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Article *in* Archives of Phytopathology and Plant Protection · April 2010

DOI: 10.1080/03235400801972293

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Screening of certain barley lines for resistance to root rot disease caused by *Fusarium graminearum*

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(*Received 10 January 2008; final version received 24 January 2008*)

This investigation was conducted in 2005/2006 and 2006/2007 to test 235 barley lines plus two varieties Giza 127 and Giza 128 for resistance and susceptibility to *Fusarium graminearum*. All screened barley lines showed varied significant degrees of infestation to root rot pathogen. A screening system is described for identifying barley lines which are effective in controlling resistant or susceptible lines. By detecting small but consistent differences in root rot severity, the bioassay proved effective in large-scale screening for partial resistance: already 335 barley lines and two varieties have been screened. We found five groups (7.12%), 22 barley lines and both varieties are resistant (R) (8.31%); 28 barley lines are moderately resistant (MR) (19.29%); 65 barley lines are moderately susceptible (MS) (27.89%); 94 barley lines are susceptible (S) and (37.39%) 126 barley lines are highly susceptible (HS). The high degree of precision makes this an invaluable tool in the understanding of pathogen aggressiveness, host specialisation and parasitic fitness. Disease scale was strongly negative and had moderate correlation with germination (−0.309** and −0.649**) under normal and disease treatment. The correlation between yield and normal and disease treatment during two seasons was strong and negative (−0.834** and −0.847**, respectively were detected).

Keywords: barley; plant breeding; root rot; disease resistance; *F. graminearum*

Introduction

Barley is one of the most important feeding crops in Egypt and many other countries in the world. It is subjected to relatively large numbers of disease during its growing season which attack all the plant parts causing serious losses in crop productivity (Mielke 1988). Among such diseases is root rot which attacks both seedlings and adult plants. It is a widespread and destructive root disease of wheat, barley, oats, and also rye and many other cereal grasses (Allam 1994; Mohamed 1996; Fernandez et al. 2007).

The disease is primarily caused by *Drechslera sorokiniana* (Sacc.) (Syn. *Helminthosporium sativum* P.K.& B.) and certain species of *Fusarium*. Other soil-borne fungi were also isolated from the infected plants showing disease symptoms

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ISSN 0323-5408 print/ISSN 1477-2906 online

DOI: 10.1080/03235400801972293
http://www.informaworld.com

(Kotlyarov and Mokhova 1990; Frissullo and Rossi 1991; Mohamed 1996). Such diseases cause considerable losses to barley yield by decreasing tiller number, kernel number per head and kernel weight (Ludwig et al. 1956). Many investigators have studied the reactions of barley cultivars to root rot disease and concluded that barley lines varied in reaction to the disease. Such variation in susceptibility among cultivars may be due to the physiological and anatomical characters of the host (Adhikary and Khan 1986; El-Meleigi 1988; Allam 1994).

In this study, breeding works could be effective by finding the best lines which are superior due to having a high yield potential with a high degree of resistance to the above-mentioned disease. Thus, the aim of the present work was to screen the available barley lines for resistance against the root rot pathogen (*F. graminearum*) under artificial infection conditions, and to determine whether strains of the *F. graminearum* complex obtained from barley seeds are pathogenic to barley and to determine their genetic lineage.

Materials and methods

Isolation and identification of the causal pathogen

Naturally diseased roots of barley showing root rot symptoms were collected from different localities of Assiut and Sohag Governorates. They were washed thoroughly with tap water and small portions of diseased tissues (2–3 cm) were surface sterilised with 3% sodium hypochlorite NaOCl for 2 min, then washed several times in sterilised water, and plated on potato dextrose agar medium (PDA) and incubated at 25°C for 7–15 days. Pure cultures of the developing fungi were obtained using single spore or hyphal tip techniques and kept in the refrigerator for further studies.

Plant material

The present study was carried out during the two successive seasons of 2005/2006 and 2006/2007 and at two locations; the first location was at the Greenhouse of Faculty of Agriculture, Assiut University, Assiut, Egypt, while the second location was at the Greenhouse of Faculty of Agriculture, Sohag University, Sohag, Egypt. The spring barley variety Scarlett (German variety) was crossed with a wild barley accession ISR42-8 (Middle East variety). The resulting F1 population was backcrossed twice (BC2) with Scarlett. The BC2 population was finally subjected to double haploid production (335 BC2DH lines). These BC2DH lines were examined for their tolerance in relation to disease. Three hundred and thirty five plus two local barley (check Giza 127 and Giza 128) lines were grown in an experiment with three replicates distributed in a randomised complete block design. Five barley seeds were sown in a 30 cm diametre and 15 cm surface, in plastic pots containing a mixture of clay/sand (3:1 v/v), with four holes pierced at the bottom for drainage, and germinated in a greenhouse set at greenhouse temperature. High-pressure sodium lamps supplemented natural sunlight with a 14-h photoperiod. Humidity was uncontrolled.

Pathogenicity tests

Pathogenicity tests of five isolates of fungal species were determined on barley cultivar under greenhouse conditions. Inoculum for each of the tested isolates was

prepared by growing the fungus in 500 ml glass bottles containing barley grains medium (100 g barley grains + 50 ml water) at 25°C for 21 days. Inoculum for each isolate was mixed thoroughly with steam sterilised clay/sand soil at the rate of 3% soil weight, and then placed in sterilised pots (30 cm diameter). Five surface disinfested grains of barley cultivar were sown in each pot. Grain disinfestations were done by dipping in 1% sodium hypochlorite for 2 min. Non-infested grains mixed with 3% sterile barley grains were used as control. Four replicates were used for each particular treatment.

Disease severity rating

After three months from sowing, plants were removed from the soil and washed thoroughly to remove soil debris and scored for root discolouration according to Allam (1994) as follows:

1 = roots without discolouration; 2 = 0–5%; 3 = 5–15%; 4 = 15–30%; 5 = 30–50%; 6 = 50–70%; 7 = 70–85%; 8 = 85–95%; 9 = 95–100% discoloured root mass.

A mean disease rating (MDR) for each replicate was calculated by multiplying the number of plants in each category by their numerical rating, adding the ratings and dividing by the total number of plants rated according to the following formula:

$$(\mathrm{MDR}) = (n \times \mathrm{I}) + (n \times 2) + \ldots\ldots (n \times 9)/n$$

where n = the total number of plants

Reaction of barley cultivars to root rot disease caused by each of F. graminearum

Three hundred and thirty five lines plus two local barley species (cv. Giza 127 and Giza 128) were tested for their reaction to root rot disease caused by *F. graminearum*. Sterilised pots (30 cm in diameter) were filled with autoclaved soils (clay) mixed with inoculum (3%). Inocula were prepared as described before. Each pot was seeded with five seeds for each cultivar. Control treatment was sown with barley cultivars Giza 127 in autoclaved tested soils mixed with disinfested barley without fungal inoculation. Pots were placed in a greenhouse in a randomised split plot design with four replications at 25 ± 5°C, and watered as needed. After three months root rot disease severity was measured for each treatment. The reaction of barley accessions with the root rot pathogen *F. graminearum* was determined according to the scale presented in Table 2. The data of season 2005/2006–2006/2007 was subjected to statistical analysis performed by the SAS software (SAS Institute 1999). The data obtained were subjected to the statistical analysis, described by Snedecor and Cochran (1969).

Results

Isolation, identification and pathogenicity of the causal pathogen

Five isolates were obtained from naturally diseased wheat roots showing root rot symptoms collected from different localities of Assiut and Sohag Governorates. They

were identified as *Fusarium moniliforme*, Shelden, *F. graminearum* Schwabe, *F. solani* (Mart), *F. oxysporum* Shelocht and *Rhizoctonia solani* Kuhn according to their morphological characteristics of mycelial and spores. Isolated fungi were tested for their pathogenicity on barley cultivar cv. Giza 127 under greenhouse conditions. Results of this study, presented in Figure 1, indicate that all tested fungi were able to infect barley plants causing root rot symptoms and reduced the surviving plants compared with non infested soil (control). They varied in their virulence. In general, *F. graminearum* caused the highest root rot severity (7.02) followed by *F. moniliforme* (6.4), whereas *F. solani* and *Rhizoctonia solani* caused the lowest (4.0 and 3.02 respectively). Accordingly, we used this result for *F. graminearum* in all the following experiments. The results obtained are in agreement with those obtained by Allam (1994), Kovalenko et al. (2002) and Surin et al. (2002) who found that the isolates from wheat and barley varied in their pathogenicity tests against wheat root rot.

Identification of the isolated fungi was carried out on 2–3 week old cultures using the morphological and microscopic characteristics of mycelium and spores according to Booth (1977) and Domsch et al. (1980).

The analysis of variance

The analysis of variance of four traits was studied and the combined analysis of variance between locations (L), disease treatment (T) and genotypes (G) were highly significant for four traits. The interactions for (L*T), (L*G), (T*G) and (L*T*G) gave highly significant differences for biomass yield and germination, while no significant differences were observed for disease severity. Results showed that the analysis of variance between replications and location R (L) were not significant for all traits (Table 1).

Three hundred and thirty five lines plus two local barleys (cv. Giza 127 and Giza 128) were screened for root rot resistance activity that had advanced to various

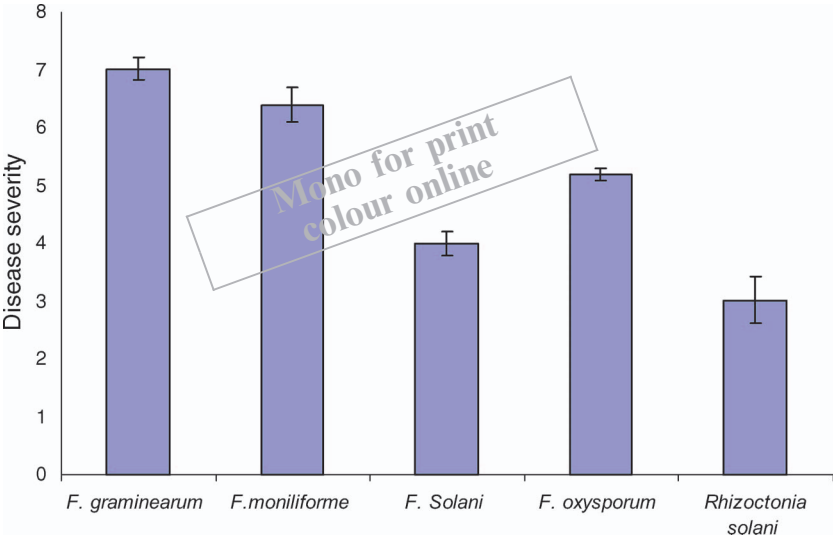

Figure 1. Pathogenicity tests of some isolates on barley cultivar cv Giza 127. Bars indicate the standard error.

stages in the screening system and were also included in a pot experiment. (7.12%) 24 barley lines were resistant (R) and two varieties, the numbers of resistance were Giza 127, Giza 128, 42, 184, 191, 181, 66, 86, 185, 178, 236, 30, 43, 41, 40, 68, 130, 299, 76, 48, 237, 62, 226 and 305; (8.31%) 28 barley lines were moderately resistant (MR), numbers 21, 28, 44, 45, 89, 140, 224, 249, 2, 29, 31, 35, 61, 64, 207, 219, 312, 9, 32, 129, 3, 12, 26, 34, 95, 180, 58 and 136, while (19.29%) 65 barley lines were moderately susceptible (MS); on other hand (27.89%) 94 barley lines were susceptible (S) and (37.39%) 126 barley lines were highly susceptible (HS) (Table 2). The reason for research is due to the impact of common root rot which has been estimated to reduce barley yields by an average of 10% on a yearly basis. Lines with resistance to common root rot and avoidance of extremely susceptible lines by breeding programs and producers would increase crop productivity. It is worth mentioning that some barley lines have their resistance characteristic classes as moderately resistant or resistant during two successive seasons. Such lines may be helpful for breeding programs due to their resistant or moderately resistant stability as well as their seed yield stability. This finding was in harmony with the results obtained by Gupta (1995), Munoz Valenzuela et al. (1996) and Abdul Wahid and El-Bramawy (2007).

The yield, biomass and germination mean for all lines were (4.0, 13.1 and 85.14%) in the Assiut location and control treatment (L1T1), which decreased to (0.67, 5.38 and 66.96%) in the Assiut location and disease treatment (L1T2); on

Table 1. Analysis of variance of traits for 337 genotypes for disease tolerance grown under disease treatments over two seasons.

		Mean Square			
SOV	D.F.	Biomass	Yield	Germination	Disease severity
L	1	682.98**	27.38**	100451.04**	37.72**
R (L)	4	0.021	0.18	0.00098	0.459
T	1	65990.31**	12311.11**	796520.77**	3788.83**
L*T	1	127.53**	24.916**	5602.106**	0.0617
G	336	56.10**	6.89**	5602.106**	6.60**
L*G	336	0.122**	0.147**	78.436**	0.00125
T*G	336	76.27**	6.784**	1901.88**	1.86**
L*T*G	336	0.166**	0.149**	77.289**	0.0012
Error	2688	0.0083	0.109	0.072	0.419

R = Replication, T = Disease treatments, L = Location, G = Barley lines.

Table 2. The scale of evaluation used through the study.

Score	Disease severity	Number of lines	Category
1	7.12%	24	Resistant (R)
2	8.31%	28	Moderately resistant (MR)
3	19.29%	65	Moderately susceptible (MS)
4	27.89%	94	Susceptible (S)
5	37.39%	126	Highly susceptible (HS)

other hand the yield, biomass and germination mean were (4.33, 14.28 and 85.07) in the Sohag location and control treatment (L2T1), that decreased to (0.68, 5.84 and 47.1%) in the Sohag location and disease treatment (L2T2), respectively. The disease severity mean for all lines were (1.12) under (L1T1), which increased to (3.06) under (L1T2), however, the disease severity means were (0.93) under (L2T1), that increased to (2.86) under (L2T2) (Table 3). The present study found that the yield, biomass and germination decrease under disease treatment. The yield and biomass for all lines were increased in the Sohag location compared to the Assiut location, while germination and disease severity were decreased in the Sohag location compared to the Assiut location (Table 3). The present study is in agreement with Ludwig et al. (1956) and Mielke (1988). Screening for resistance against root rot will help identify barley genotypes with resistance as well as high yield potential which can be directly used for large scale cultivation. In addition, it will help in selection programs for incorporating resistance in agronomically suitable lines. Highly significant variations were noticed among screened barley genotypes concerning the degree of infestation with *F. graminearum* as well as the grain yield.

The simple Pearson's correlation are presented in Tables 4 and 5. There are three levels of correlation: <0.2 was weak, from >0.2 to <0.5 was moderate, and more than >0.5 was strong (Hamam 2004; Hamam and Salman 2007). The yield was positive and moderate, correlated with biomass (0.406**, 0.309**, 0.406** and 0.344**) under Assiut location and Control treatment (L1T1), Assiut location and disease treatment (L1T2) Sohag location and Control treatment (L2T1) and Sohag location and disease treatment (L2T2) respectively. Correlations were weak between yield and germination (-0.069*, -0.136**, -0.074* and -0.133**) under (L1T1), (L1T2) (L2T1) and (L2T2) respectively. Disease severity resulted in a weak correlation with yield (0.093*, 0.145**, 0.093 and 0.165**) under (L1T1), (L1T2)

Table 3. Means of disease severity, germination, yield and biomass under two locations and two disease treatments over two years.

Locations and treatments	Disease severity	Germination (%)	Yield/plants	Biomass
L1T1	1.12	85.14%	4.00	13.10
L1T2	3.06	66.96%	0.67	5.38
L2T1	0.93	85.07%	4.33	14.28
L2T2	2.86	47.10%	0.68	5.84

L1T1 = Assiut location and control treatment, L2T1 = Sohag location and control treatment, L1T2 = Assiut location and disease treatment, L2T2 = Sohag location and disease treatment.

Table 4. Pearson's correlation coefficients (r) between four studied traits with locations, disease treatments and lines.

		Treatment		
Parameters	Locations	Control	Disease	Lines
Biomass	0.078*	-0.772**	-0.773**	-0.063*
Yield	0.039*	-0.839**	-0.847**	-0.046*
Germination	-0.069*	0.699**	0.698**	0.077*
Disease severity	-0.168**	-0.309**	-0.649**	-0.119**

Table 5. Pearson's correlation coefficients (r) between biomass, yield, germination and disease scale under two locations and two disease treatments over two years.

Parameters	Locations and treatments	Biomass	Yield	Germination	Disease severity
Biomass	L1T1				
	L1T2				
	L2T1				
	L2T2				
Yield	L1T1	0.406**			
	L1T2	0.309**			
	L2T1	0.406**			
	L2T2	0.344**			
Germination	L1T1	−0.066*	−0.069*		
	L1T2	−0.147**	−0.136**		
	L2T1	−0.069*	−0.074*		
	L2T2	−0.149**	−0.1336**		
Disease severity	L1T1	−0.006	0.093*	−0.283**	
	L1T2	0.139**	0.145**	−0.583**	
	L2T1	−0.002	0.093*	−0.2923**	
	L2T2	0.141**	0.165**	−0.552**	

L1T1 = Assiut location and control treatment, L2T1 = Sohag location and control treatment, L1T2 = Assiut location and disease treatment, L2T2 = Sohag location and disease treatment.

(L2T1) and (L2T2) respectively. Weak correlations between biomass and germination (−0.066*, −0.147**, −0.069* and −0.149**) under (L1T1), (L1T2) (L2T1) and (L2T2) respectively were observed. Disease severity resulted in weak correlation with biomass (0.006, 0.139**, 0.002 and 0.141**) under (L1T1), (L1T2) (L2T1) and (L2T2) respectively. Germination resulted in negative moderate and strong correlation with disease scale (−0.283**, −0.583**, −0.292** and −0.551**) under (L1T1), (L1T2) (L2T1) and (L2T2) respectively. The correlation between location, lines and (biomass, yield, germination and disease) scale over two seasons was weak and negative. Strong negative correlation (−0.772**, −0.839**, 0.699** and −0.309**), (−0.773**, −0.847**, 0.698** and −0.649**) between disease severity and (biomass, yield, germination and disease severity) were detected under normal and disease treatment, respectively. Amir et al. (1991) found a greatly reduced yield under root rot. The associations between yield and other traits under disease treatments help the breeder in identifying most traits which can be considered as selection criteria for improving disease tolerance in barley. Thus, screening for one or more traits which correlated with yield may be amenable for yield improvement under control or disease treatment. The results obtained are in agreement with this reported by Kuroli (1983) and Pillen et al. (2003).

In conclusion, the results suggest that pot bioassays on mature kernels appear to be, after additional testing and standardisation, a useful tool for screening barley plants with a superior level of protection against *Fusarium graminearum*. It is important to know that some barley lines have their resistance characteristic classes as moderately resistant or resistant during the two successive seasons. The line Nos. 21, 28, 44, 45, 89, 140, 224, 249, 2, 29, 31, 35, 61, 64,207, 219, 312, 9, 32,129, 3, 12, 26, 34, 95, 180, 58 and 136 can be used as a source of *F. graminearum* resistance and the line numbers of resistance of Giza 127, Giza 128, 42, 184, 191, 181, 66, 86, 185, 178,

236, 30, 43, 41, 40, 68, 130, 299, 76, 48, 237, 62, 226 and 305, proved highly root rot resistant under Upper Egypt conditions. Generally, such lines might be useful for breeding programs due to the stability of their resistance as well as satisfactory yield. Selection is a good criterion for choosing barley lines that have a high chance for satisfactory resistance characteristics to root rot pathogen, *F. graminearum*, and high yield potential.

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