter, 12 cm long) were filled with a soil-sand mixture which was sterilized by dry heat, two weeks before the start of the experiment. Two germinated seed were placed on the top of a cylinder, after which a 5 cm long cylinder with a slightly smaller diameter was pressed 1 cm deep in the soil and filled up with the soil-sand mixture. This method ensured an equal planting depth and facilitated note taking.

The seedling test was repeated twice. Four trays with 64 cylinders each were used per test. Out of these 64, 48 (6 for each variety) were planted with inoculated seed and 16 (2 for each variety) were planted with noninoculated seed which was germinated on filter paper moistened with distilled water. The soil was watered to full capacity and the trays placed in a Conviron E-7 growth chamber with 14 hr light per day. Temperature was set at 20°C during light and 16°C during dark. Discoloration of the SCIs was scored after emergence of the third leaf, using the same scale as for the field evaluation. As with the field test, a percentage was given to each scoring class and the tray mean of the six cylinders planted with inoculated seed was used for the statistical analysis.

Results

Differences among varieties in root rot symptoms in the field. A significant difference among varieties existed in both yield trials ($p < 0.001$ in Trial 1 and $p = 0.026$ in Trial 2). Variety averages of percentage discolored SCI was 29.3 in the first trial and 28.2 in the second. A combined analysis over the checks was performed as suggested by Singh (11). This analysis showed no difference in variance between both experiments, while overall means over the five check varieties was 31.0 for the first trial and 28.8 for the second. A standard error of 4.1 for the corrected entry means was calculated using the pooled error term. Table 1 shows the performance of the 45 entries (two trials of 20 entries and 5 common checks). The evaluated lines could be divided into 10 pure-line selections of barley landraces from Syria and Jordan and 3% breeding lines. Of the breeding lines 10 were derived from crosses with the landrace Arabi abiad. The number in each category was too small to perform a statistical test, but it can be noted that none of the landrace lines had a severity of above 40%.

Fungi associated with subcrown internode discoloration. *C. sativus* was more frequently isolated from affected SCIs than *Fusarium spp.* on both the general growth medium and on the SCA. SCIs rated as «Severe» yielded a high frequency of *C. sativus,* even on the SFA (Table 2). SCIs rated as «Clean» were not completely free of either of the two pathogens, indicating that the fungi may be present in or on the tissue even in the absence of disease lesions. *Fusarium oxysporum* and *F. sambucinum* were isolated,

Table 1. Subcrown internode discoloration of 45 barley lines grown during 1987 - 88 in Breda, Syria.

Percentage* discoloration	Breeding lines	Crosses with Arabi abiad	Landrace lines	Total
10	0	0	0	0
20	4	2	1	7
30	9	4	6	19
40	9	3	3	15
50	2	1	0	3
> 50	1	0	0	1
Total	25	10	10	45

* Categories are indicated by the upper limit. Number of lines in each category is given.

especially on the Selective Fusarium Agar (SFA). However, a relatively low frequency of *Fusarium spp.* were isolated from severely affected tissue, and the fungus seems to be more associated with clean and slightly affected plant tissue. Based upon recovery of fungi on the three semiselective media, it can therefore be concluded that *C. sativus* was the primary pathogen responsible for discoloration of the subcrown internode tissue of barley plants in this trial.

Seedling tests. *C. sativus* isolates from Breda were capable of producing root rot symptoms on barley seedlings, while plants from noninoculated seed were all clean. Table 3 lists the results of the two different seedling tests. A reasonable correlation existed between the results of the first test and the rating of adult plants in the field in Breda. However, results were different in the second seedling test. Combined analysis over both experiments, showed significant differences between experiments ($F = 9.9$, $p = 0.02$) and among varieties ($F = 18.7$, $p < 0.001$). However the significant interaction between varieties and experiments ($F = 7.6$, $p < 0.001$) shows the poor repeatability of these seedling tests, eventhough the experiments were conducted under controlled environment conditions.

Table 2. Frequency of *Cochliobolus sativus* (Cs) and *Fusarium spp.* (Fs) isolated on three different media from subcrown internode tissue of barley grown at Breda, Syria and classified into four disease categories.

	SCA				PDA				SFA			
	Cs	Fs	Mx	Tot*	Cs	Fs	Mx	Tot	Cs	Fs	Mx	Tot
Clean	19	0	19	38	24	12	24	60	6	47	29	82
Slight	69	0	19	88	39	22	17	78	6	41	24	71
Moderate	58	0	42	100	11	17	61	89	18	29	29	76
Severe	58	0	37	95	37	5	32	74	30	25	90	90

* «Mx» indicates mixed infections of *C. sativus* with *F. oxysporum* and/or *F. sambucinum*. «Tot» indicates the percentage of subcrown internodes yielding any of the pathogens. SCA: Semiselective media for *Cochliobolus sativus* PDA: General growth media. SFA: Semiselective media for *Fusarium* spp. (media composition described in the test).

Table 3. Mean percentage for root rot infection of subcrown internode (percentage discolouration) in uninoculated field plots and in inoculated growth chamber experiments.

Variety	Field	Growth chamber		
		Exp 1	Exp 2	mean
Tadmor	-	4	2	3 a*
Arizona 5908/Aths/ Lignee 640	14	13	14	13 b
Arabi aswad	30	21	8	14 b
SLB 39 - 60	12	19	29	24 c
Sawsan/ Lignee 527/Arar	52	47	9	28 c
ER/Apm/ Belle| Maf 102	15	31	26	28 c
Kv/Mazurka	-	57	19	38 d
JLB 06 - 38	37	48	44	46 d
Average		30	19	24

*) LSD between varieties means = 9.2, different letters indicate differences larger than the LSD value.

The poor correlation between the performance of varieties in the seedling test and in the field supports the conclusions of Stack (13) that seedling tests are not suitable to detect varietal differences in resistance to *C. sativus*.

Discussion

Conclusions from this study are only valid for a specific site during a specific year. However, the high occurrence of root rot symptoms was present during a season with a relatively high rainfall (415 mm vs long-term annual rainfall of 269 mm). Drought is believed to increase the severity of

root rot (8) and disease pressure may be higher in years with average rainfall. Piening *et al.* (8) calculated yield loss coefficients for the disease classes used here, based on a three-year survey in the prairie provinces of Canada. Using their coefficients, yield losses due to root rot in this experiment would range between 8% for the least affected and 23% for the most affected entry. However, extensive crop loss studies in the target environment should be conducted before further conclusions are drawn.

C. sativus was ranked as the most important root rot pathogen in this study, while *Fusarium spp.* were found to be more associated with symptomless root tissue. *Fusarium spp.* are known to invade host tissue previously infected with *C. sativus,* while the reverse rarely occurs (14). However, the relative importance of *Fusarium spp.* could be different in other years, in other locations, or in a different rotation scheme. *Fusarium oxysporum,* one of the *Fusarium spp.* isolated from barley plants in this study, is reported to be a pathogen on legume plants (6). No tests with these isolates were conducted on legumes used in rotation with barley.

In general, landrace lines showed a lower level of disease than breeding lines and the landrace line «Tadmor» was the most resistant entry in the seedling tests. Differences among and within barley landraces from Syria and Jordan in resistance to leaf diseases were previously reported (15) and may be present for root rot as well. Large differences were present among the evaluated breeding lines. The multi-location yield testing over several years (used by ICARDA's Barley Improvement Project to evaluate its breeding material for yield and other characteristics) will expose material to root rot. However, the presence of highly susceptible lines in the material evaluated might be an indication that breeders cannot rely on natural selection pressure, as it is not sufficiently uniform (2). Alternatively it might indicate that root rot has little effect on yield. If crop loss studies show that this disease is a yield limiting factor in dryland barley cultivation in West Asia, a varietal testing program based on artificial inoculation will need to be established.

Acknowledgment

Part of the work was supported by an USAID grant for research on barley diseases and related breeding strategies.

الملخص

فان لور. يوب ووليام جراي، وليانغ كو، وزياد عملندار. 1991 . حول ظهور تعفّن الجذور على الشعير في أحد المواقع التجريبية في شمال سورية والاختلافات الصنفية في المقاومة لقطر *Cochliobolus sativus* (Ito Kurib.) Drechsl ex Dastar) مجلة وقاية النبات العربية 9 (2) : 129 - 133 .

تم تحت ظروف العدوى الطبيعية في شمال سورية، تقويم ما مجموعة 45 سلالة من الشعير لأعراض تعفّن الجذور. وقد وجدت فروقات معنوية بين الأصناف في مدى تلوّن السلاميات تحت التاجية. ووجد أن تلك الأعراض مترافقة مع الفطر *C. sativus* بشكل أساس، ومع الفطر *Fusarium oxysporum* و *F. sambucinum* بشكل أقل. وقد أكدت اختبارات البادرة القدرة الإمراضية لعزلات *C. sativus* على أن الفروقات في درجات المرض بين الأصناف في اختبارات البادرة لم تكن مماثلة لتلك الخاصة بالنباتات البالغة في الحقل.

كلمات مفتاحية: تعفّن الجذور، الشعير، *Cochliobolus sativus, Fusarium spp.*

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