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A Simple method for determining barley reaction to spot blotch disease

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A simple method was used to determine the reaction of 14 barley genotypes to *Cochliobolus sativus*, the causal agent of barley spot blotch disease. In this assay, five transparent centered circles ranged from resistant to very susceptible, was used to screen barley genotypes for resistance to the major virulent pathotype Pt4 in barley populations in Syria. The center of circles placed on the started point of lesion and its extended through the circle could give numerical values for each genotype. The method allows low, intermediate and absolute levels of resistance to spot blotch to be determined. The transparent circles method was repeatable and the disease scores obtained were correlated (r = 0.97, P < 0.001) with those obtained by the seedling assay. The established method is both simple and precisely, it could make a significant contribution in many types of studies in plant pathology breeding programs.

Key words: *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 large economic losses in cereal farming in North America (Mathre, 1990), Australia (Meldrum *et al.*, 2000) and Middle East (van Leur *et al.*, 1997). The economic damage caused by SB can be avoided by using large amounts of fungicides or by planting resistant genotypes, with the latter option being the more economically and environmentally appropriate solution (Gupta *et al.*, 2001). However, development of stable forms resistant to foliar diseases depends upon identification of resistances effective against the prevalent isolates in barley growing areas (Ghazvini and Tekauz, 2008).

Barley reaction to SB is commonly 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; Arabi and Jawhar, 2010). Inconsistencies between individuals using this subjective rating system, however, are a source of experimental error (Ghazvini and Tekauz, 2008). Arabi and Jawhar (2007) have used a detached seedling first leaf technique for evaluating barley resistance to SB. This method is 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.

Reliable laboratory screening technique can increase the effectiveness of plant disease resistance breeding programme by reducing the unnecessary

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expense and inconvenience of field-testing. 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.

Materials and Methods

Host genotypes

The 14 barley cultivars used in this study were chosen for their reaction to SB from highly susceptible to highly resistant and diverse origins (Table1). The universal susceptible cultivar WI2291 from Australia was included in each set as checks.

Isolation of fungus and inoculation

After an extensive screening for over ten years in the greenhouse and in the laboratory the *C. sativus* pathotype (Pt4) was proved to be the most virulent isolate to all barley genotypes available so far (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 x 10^4 conidia/mL using hemacytometer to provide estimates of the inoculum concentration. A surfactant (polyoxy-ethylene-20-sorbitan monolaurate) was added (100 µL/L) to the conidial suspension to facilitate dispersion of the inoculum over the leaf surfaces.

Seedling experiment

Barley seeds of each genotype were surface-sterilized 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 pathotype Pt4. Pots were placed in a growth chamber at temperatures 22±1°C (day) and 17±1°C (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. Inoculated plants were kept in a mist chamber at 20 °C the darkness to maintain relative humidity at 100% for 48 h.

A transparent circles protocol

A transparent circles method was classified into five centered circles ranged from resistant (center circle; 0.3-0.1mm) to very susceptible (outer circle; < 9mm of lesion size). In this method, a center of circles placed on the started point of the lesion and its extended through the circle could give a degree barley reaction to the disease. The experiment was repeated five times.

Disease assessment

The infection response based on the measurement of individual lesion size (dimension; mm) for each second leaf was assessed 10 days after inoculation using five circles infection response rating scale for transparent circles method, and 0 to 5 scale (Steffenson and Fetch, 1996) for seedling test. Each leaf was assessed separately and the assessments were performed by the same person in all experiments. The experiment was repeated five times.

Statistical analysis

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 circle assay and seedlings was examined by studying the correlation among genotypes means in all different experiments using STAT-ITCF program (Anonymous, 1988).

Results and Discussion

A wide range of disease severity was observed among genotypes in each test (Table 1). A five-circle infection response rating scale was developed based on the relative size of lesions (presence of necrosis and chlorosis) observed on barley genotypes infected with *C. sativus* pathotype Pt4 (Table 1). Central circle (A) consisted of minute round and small oblong necrotic lesions without chlorosis ranged from 0.3 to 2.0 mm. Circle 2 (B), consisted of larger round to oblong necrotic lesions approximately > 2 to 4 mm with very slight diffuse marginal chlorosis. Circle 3 (C), consisted of medium-sized elliptical to somewhat ovate necrotic lesions with distinct but restricted chlorotic margins ranged > 4 to 6 mm. Circle four (D), consisted of larger ovate necrotic lesions surrounded by large chlorotic halos approximately > 6 to 9 mm. Circle five (F), consisted of consisted of elliptical to elongated elliptical necrotic lesions with distinct chlorotic margins and expanding diffuse chlorosis > 9 mm (Fig. 1).

The reactions of the 14 barley genotypes tested for SB resistance for both transparent circle and seedling methods are reported in Table 2. Significant correlations were found ($P < 0.001$) between transparent circle values and seedling ($r = 0.97$) 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). 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 and Golf were moderately resistant and Banteng and Bowman were resistant (Table 2). The ratings of genotypes shown to be either resistant or susceptible to SB were quite consistent among different tests.

Table 1. Spot blotch symptoms and development on 14 barley genotypes using transparent circles method

Genotype		SB	
	Source	Symptoms	Disease development
WI2291	Australia	E	Up to circle five
Arabi Abiad	Syria	E	Up to circle five
AECS 76	Syria	D	Up to circle four
AECS 71	Syria	C	Up to circle three
Furat-2	Syria	D	Up to circle four
Thibaut	France	C	Up to circle three
Selina	France	E	Up to circle five
Arizona	USA	D	Up to circle four
Bowman	USA	B	Up to circle two
Arrivate	USA	E	Up to circle five
CI-5791	Ethiopia	E	Up to circle five
Golf	England	B	Up to circle two
Smash	Belgium	E	Up to circle five
Banteng	Germany	A	Up to circle one

A: Small round to oblong dark brown necrotic lesions
B: large round to oblong necrotic lesions
C: Lesions consisting of light brown with whitish gray centers and chlorotic margins
D: Large ovate necrotic lesions surrounded by large chlorotic halos
E: Solid dark brown necrotic lesions with expanding chlorosis and necrosis

Several factors have been shown to influence the susceptibility of barley to SB under field conditions (Nutter *et al.*, 1985; 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 circles method 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.

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 susceptibility circles method (B)

Genotype	A	B
		Lesion size (mm)
WI2291	4a[x]	VS (12 × 5)
Arabi Abiad	4a	VS (11 × 4)
AECS 76	3b	S (8 × 5)
AECS 71	2c	MS (6 × 3)
Furat-2	4a	VS (9 × 4)
Thibaut	2c	MS (4 × 3)
Selina	4a	VS (9× 4)
Arizona	4a	VS (9 × 4)
Bowman	2c	R (2 × 3)
Arrivate	3b	VS (9 × 5)
CI-5791	4a	VS (9 × 4)
Golf	2c	MR (4 × 3)
Smash	4a	VS (11 × 4)
Banteng	(1-0)d	R (0 × 1)

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

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 minimized.

The transparent circles method is both simple and rapid; it enables the resistance of all plants to be evaluated rapidly under uniform conditions. Furthermore, it helps to quantify differences of SB categories to more accurate and simplify greenhouse and field assessments. Therefore, this method could be useful for many types of studies on this disease; additionally, it might be used for various foliar diseases after adjusting the distances between circles due to the lesion size of each.

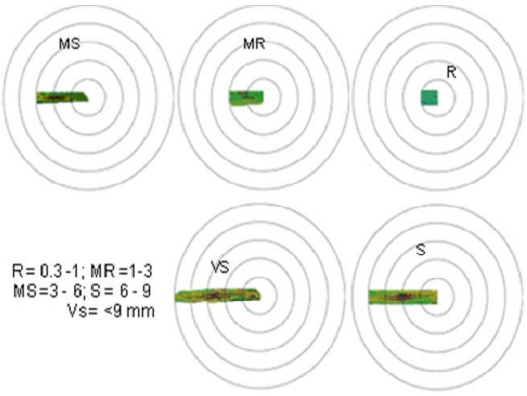

Fig. 1. SB scale as revealed by using five transparent circles

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