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A Simple approach to assess common root rot severity incidence data in wheat

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Abstract: Common root rot (CRR) of wheat, caused by *Cochliobolus sativus*, produces discoloration of the subcrown internodes (SCIs) and is directly related to yield losses. It is critical to clearly define and standardize the CRR assessment methods to avoid subjectivity and variability between assessors. Therefore, in this study, a comparison between the incidence (I; proportion of diseased SCIs) and the severity (S; proportion of SCI showing CRR symptoms) was investigated to explore the possibility of simplifying disease rating. Assessments were made visually at multiple sample sites in artificially- and naturally-inoculated research and production fields for three growing seasons. Significant differences (*P* = 0.001) in mean I and S values were found among cultivars, with values being consistently higher in the susceptible ones. However, CRR severity increased linearly as incidence increased in both *Triticum durum* and *T. aestivum* wheat. Their slopes and intercepts of the I–S relationship were consistent over the three growing seasons. This result may be considered a significant contribution for CRR assessment in wheat breeding programs.

1. Introduction

Common root rot (CRR) caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker), is consistently one of the most damaging diseases of wheat and barley worldwide (Gurung *et al.,* 2013; Fernandez *et al*., 2014). CRR is considered economically important because it can cause marked reduction in yield and quality of the crop (Kumar *et al.,* 2002). This disease produces a brown to black discoloration of the subcrown internode (SCI), therefore presence and severity can be determined by pulling up plants and examining SCI for disease (Kokko *et al.,* 1995; Mathre *et al.,* 2003).

Efforts to minimize the impact of CRR have been centered around the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application (Fernandez and Conner, 2011; Burlakoti *et al.,* 2013). From a management perspective, the comparison of CRR epidemics across years and locations is necessary to determine the effects of the environment on the efficacy of a given management approach, under similar environmental conditions, and to develop or recommend management strategies or decision thresholds (Fernandez *et al*., 2014).

The first step to quantify the effect of CRR is to develop a key that clearly defines and standardizes the assessment methods to avoid subjectivity and variability between assessors. Therefore, CRR evaluation methods need to easily provide objective measurements so that data from different sources are comparable, and provide an adequate sample of the crop for assessment (Mathre *et al.,* 2003). Reaction of wheat to CRR is commonly measured either by incidence (I, proportion of SCI units diseased) or severity (S, proportion of SCI showing CRR symptoms). However, incidence is a binary measurement (Madden and Hughes, 1999), meaning it is a measure of only one of two possible states, diseased or not diseased. Moreover, in spite of the drawback, however, severity is often considered a more important and useful measure of disease intensity than incidence to evaluate yield loss and to deterimine the effectiveness of disease management strategies (Fernandez *et al.,* 2009).

Since measurements of incidence are more easily acquired and more reliable than measurements of severity, and severity is more useful than incidence for certain objectives, a quantitative relationship between incidence and severity would greatly facilitate the evaluation of disease intensity when accurate assessments of severity are not available or possible (Seem, 1984; Fernandez *et al.,* 2014). Therefore, in this study, the I-S relationship of CRR was investigated to explore the possibility of simplifying disease assessment.

2. Materials and Methods

Disease assessment sites

In order to acquire data from CRR epidemics of different intensities and to represent a range of environmental, cropping, and management conditions likely to influence

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the development of CRR, three different locations with several research plots and production fields were selected for CRR assessment in three growing seasons (Table 1).

Inoculum preparation

The *C. sativus* isolate (Pt4) has been proved to be one of the most virulent isolates to all barley and wheat genotypes available so far (Arabi and Jawhar., 2002). In the present study, the fungal mycelia were transferred from a stock culture into Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) with 13 mg/I kanamycin sulphate and incubated for 10 days at 21±1°C in the dark.

Host genotypes

The ten wheat cultivars (six *Triticum durum* and four *T. aestivum)* used in this study were chosen for their wide genetic variability for *C. sativus* reaction from highly susceptible to highly resistant (Table 2). The local susceptible landrace Salamoni was included in each set as check.

Experimental design

Seeds were artificially inoculated with Pt4 isolate following the procedure set out by Arabi and Jawhar (2013). The experimental design was a randomized complete block design with three replicates. The seeding depth was 6 cm (Kokko *et al.,* 1995). Plot area was 1 x 1 m with a 1 m buffer. Each plot consisted of five rows 25 cm apart with 50 seeds sown per row. Experimental design, cultural practices, and inoculation methods were performed as described by Arabi and Jawhar (2002). Weeds were controlled by pre- and post emergence herbicides as appropriate. Soil fertilizers were drilled before sowing at a rate of 50 kg/ha urea (46% N) and 27 kg/ha superphosphate (33% P).

Disease assessment

In each field/plot, I and S were estimated visually at several systematically selected sampling sites, 20-25 subsampling from each row were taken at random from each replication.

Incidence (I) was recorded as the proportion of diseased SCIs (number of SCIs with nonzero severity divided by the total number of plants sampled). Severity (S) was recorded as infected SCIs expressed as a proportion of the total area.

Statistical analysis

Data for I and S were analyzed by analysis of variance (Newman-Keuls test), using the STAT-ITCF program (ITCF, 1988). The assumption of coincidence for each year was tested using the ANOVA procedure implemented in the software package Statistica 6.1. Years were set as the categorical variable and coincidence was tested by simultaneously checking the year's effect combined with its interaction with the incidence. For all experimental data, each pair of I and S values from each sampling site was considered an observation for data analysis. The experimental data were edited to remove observations with no diseased plants (i.e., I = 0 and S = 0), since the I-S relationship is only defined when disease is present.

3. Results

Significant differences ($P = 0.001$) in mean I and S values were detected, with values being consistently higher in the susceptible cultivars for the three growing seasons (Table 2). The data show that the highest mean I and S were recorded in the *T. aestivum* landrace Salamoni (I and S =100), whereas the lowest was found in the *T. durum* landrace Horani (I and S ≈ 7). In general, the *Triticum durum* genotypes were more resistant than *T. aestivum* (Table 2), in agreement with data presented by Bhandari and Shrestha (2004).

Additionally, the data demonstrate that S increased linearly as I increased (Fig. 1). There was no difference in the slopes and intercepts of the I-S relationship among the three years, as was shown by the coincidence test ($F_{3, 32} = 0.309$, $P = 0.585$). In some cases I = S for one or more observations such as in the susceptible landrace Salamoni (Table 2). This can be explained by the fact that when all plants in the sample are diseased, there is no longer any information on the magnitude of (mean) S in relation to I, other than being larger than the (mean) S when some plants are disease-free. In this extreme situation, I was equal to S for wheat CRR reaction. These findings are in agreement with the results of Paul *et al.* (2005) for fusarium head blight on winter wheat.

The overall response to CRR for the three growing seasons considered in this study differed with the differences

Table 1 - Range of magnitude of environmental conditions encountered during three growing seasons (2011, 2012 and 2013)

Location	No. fields	Temperature (°C) [z]	Relative humidity (%)[z]	Average rainfall (mm) [y]	Altitude (m)	Directions
Draa (south)	4	33-39	40-51	256	716.5	36°06'23.86" E 33°06'55.71" N
Allepo (north)	4	25-36	41-79	360	297.4	33°55'56.99" E 36°01'31.14" N
Hassaka (north east)	5	35-48	35-42	228	313.9	40°40'02.31" E 36°31'53.73" N

[z] Average during April, May and June.

[y] Average from November to April.

Table 2 - Mean common root rot disease incidence (I) and severity (S) of the most frequently grown wheat cultivars in Syria under field conditions for 3 years, combining data for three locations

Cultivar						
	S	I	S	I	S	I
Triticum aestivum						
Sham 2	15.20 e	20.00 e	22.50 de	20.50 e	18.30f	17.60 e
Bouhouth 4	33.16 d	40.00 d	25.40 d	33.00 d	22.50 e	30.00 d
Bouhouth 6	42.60 c	50.50 cd	48.20 c	47.60 c	40.30 c	43.00 c
Salamoni (Landrace)	95.50 a	100.00 a	90.07 a	100.00 a	89.30 a	91.00 a
Doma 4	60.16 bc	73.00 c	52.90 c	50.60 c	40.50 c	39.00 c
Mexipak	66.50 b	80.00 b	70.30 b	78.30 b	63.20 b	86.20 b
T. durum						
Doma 1	31.13 d	33.00 de	27.00 d	30.00 d	35.70 d	39.50 c
Sham 3	10.30 e	18.00 e	9.10 e	15.10 f	12.20 g	18.30 e
Horani	7.50e	10.60f	7.80e	8.50fg	5.50h	9.67f
Bouhouth 7	30.06 d	38.00 d	22.33 de	25.60 de	31.70 d	33.50 d
LSD	8.83	6.11	7.42	5.3	4.09	4.01

Values followed by different letters columns are significantly different at P= 0.001 according to Newman-keuls test. LSD: Least Significant Difference at P<0.05.

in susceptibility levels of the cultivars. However, cultivars that are resistant to CRR may in fact have different resistance response to the spread of the fungus within the infected plants. Hence, for any given I value, a wide range of S values may be observed across cultivars. McRoberts *et al.* (2003) reported that incidence severity analysis was directly useful in evaluating resistance response.

In particular, the I–S relationship could be used to draw conclusions about the relative rate of disease increase among cultivars with different levels of resistance.

4. Discussion and Conclusions

Our results show that neither differences in weather conditions for the three growing seasons, nor geographical locations resulted in any different patterns in the I-S relationship. Although the locations were up to 50 km apart, it appeared that within a climatologically similar region, I-S relationships did not show distinct differences among sites. Moreover, in this study, the number of plants sampled and the small distance among locations did not affect the I-S relationship either.

We undertook this study to determine an I-S relationship for CRR and then to establish whether that relationship would remain the same for different years, locations and cultivars. The results reveal a positive correlation between CRR parameters I and S in wheat which was consistent among seasons and locations. However, characterizing the functional relationship between I and S is still critically important, because through this relationship researchers can identify the cultivars with unusually large or small S for a given I (McRoberts *et al.,* 2003), or through covariance analysis (when there are several pairs of I-S points for each cultivar), identify cultivars with an unusual I-S relationship compared with others. Moreover, the es-

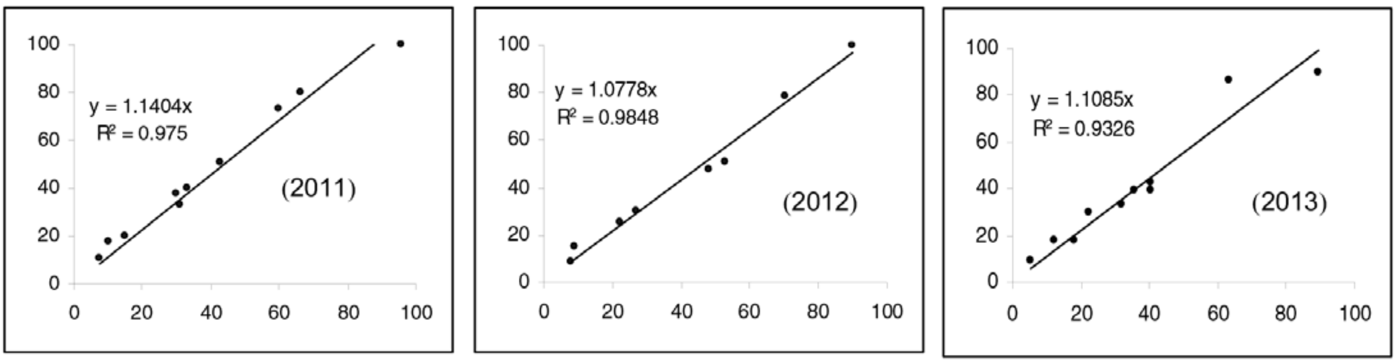

Fig. 1 - Relationship between incidence (I; proportion of diseased SCIs) and severity (S; proportion of SCI showing CRR symptoms) of wheat common root rot for three growing seasons.

timation of mean I from S would substantially reduce the work load in CRR quantification in field surveys and treatment comparisons.

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