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Resistance and tolerance reactions of barley doubled haploid lines to common root rot pathogen (*Cochliobolus sativus*)

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Abstract

Common root rot (CRR), caused by the fungal pathogen *Cochliobolus sativus*, is an important disease of barley worldwide. The current CRR rating scale does not sufficiently represent a cultivar's true performance, as it neglects tolerance of the cultivar. Since, resistance reflects the ability of a plant to restrict the pathogen infection, or inhibition pathogen growth throughout the plant, whereas, tolerance is the ability of the plant to yield, despite being infected. Therefore, a more informative CRR rating system is required to address this issue. Keeping in view this objective, two doubled haploid (DH) populations were tested in this investigation using both resistance and tolerance strategies. Results demonstrated significant differences among DH lines with a broad spectrum of disease responses ranging from high to low levels based on the percentage of subcrown internode discoloration rating scale. However, even though B08-AS-2 had a high CRR infection level (77.4%), it had a highest grain yield per plant (15.5g), similarly with B08-AS-4, 7 and 18 lines. However, the most resistant lines B08-AS-12 and 16 did not give a high yield. Furthermore, the correlation between resistance and tolerance to CRR was r =0.17, P<0.01, indicating that reaction of barley genotypes to this disease should therefore consider not only the resistance rating of a line, but also its tolerance which can reflect a genotype's true performance.

Keywords: Barley (*Hordeum vulgare* L.), common root rot, *Cochliobolus sativus*, resistance, tolerance, yield.

Introduction

Cochliobolus sativus (Ito & Kuribayashi) Drechs. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoemaker), the causal agent of common root rot (CRR), is one of the most important pathogens of barley (*Hordeum vulgare* L.) worldwide. CRR is considered economically important as it can cause significant reduction in both yield and quality of the crop [1,2].

The disease produces a light to dark-brown discolouration of the subcrown internode (SCI), extending further up the stem in more susceptible cultivars. This discolouration is directly related to the invasion of the barley tissue by the *C. sativus* during the infection phase [3], therefore, the level of resistance can be determined by examining the degree of SCI discolouration [4,5]. While time-consuming, this strategy is useful in determining the amount of fungus in the plant, and thus the relative level of susceptibility without measuring the plant yield, despite being infected.

The use of barley resistant genotypes remains the most suitable and economical manner to control this disease. However, there are presently insufficient germplasm resources with resistance to CRR [6,7], therefore, screening of a larger number of genotypes is needed to identify new resistant sources. Significant variation exists in barley genotypes performance under CRR, representing opportunities for an intervention in disease management [8,9], however, once a barley plant becomes infected, the resistance and tolerance of the genotype determine how the plant responds.

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Resistance and tolerance represent two important mechanisms that plants evolved to protect themselves from pathogens [10]. Resistance to a pathogen is usually defined as the ability of a plant to restrict the infection by the pathogen, or restrict its growth throughout the plant, by contrast, tolerance is usually measured as the ability of the plant to yield, despite being infected [11]. No studies for the reactions between CRR- barley resistance and tolerance were so far reported in the plant pathology literature.

C. sativus populations are extremely diverse due to a high degree of sexual reproduction and large effective population sizes. As a result, the pathogen has the capacity to adapt rapidly to both fungicides [12] and host resistances [3] as a result of strong directional selection favoring particular pathogen genotypes. Therefore, CRR resistance has been widely discussed and used to describe how a variety will perform under the infection conditions. Importantly, tolerance equally impacts a cultivar response to root rot but is often not considered. Hence, accurate measurement under CRR conditions is crucial to enhance our capacity for selection based on a cultivar's true performance. Here we used two barely DH populations to determine their performance under CRR infection using both the resistance and tolerance strategies.

2. Materials and Methods

2.1. Plant material

Two barley double haploid populations; 34 out of 150 lines produced according to Kasha and Kao [13] were used in this study (Table 1). The lines were produced through three resistant-by-susceptible barley crosses made between three parents possessing different CRR reactions [14]. Arabi Abiad is a Syrian local cultivar, PK36-130 is a Pakistan cultivar and IC-9 is a new cultivar developed at ICARDA (International Center for Agricultural Research in the Dry Areas).

2.2. Inoculum preparation

The virulent *C. sativus* CRR16 strain [9] was used for artificial screening in this study. Mycelia were grown in Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) for 10 days at 20 ± 22 °C in the dark. Conidia were collected by flooding the plate with sterile distilled water and scraping the colony surface with a flame- sterilized glass slide, and conidial suspension was adjusted to 5 X 10^5 conidia/mL.

2.3. Field trials

Trails were conducted at a site approximately 55 km south of Damascus for two years, under annual rainfall range 200- 250 mm. Seeds were inoculated by mixing peat-gum-conidia inoculums according to the method described by Van Leur [1], and sown at a depth of 6 cm to induce the formation of long subcrown internodes [5] with three replicate plots. Each was 1 x 1 m with a 1-m wide border. Each plot consisted of six rows 30 cm apart with 50 seeds/row. Soil was fertilized with 50 kg/ha urea (46% N) and 27 kg/ha superphosphate (33% P).

2.4.Yield assessment

The four central rows of each replicate plot were harvested at maturity stage to evaluate grain yield (g/plant).

2.5. CRR estimation

Estimations were made visually 7 weeks post inoculation by measuring the percentage of SCIs showing CRR symptoms with a scale used by Ledingham et al. [15], where the plants are classified as follows: Resistant 1 to 25%, moderate susceptibility 26 to 50%, and susceptible (greater than 50%) of the SCIs covered by CRR lesions.

2.6. Statistical analyses

Data were analyzed using the STAT-ITCF statistical programme. Differences between means were evaluated for significance by using Newman-Keuls test at 5% probability level [16].

3. Results and Discussions

In this work, three barley parents with different levels of resistance to *CRR* were used. As shown in Figure 1, the disease caused more severe infection on the susceptible parent 'Arabi Abiad' as compared with the resistant ones. Furthermore, the discoloration and necrosis of the SCIs were typically observed in infected plants (Fig. 1) which is in agreement with our previous field observations [9].

According to the scale described by Ledingham et al. [15], reactions of the 34 progeny lines to CRR under field tests were classified into three groups. However, significant differences ($P < 0.05$) in mean severity values were detected among different lines, and a continuum of genotypic reactions to the virulent strain CRR16 from resistant to susceptible was observed (Fig. 2).

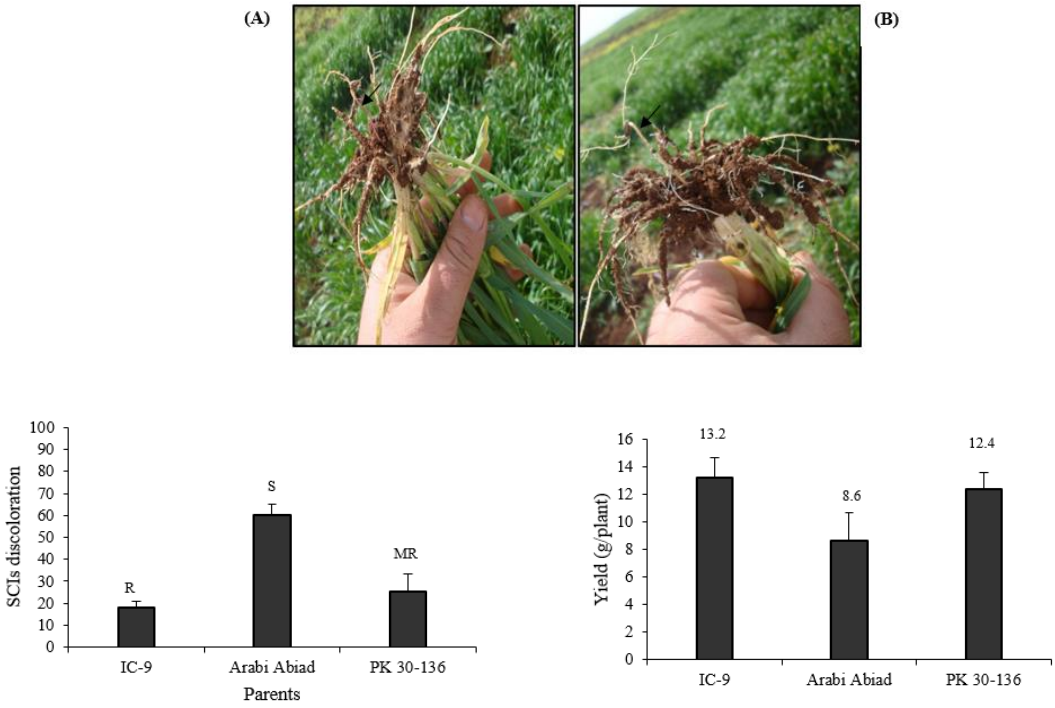

Figure 1. A. CRR symptoms on the barley resistant (right) and susceptible (left) DH lines. **B.** Yield and frequency of disease reactions incited on the barley parents, 7 weeks after *CRR* infection according to the scale of Ledingham et al. (1973). R: resistant, MR: moderately resistant and S: susceptible.

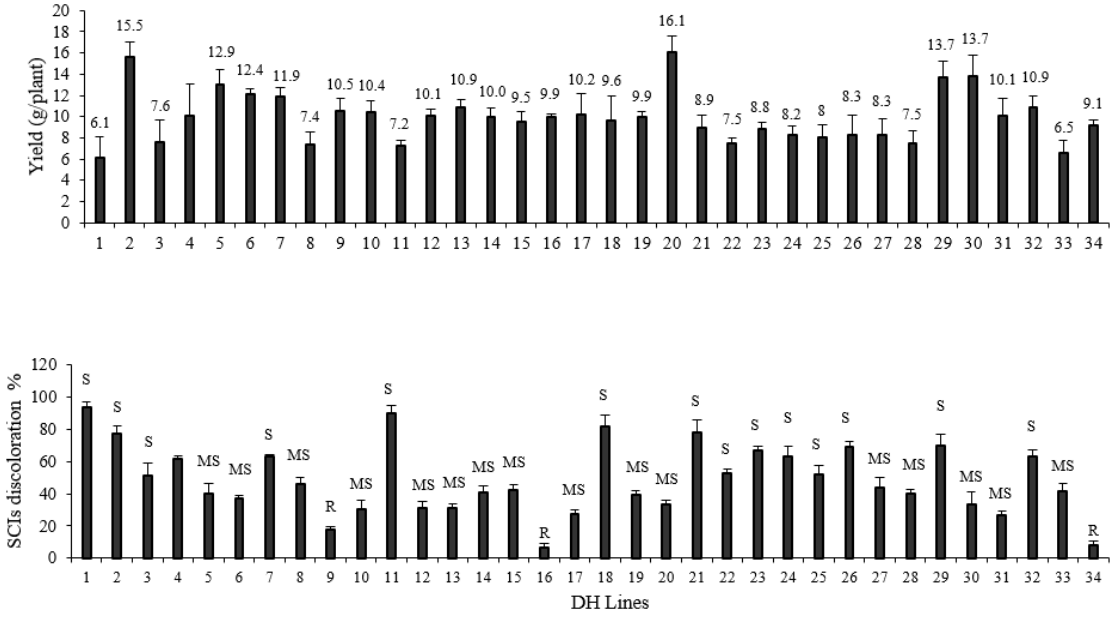

Figure 2. Frequency distribution of CRR infection response of 34 DH barley line which were resistant (R), moderately susceptible (MS) and susceptible (S) according to the scale described by Ledingham et al. 1973. Data were obtained from three crosses of barley parent.

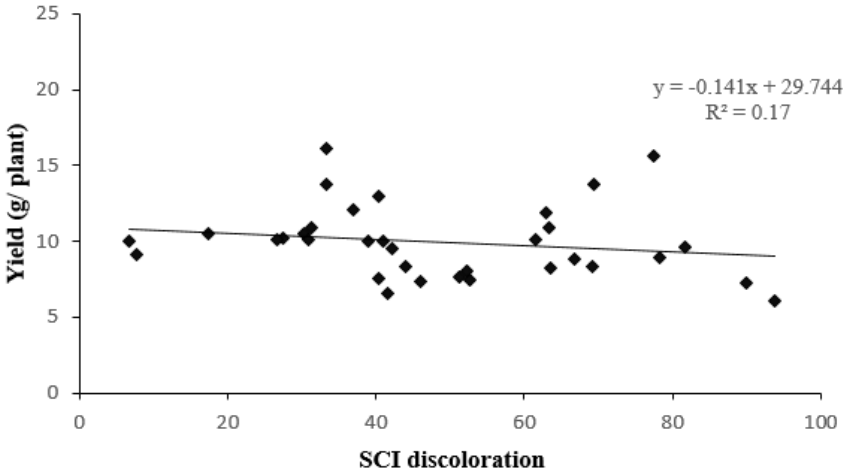

Figure 3. Relationship between SCI discoloration and yield (g/plant) of barley DH lines.

Data showed that even though B08-AS-2 had a high CRR infection level (77.4%), it had a highest grain yield per plant (15.5g), similarly with B08-AS-4, 7 and 18 lines. Whereas, the most resistant lines B08-AS-12 and 16 did not give a high yield (Fig. 2). A correlation (r =0.17, P<0.01) was found between resistance and tolerance to CRR (Fig. 3), indicating that reaction of barley genotypes to this disease should therefore consider not only the resistance rating of a line, but also its tolerance which can reflect a genotype's true performance. Our results are in agreement with those of Mikaberidze and McDonald [17] on wheat, and with Politowski and Browning [18] on oat. Our data can be also supported with the results of Davies [19] on crown rot caused by the fungus *Fusarium pseudograminearum.*

It has been reported that barley plant may be able to compensate the reduction in one yield component and continue to yield well in spite of CRR infection [15, 20]. So, the ability of barley genotypes to yield well when damaged by CRR may indicate a level of tolerance to the disease. Here, it might explain that in spite of CRR infection, barley plants have the ability later to develop yield components to compensate for initial tiller reduction and also by inherent tolerance to the disease.

However, identification of new sources of resistance to CRR and their introduction into cultivated crops is a very important element of breeding programs [21], therefore, barley breeders need to identify whether a CRR yield loss amount can be attributed to resistance or tolerance in the tested lines.

One modern helping method is to use molecular markers which can alleviate some of these issues, by providing breeders with a rapid method of determining whether certain genes or combinations of genes are present in a breeding line which confer improved resistance or tolerance to CRR. Whilst molecular markers are particularly effective for some traits, markers associated with CRR have some difficulties. There are only a few markers available for a few number of resistance QTLs only, and these are often unreliable, and only loosely linked with the trait [22]. However, along with work on molecular markers, the current screening methods are very time consuming and thus expensive to run and have relatively low throughput.

On the other hand, different factors have been shown to influence the barley SCIs discoloration under field conditions [23] which should be carefully considered during CRR evolutions. For instance, the soil might contain other fungal pathogens and bacteria that can cause SCI rotting, which can lead to a misdiagnosis of disease symptoms with *C. sativus*. In addition, high soil moisture may also lead to SCI discoloration similar to that caused by *C. sativus* infection.

4. Conclusion

The present study demonstrated that precise measurement and classification of cultivars performance under barley CRR infection is essential for selection. A relationship was found between resistance and tolerance (r =0.17, P<0.01), this might suggest that resistance and tolerance can be combined to achieve an additive effect for obtaining a cultivar's true performance during barley- CRR evaluations.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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