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Barley reaction to *Cochliobolus sativus* based on detached first leaf

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Abstract. Spot blotch, caused by *Cochliobolus sativus*, is a common foliar disease of barley that is controlled primarily through the development of resistant cultivars. An assay involving the inoculation of a detached first leaf (DFL) maintained *in vitro* was used to determine the reaction of five barley genotypes to the disease. The estimation of the infected leaf area enabled discrimination among barley genotypes differing in their susceptibility to the pathogen. Significant correlations were found ($P = 0.001$) between *in vitro* DFL values in both seedling ($r = 0.89$) and adult ($r = 0.95$) plants. The established assay using *in vitro* DFL enables a fast assessment of the susceptibility of barley to spot blotch and should be useful for many types of studies on this disease.

Cochliobolus sativus (anamorph: *Bipolaris sorokiniana*), a foliar pathogen, causes spot blotch of barley which is responsible for large economic losses in some barley growing areas. Although fungicides can be effective in reducing severity of this disease, the most effective and environmentally sound means of control is by the use of resistant cultivars (Wilcoxson *et al*. 1990).

Barley reaction to *C. sativus* is often evaluated at the seedling stage in the greenhouse and at the adult plant stage in the field by determining the visible disease symptoms as a percentage of infected leaf area (Fetch and Steffenson 1999). This method is time-consuming and several environmental interactions make it impossible to obtain error-free estimates (Gilchrist *et al*. 1995).

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 laboratory screening technique for reducing the inconvenience of field tests. Therefore, the aim of the present study was to test an *in vitro* procedure that would reliably determine differences in spot blotch infection levels using detached first leaves in a simple and rapid way to solve the assessment of barley susceptibility to *C. sativus* in the field.

The five barley genotypes, including the universal susceptible cultivar 'WI 2291' from Australia, used in this study (Table 1) were selected from a preliminary spot blotch differential set, to cover a spectrum of resistances to *C. sativus*.

Seven isolates of *C. sativus*, selected on the basis of cultural morphology and virulence (Arabi and Jawhar 2003), were used. Each isolate was grown on potato dextrose agar (PDA; DIFCO, Detroit, MI) for 10 days at $22 \pm 1°C$ in the dark. After 10–12 days, conidia were collected by flooding the plate with 10 mL of sterile distilled water and then scraping the agar surface with a glass slide to dislodge the conidia. Equal volumes of conidial suspension from each isolate were combined and filtered through a double layer of cheesecloth. The conidial suspension was adjusted to 2×10^4 conidia/mL using haemocytometer 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.

Seeds were surface-sterilised with 5% sodium hypochlorite solution for 5 min and then washed three times in sterile distilled water. They were sown into plastic flats ($60 \times 40 \times 8$ cm) filled with sterilised peatmoss, and arranged in a randomised complete block design with three replicates. Each experimental unit consisted of two rows of 18 seedlings per genotype. A full replicate consisted of the plots of five genotypes; this full test was repeated three times. Flats were placed in a growth chamber at temperatures $22 \pm 1°C$ (day) and $17 \pm 1°C$ (night) with a daylength of 12 h and a relative humidity of 80–90%.

Inoculation was performed at the two-leaf stage (Zadoks GS 11–12; Zadoks *et al*. 1974) by uniformly spraying 0.2 mL per plant of conidial suspension with a handheld spray bottle. Plants were then placed in the dark at 95–100% RH for the first 18 h.

The barley genotypes were inoculated by the conidial suspension (2×10^4 conidia/mL) when the awns of most plants were emerging (Zadoks GS 49). This inoculum was applied at a rate of 48 mL/m^2 as recommended by Nutter *et al*. (1985). Plants were inoculated during the evening when the environmental conditions were predicted to be favourable for dew formation.

The first fully expanded leaf for each of the five genotypes was removed from the plant and immersed in 5% sodium hypochlorite solution for 1 min, rinsed three times (5 min each) in sterile distilled water and dried using sterilised filter paper. The leaves were then placed onto 1.5% water agar supplemented with 80 mg/L of benzimidazole in a sterile plastic Petri dish. The experimental design was a randomised complete block with five replicates. Each replicate consisted of five Petri dishes of three leaves for one cultivar. Three drops of the conidial suspension were distributed uniformly onto each leaf. Drops of sterile

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Table 1. Comparative disease severity ratings (Steffenson and Fetch 1996) using three different methods of assessment of *Cochliobolus sativus* on different barley genotypes

Values within a column followed by different letters are significantly different at $P < 0.001$ according to Newman–Keuls test. ***, $P < 0.001$; n.s., not significant

Genotype		Detached first leaf	Seedling	Adult plant
Arabi Abiad		89.00a	98.00a	91.60a
WI2291		82.30b	85.44b	81.00b
Arrivate		57.63c	72.96c	69.17c
AECS 76		23.97d	46.80d	41.53d
Thibaut		14.97e	17.04e	20.63e
Source of variance	d.f.		m.s.	
Genotype	4	3347.84***	4814.17***	2558.42***
Replicate	2	18.07 n.s.	8.45 n.s.	22.9 n.s.
Residual	8	14.56	15.2	3.43

distilled water were similarly placed on one leaf as a control. The Petri dishes were incubated for 5 days at 20–22°C (day) and 16–18°C (night) with a daylength of 12 h. The experiment was repeated five times.

The infection response in the seedling assays was assessed on the second leaves of plants 14 days after inoculation, whereas the infection response of the adult plants in the field were assessed on the upper three leaves of plants at the early to mid-dough stage of development (Zadoks GS 83–85). Five days after inoculation, the DFL infection response was assessed. The 0–100 scale of Steffenson and Fetch (1996) was used in all three methods.

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

A wide range of disease severity was observed among genotypes in each test (Table 1). The estimation of the infected leaf area enabled the discrimination among barley genotypes differing in their susceptibility to the pathogen. Significant correlations were found ($P = 0.001$) between *in vitro* DFL values and both seedling ($r = 0.89$) and adult plants ($r = 0.95$).

The analysis of variance of results from different tests demonstrated a significant ($P = 0.05$) test × genotype interaction (Table 1). Comparisons of disease severity on DFL assays, seedlings and adult plants by correlations of genotypes means, showed reasonably good agreement among the three methods. The data showed that the cultivars WI 2291, Arabi Abiad and Arrivate were significantly more susceptible to spot blotch than most of the other genotypes, whereas, the two genotypes AECS 76 and Thibaut were resistant (Table 1). Resistance to spot blotch in Thibaut has been previously reported by Arabi and Jawhar (2003). The ratings of genotypes shown to be either resistant or susceptible to spot blotch were quite consistent among different tests.

Clearly, the study shows that resistance to *C. sativus* in the FDL involves similar reactions to those controlling spot blotch in seedlings and adult plants. This result is in agreement with Mumford (1966) and contrasts with others (e.g. Steffenson and Fetch 1996) who found a weak correlation between seedling and adult plant reaction to spot blotch. However, although infection response at the adult plant stage is usually the key indicator in resistance screening, the DFL method has proven valuable for spot blotch genetic studies in barley (Arabi 2005).

Fetch and Steffenson (1999) reported that infected primary leaves with *C. sativus* senesced more rapidly than infected second or third leaves of barley. However in barley, the first leaf of the germinating seed is considered to be an interesting embryonic organ for studying the metabolic events triggered by germination, such as syntheses and relationship of protein, RNA and DNA with respect to the state of the genome (Ahmed and Kamra 1975).

In field tests, high inoculum densities make it difficult to reliably assess the type and the size of individual lesions. However, low inoculum densities can result in insufficient number of lesions for assessing the infection responses of barley lines. In the DFL *in vitro* assays, amount of inoculum was controlled and consistently produced sufficient numbers of well separated lesions for assessing infection responses on barley plants. Furthermore, the DFL *in vitro* assays enabled the study of lesion development at a suitable position on the leaf. Fetch and Steffenson (1999) recommended that the spot blotch 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. In addition, in our method, the DFLs are surface sterilised which eliminates most of the foliar saprophytes.

The presence of significant positive correlations among DFL, seedling and adult plant resistance to *C. sativus* indicate a need for further studies at the molecular level to understand this relationship. Such information would be useful to plant breeders because DFL testing for spot blotch reaction requires only 5 days, whereas tests of seedlings and adult plants require ~25 and ~90 days, respectively.

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