Plant Pathol. J. 26(4) : 421-423 (2010)
DOI: 10.5423/PPJ.2010.26.4.421

The Plant Pathology Journal

Notes

Greenhouse Method for Assessing Spot Blotch Resistance in Barley

Mohammad Imad Eddin Arabi* and Mohammad Jawhar
Department of Molecular Biology and Biotechnology, AECS, P. O. Box 6091, Damascus, Syria
(Received on July 27, 2010; Accepted on November 3, 2010)

New sources of barley (*Hordeum vulgare* L.) resistant to spot blotch, caused by *Cochliobolus sativus*, are needed to provide effective resistance because of the rapid change pathotype patterns of *C. sativus* in fields. The purposes of our study were to develop a method to screen barley for resistance to spot blotch disease and then use this methodology to screen barley genotypes for resistance to the major virulent pathotype Pt4 in barley populations in Syria. A transparent tape method, in which a conidial suspension of *C. sativus* was dropped onto transparent tape and placed, treated-side down, on the second leaf surface of barley plants. Disease symptoms of fungus were easily detected on the leaves covered by the transparent tape after 48h of inoculation. The transparent tape method was repeatable and the disease scores obtained were correlated ($r = 0.91$, $P = 0.001$) with those obtained by the seedling assay. This method may be beneficial in various plant pathology breeding programs.

Keywords : *Cochliobolus sativus*, *Hordeum vulgare* L., resistance test, spot blotch

Cochliobolus sativus (Ito & Kurib.) Drechsl. ex Dast. [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], the cause of spot blotch (SB), is a common foliar pathogen of barley (*Hordeum vulgare*), a disease responsible for heavy crop losses (Wilcoxon et al., 1990; Kumar et al., 2002). Although fungicides can be effective in reducing SB severity (Kiesling, 1985), the most effective and environmentally sound means of control is the use of resistant cultivars. However, development of stable forms resistant to foliar diseases depends upon identification of resistances effective against the prevalent isolates in barley growing areas (Gupta et al., 2001).

A fast, accurate, and precise screening method that can be used to identify sources of tolerant, partially resistant, and pathotype specific resistant barley germplasm would aid in the development of cultivars for optimal control of *C. sativus*. Resistance in barley to SB is often evaluated at the seedling and adult plant stages by determining the visible disease symptoms as a percentage of infected leaf area (Fetch and Steffenson, 1999; Ghazvini and Tekauz, 2008). This method is time-consuming and several environmental interactions make it impossible to obtain error-free estimates (Gilchrist et al., 1995). Recently, a detached seedling first leaf technique (Arabi and Jawhar, 2007) has been used for evaluating barley resistance to SB. However, this method based on testing detached leaves *in vitro* far away from the mother plant, and the assessment should be done during short time using artificial media, antibiotics and Petri dishes.

In classifying the disease reactions of barley lines for breeding purposes or for studies on host parasite genetics, it is important to have a reliable screening technique for reducing the inconvenience of field tests. The objectives of our research were to develop a *C. sativus* screening method for barley and then screen barley genotypes for resistance to the major virulent pathotype Pt4 in barley populations in Syria.

Inoculum preparation. The major Syrian pathotype *C. sativus* (Pt4) used in the study was the most virulent of 117 isolates collected in 1998 and 2004 from naturally infected barley in different regions of Syria, as described by Arabi and Jawhar (2003). The fungal mycelia were transferred from a stock culture into Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) with 13 mg/l kanamycin sulphate and incubated for 10 days at 21 ± 1°C in the dark. Then, conidia were collected with 10 mL of sterile distilled water. The conidial suspension was adjusted to 2×10^4 conidia/mL using hemacytometer counts of conidia to provide estimates of the inoculum concentration. A surfactant (polyoxyethylene-20-sorbitan monolaurate) was added (100 μL/L) to the conidial suspension to facilitate dispersion of the inoculum over the leaf surfaces.

Host genotypes. Fifteen barley genotypes were randomly chosen and tested with *C. sativus* pathotype Pt4 in a transparent tape inoculation method. Universal susceptible control (cv. WI 2291) from Australia was included in the experiment.

Seedling experiment. Barley seeds were surface-sterilized

*Corresponding author.
Phone) 00963-11-2132580, FAX) 00963-11-6112289
E-mail) ascientific@aec.org.sy

with 5% sodium hypochlorite (NaOCl) for 5 min and then washed three times in sterile distilled water. They were planted in pots filled with sterilized peatmoss, and arranged in a randomized complete block design with three replicates. Each experimental unit consisted of 10 seedlings per genotype. A full replicate consisted of 10 pots inoculated with Pt4 isolate. Pots were placed in a growth chamber at temperatures 22 ± 1ºC (day) and 17 ± 1ºC (night) with a daylength of 12h and a relative humidity of 80-90%.

Plants were inoculated at growth stage (GS) 12 (Zadoks et al., 1974) by uniformly spraying each plant with 0.2 mL of conidial suspension with a hand-held spray bottle. Plants were then placed in the dark at 95-100% R.H. for the first 18 h.

A transparent tape protocol. Segments of transparent tape (2-3 cm long) were dropped with 5-µl of conidial suspension, and then placed over healthy second leaves. Ten pots of 10 seedlings per genotype were inoculated. The experimental design was a randomized complete block with three replicates. Drop of sterile distilled water were similarly placed on one transparent tape as a control. The pots were incubated for 5 days under similar conditions as above. The experiment was repeated five times.

The infection response based on the measurement of individual lesion size (dimension; mm) for each second leaf was assessed 14 days after inoculation for seedling assay, and five days after inoculation in transparent tape method. The 0-4 scale was used in both methods, where the scores 0, 1, 2, 3 and 4 indicate resistant and increasingly susceptible phenotypes.

Data of different tests were analyzed to determine whether there was a significant test x genotype interaction. The relationship among disease ratings on the transparent tape assay and seedlings was examined by studying the correlation among genotypes means in all different experiments using STAT-ITCF program (Anonymous, 1988).

As a preliminary experiment, the transparent tape inoculation method developed in barley was used to inoculate five barley genotypes at densities ranging from 2×10^4 to 2×10^6 conidia ml^{-1} (Table 1). Disease symptoms of fungus were easily detected on the leaves covered by the transparent tape after 48h of inoculation. The lesion sizes increased along with the increase in conidia concentration, which varied due to the resistant level of each genotype (Table 1). The result reflects the suitability of transparent tape method under different densities of inoculum.

The reactions of the 15 five barley genotypes tested for SB resistance for both transparent tape and seedling methods are reported in Table 2. Significant correlations were found ($P = 0.001$) between transparent tape values and seedling ($r = 0.91$) experiments. Differences in severity levels were detected among the different genotypes, with the severity values being consistently higher in the susceptible genotypes in both experiments (Table 2). With the standard inoculation method, all pilot genotypes showed different susceptibility and had a disease score of 4 at an inoculation density of 2×10^4 conidia ml^{-1} 14 days after inoculation under controlled conditions, the same as the results obtained by the transparent tape method. Moreover, the experiments were repeated five times and showed similar outcomes. The results of one experiment are presented here (Table 2).

Inoculation with the *C. sativus* virulent pathotype Pt4 increased the size of lesion on susceptible genotypes in transparent tape and seedling experiments. Both methods show that Thibaut, Golf, Bowman and AECS 71 were moderately resistant and Banteng was resistant (Table 2). The ratings of genotypes shown to be either resistant or

Table 1. Effect of inoculation density on lesion size (mm) using the transperant tape method

	Inoculum concentration (conidia ml^{-1})		
	2×10^4	2×10^5	2×10^6
Genotype			
WI2291	14 × 6	17 × 8	21 × 11
Arabi Abiad	10 × 5	12 × 6	15 × 9
Arrivate	13 × 4	14 × 6	17 × 7
Thibaut	6 × 2	7 × 2	9 × 5
Banteng	0 × 1	1 × 1	2 × 2

Table 2. Disease rating and initial lesion size (mm) of barley reaction to SB (2×10^4 conidia/ml) using the standard seedling inoculation test (A) and the transparent tape method (B)

Genotype	Source	Disease rating		Lesion size (mm)	
		A	B	A	B
WI2291	Australia	4ax	4a	16 × 6	14 × 6
Arabi Abiad	Syria	4a	4a	11 × 4	10 × 5
AECS 83	Syria	4a	4a	10 × 4	9 × 5
AECS 76	Syria	3b	3-4b	10 × 5	13 × 4
AECS 71	Syria	2c	2c	6 × 3	6 × 2
Furat-2	Syria	4a	4a	9 × 4	8 × 5
Thibaut	France	2c	2c	6 × 3	6 × 2
Selina	France	4a	4a	11 × 4	9 × 5
Arizona	USA	4a	4a	9 × 4	8 × 5
Bowman	USA	2c	2c	6 × 3	6 × 4
Arrivate	USA	3b	3-4b	10 × 5	13 × 4
CI-5791	Ethiopia	4a	4a	10 × 4	11 × 3
Golf	England	2c	2c	6 × 4	6 × 4
Smash	Belgium	4a	4a	11 × 3	9 × 5
Banteng	Germany	(1-0)d	(1-0)d	0 × 1	0 × 1

[x] Values within a column followed by different letters are significantly different at $p < 0.001$ according to Newman-Keuls test.

susceptible to SB were quite consistent among different tests.

Several factors have been shown to influence the susceptibility of barley to SB under field conditions (Meldrum et al., 1999). These researchers demonstrated that susceptibility is related to both inoculum level and climate conditions. In our transparent tape experiments, the level of inoculum was controlled. Therefore, precise assessment could be made on the barley reaction to SB disease. Furthermore, the transparent tape assays enabled the study of lesion development at a suitable position on the leaf. Fetch and Steffenson (1999) recommended that the SB responses should be assessed on a sample of lesions from the central portion of the leaf blade, excluding the point at which the leaf bends downwards.

C. sativus is pathogenic to many gramineous hosts; many researchers are therefore studying the diseases caused by this fungus (Tinline, 1988; Kumar et al., 2002, Ghazvini and Tekauz, 2008). In particular, abundant research on its pathogenicity and on plant-pathogen interactions at the macro and molecular levels has been conducted in barley. Since the fungus can infect barley at any growth stage and causes serious yield loss, the risk of escape of highly pathogenic test isolates from glasshouses into barley production areas needs to be minimised.

The transparent tape method is both simple and rapid; it enables the resistance of all plants to be evaluated rapidly under uniform conditions. Under field testing the inoculum is not uniformly distributed and infection levels may fluctuate widely, requiring evaluation of resistance over several growing seasons. Moreover, this method can help to replicate infection assays, and to test more than one isolate on the same plant without using surfactant. The new source of resistance to barley SB Banteng identified in this study, confer resistance to SB populations present in Syria. Therefore, they can make a significant contribution to the diversity of *C. sativus* resistance gene pool available to barley breeders.

Acknowledgments

The authors thank the Director General of AECS and the Head of the Molecular Biology and Biotechnology Department for their continuous support throughout this work. Thanks also extended to Dr. A. Aldaoude for critical reading of the manuscript.

References

Anonymous, 1988. STAT-ITCF, Programme, MICROSTA, realized by ECOSOFT, 2nd Ver. *Institut Technique des Cereals et des Fourrages Paris*, pp. 55.

Arabi, M. I. E. and Jawhar, M. 2003. Pathotypes of *Cochliobolus sativus* (spot blotch) on barley in Syria. *J. Plant Pathol.* 85: 193-196.

Arabi, M. I. E. and Jawhar, M. 2007. Barley reaction to *Cochliobolus sativus* based on detached first leaf. *Austral. Plant Pathol*. 36:475-477.

Fetch, T. C. and Steffenson, B. J. 1999. Rating scales for assessing infection responses of barley infected with *Cochliobolus sativus*. *Plant Dis*. 83:231-217.

Ghazvini, H. and Tekauz, A. 2008. Host pathogen interactions among barley genotypes and *Bipolaris sorokiniana* isolates. *Plant Dis*. 92:225-233.

Gilchrist, S., Vivar, F. L. H., Gonzalez, C. and Velaquez, C. 1995. Selecting sources of resistance to *Cochliobolus sativus* under subtropical conditions and preliminary loss. *Rachis* 14:35-40.

Gupta, S., Loughman, R., Platz, G. J., Lance, R. and Jones, M. 2001. Journey of net blotch from pathotype diversity to useful resistance in barley. Proceedings of the 9th Australian Barley Technical Symposium, Melbourne, Australia.

Kiesling, R. L. 1985. The disease of barley. In. Rasmusson, D. C. (ed) barley. American Society of Agronomy. Madison. Wis. USA. pp. 269-312.

Kumar, J., Schafer, P., Huckelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S. and Kogel, H. K. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Mol. Plant Pathol*. 3:185-195.

Meldrum, S. M., Ogle, H. J. and Platz, G. J. 1999. The effects of wet period, spore concentration and genotype on infection of barley by *Bipolaris sorokiniana. Proceeding of the 9th Australian Barley Technical Symposium*, Melbourne Australia.

Tinline, R. D. 1988. *Cochlliobolus sativus*, a pathogen of wide host range. Pages. 113-122. in: Advances in Plant Pathology. Vol. 6 D.S. Ingram and P.H. Williams, eds. Academic Press, London.

Wilcoxon, R. D., Rasmusson, D. C. and Miles, M. R. 1990. Development of barley resistance to spot blotch and genetics of resistance. *Plant Dis*. 74:207-210.

Zadoks, J. C., Chang, T. T. and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. *Weed Res*. 14:415-421.