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Aggressiveness of four Fusarium head blight species on wheat cultivars

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Abstract: Aggressiveness of four Fusarium head blight species (*F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiesti*) was studied on six Syrian wheat cultivars under controlled conditions. Two aggressiveness criteria: diseased-head severity (DHS, Fusarium infection) and disease development (DD, Fusarium spread) were visually estimated as percentage of heads showing Fusarium symptoms in wheat cultivars at the soft dough stage. Results showed significant differences among fungal isolates and wheat cultivars for the two tested criteria. The mean values of DHS evaluations ranged from 33.27 to 45.49% among fungal isolates, and from 29.62 to 42.22% among tested cultivars. The mean DD rating varied from 25.58 to 35.43% among fungal isolates, and from 25.33 to 34.01% among tested cultivars. Results in the current research highlighted that the level of resistance in Syrian cultivars to *Fusarium* species is characterized with low to moderate DHS and DD evaluations (%). Also, the results were comparable with those previously obtained using the same fungal isolates and wheat cultivars *in vitro*. The current study confirmed the suitability of *in vitro* method to be used as fast and reliable test to analyze aggressiveness in *Fusarium* species.

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1. Introduction

Fusarium head blight is one of the most destructive global diseases of wheat. In infected plants, it leads to kill the developing seed (prematurely bleached spikes) within moist conditions and moderate temperatures prevail during flowering. Since it was identified in 1884, severe epidemic outbreaks caused quantitative losses in yield of up to 50-75% (Parry *et al.,* 1995; McMullen *et al.,* 2012). It also reduces grain quality due to contamination of harvest with large amount of mycotoxins that cause toxicities to human and livestock (Maresca, 2013). At least seventeen *Fusarium* species with several habitats and types of mycotoxins produced have been associated with *Fusarium* species (Parry *et al.,* 1995). *Fusarium graminearum* is the main causal agent of this disease and has been subdivided into at least 11 cryptic species (O'Donnell *et al.,* 2004). Other species can cause Fusarium disease on a lesser scale such as *F. avenaceum*, *F. culmorum*, *F. solani*, *F. equiseti*, *F. verticillioides* and *F. poae*

(Xu *et al.,* 2008).

Understanding the interaction between wheat plants and Fusarium populations requires more detailed knowledge about the variation of aggressiveness (Wu *et al.,* 2005). Van der Plank (1968) defined aggressiveness as a quantitative ability of an isolate to cause disease on a susceptible host plant in a non-race-specific pathosystem. Aggressiveness is an important factor determining the potential ability of Fusarium isolates to cause Fusarium epidemics. Variability of quantitative component of pathogenicity in *F. graminearum* has been the subject of several studies (Parry *et al.,* 1995; Leonard and Bushnell, 2003; Wu *et al.,* 2005; McMullen *et al.,* 2012). However, other Fusarium species have attracted less pathogenic analyses (Xu *et al.,* 2008; Bakri *et al.,* 2012; Sakr, 2017). Fusarium resistance in wheat plants is conferred by quantitative trait loci (QTL) detected on all chromosomes (Loffler *et al.,* 2009). It is necessary to combine type I (resistance to initial infection) and type II (resistance to spreading) to get Fusarium resistant wheat plants (Loffler *et al.,* 2009).

In Syria, about 1.7 million hectares were sown to wheat, with an annual production of 3.9 million tons in 2011. Host-pathogen interactions were evaluated for several local wheat cultivars inoculated with fungal isolates of different species associated with *Fusarium* species, and differential reactions on cultivars were detected (Alazem, 2007; Talas *et al.,* 2011; Bakri *et al.,* 2012). Recently, Sakr (2017) analyzed aggressiveness of four Fusarium species *in vitro*, and significant differences were detected between pathogen isolates and wheat genotype. In order to underline pathogenic variation for *Fusarium* species collected from Ghab Plain, one of the principal Syrian wheat production areas, the objectives of the current study were to (1) evaluate aggressiveness of four isolates [F2 (*F. culmorum*), F27 (*F. verticillioides*), F35 (*F. solani*), and F43 (*F. equiesti*)] on six wheat Syrian cultivars under controlled conditions, and (2) compare results previously obtained by Sakr (2017) *in vitro* with the current data from floret inoculation under controlled conditions.

2. Materials and Methods

Fungal isolates and inoculum production

The fungal isolates of four *Fusarium* species [*F. culmorum* (F2), *F. verticillioides* (F27), *F. solani* (F35), and *F. equiesti* (F43)] were collected in 2015 from naturally wheat spikes exhibiting Fusarium symptoms from different locations of Ghab Plain in 2015. Isolates were identified morphologically according to Nelson *et al.,* (1983). The cultures were maintained in sterile distilled water at 4°C and freezing at -16°C until needed.

For inoculum preparation, four to six agar plugs out of the stored isolates were put over the surface of PDA in 9-cm Petri dishes and incubated for 10 days, at 22°C in the dark to allow mycelial growth and sporulation. Ten ml of sterile distilled water were added to each dish, and the resulting spore suspensions were adjusted to 5×10^4 spores/ml for inoculation following a count in a hemacytometer (Bakri *et al.,* 2012).

Wheat cultivars and growth chamber conditions

In the current study, aggressiveness for the four Fusarium head blight isolates was measured on six wheat cultivars previously analyzed *in vitro* ('Cham1', 'Cham7', 'Acsad65', 'Cham4', 'Cham6' and 'Douma4', most cultivated in different Syrian areas) under controlled conditions.

Wheat seeds were surface-sterilized with 5% sodium hypochlorite solution for 8 min and then washed six times in sterile distilled water (Purahong *et al.,* 2012). They were sown into plastic pots (15-cm) filled with 2 kg of sterilized soil (ten seeds per pot), and arranged in a complete randomized design with three replicates. Three plots per replicate were left non-inoculated as control treatment. Pots were placed in a growth chamber operated at 20°C during day and night with an 16-h photoperiod. Following emergence, plants were thinned to three per pot and nitrogen fertilizer was applied twice at two dates: emergence and tillering.

Aggressiveness tests

At 10-14 days after heading, spore suspensions of the four Fusarium head blight isolates or sterile distilled water (control) were sprayed one time into flowering spikes. Six flowering spikes were randomly selected within each replicate of the six cultivars. After the inoculum dried for 30 min, inoculated spikes were then kept covered for 48 h using polythene bags to ensure 100% RH.

Head blight symptoms were evaluated as percentage of spikes showing Fusarium symptoms after 7, 14, and 21 days, when plants were at the soft dough stage. Fusarium disease severity was visually estimated *in situ* for each inoculated spike using the Xue's *et al.,* (2004) scale. This scale includes nine levels of incidence expressed in percentage of bleached spike area on a head: 0 (no visible Fusarium symptoms) to

9 (severely diseased, spike dead). Each head was assessed separately in all experiments.

The values of diseased-head severity as percentage of infected spikes measured 21 days after inoculation (DAI) were considered, for each cultivar, a parameter to determine initial infection. The values of disease development calculated by the means of each evaluation; 7, 14 and 21 DAI over the estimation time were considered, for each cultivar, a parameter to determine pathogen spreading.

Statistical analyses

Statistical analyses of aggressiveness data were performed using StatView, 4.57® Abacus Concepts, Berkley, Canada. Before statistical analysis, the percentages were transformed using the Arcsines function. A complete randomized design with two factors (Fusarium isolate and wheat genotype) and 3 replications was used for aggressiveness analysis. Fisher's LSD test was used to compare the means at $P < 0.05$.

3. Results and Discussion

Understanding the interaction between Fusarium head blight species and wheat plants requires knowledge of the variation of quantitative component of pathogenicity (Wu *et al.,* 2005). With this in mind, aggressiveness variability for four local *Fusarium* species was analyzed by using a floret inoculation in a growth chamber on six wheat cultivars most cultivated in different Syrian areas.

Differences in aggressiveness of four *Fusarium* species (*F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiesti*) are indicated when isolates vary in the amount of damage that they cause in wheat plants. The results demonstrated that none of the six tested cultivars was immune from disease. However, typical Fusarium symptoms induced by the four isolates (F2, F27, F35 and F43) were clear and easy to score in the inoculated spikes, while no symptoms were present in the control (Fig. 1). The mean values of diseased-head severity (DHS) ranged from 33.27% to 45.49% as compared with 0% for the control treatment (Table 1). There were significant differences among four isolates (F isolates=4.376; Probability=0.0084). The mean values of disease development (DD) varied from 25.58% to 35.43% (Table 1). Significant differences among four isolates (F isolates=4.257; Probability=0.0096) were detected. Results in the current study showed that there was no interaction between Fusarium isolates and host plant for the two

Fig. 1 - Fusarium head blight symptoms on spike of Syrian wheat cultivar Cham4 inoculated with isolate F35 (*Fusarium solani*) compare with control (water).

parameters. This indicates to non-race-specific interaction described for this pathosystem (Loffler *et al.,* 2009).

High diseased-head severity and disease development values represent high aggressiveness (Parry *et al.,* 1995; Wu *et al.,* 2005; Xu *et al.,* 2008). Results shown in Table 1 indicate that the isolate F35 of *F. solani* was the most aggressive with a mean value of DHS of 45.49% and DD of 35.43%; followed by other tested isolates. These results are in accordance with previous analysis on the aggressiveness of theses isolates *in vitro*; Sakr (2017) observed that the isolate F35 was the most aggressive one, followed by other analyzed isolates. The current study confirmed the suitability of *in vitro* modified Petri-dish method to be used as fast and reliable test to analyze aggressiveness in Fusarium species. Results in the current study are comparable with those found by Alazem (2007) and Bakri *et al.,* (2012) for *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiesti* in which significant differences were detected for aggressiveness among fungal isolates in each Fusarium head blight species in a growth chamber.

The mean value of DHS and DD rating for six

Table 1 - Diseased-head severity and disease development scores in % among isolates of four Fusarium head blight species measured on six Syrian wheat cultivars

Isolate	'Cham1'	Cham7'	Acsad65'	Cham4'	Cham6'	Douma4'	Mean
Diseased-head severity scores (%)							
F2	25.92	25.92	29.62	37.03	44.44	44.44	35.24
F27	29.62	29.62	37.03	33.33	37.04	36.66	33.27
F35	33.33	33.33	40.74	58.88	47.77	47.77	45.49
F43	29.62	29.62	44.44	40.00	37.04	40	37.40
Mean	29.62	29.62	37.96	42.31	41.57	42.22	
	F isolates=4.376; Probability=0.0084						
	F cultivars=2.907; Probability=0.0226						
	F interactions=0.601 NS; Probability=0.8594						
Diseased development scores (%)							
F2	23.56	24.69	21.16	30.86	40.4	29.62	28.38
F27	24.69	23.56	28.49	25.64	24.69	26.45	25.58
F35	30.30	31.74	29.10	45.58	32.09	43.77	35.43
F43	22.79	25.64	37.03	33.95	26.45	29.10	29.16
Mean	25.33	26.41	28.94	34.01	30.91	32.23	
	F isolates=4.257; Probability=0.0096						
	F cultivars=1.870; Probability=0.1171						
	F interactions=1.199 NS; Probability=0.3051						

Fusarium head blight incidence scores were evaluated as percentage of spikes showing *Fusarium* species symptoms using the Xue's *et al*., (2004) scale.
F tests (P<0.05), NS= not significant.

wheat cultivars (Table 1) reflects the ability of the same isolate of the pathogen (F2, F27, F35 and F43) to distinguish different levels of resistance as observed for the same pathosystem (Alazem, 2007; Talas *et al.,* 2011). Also, the resistance of a given wheat cultivar is not related to a certain Fusarium species (Table 1). Significant differences were underlined for DHS (F cultivars=2.907; Probability=0.0226) and DD (F cultivars=1.870; Probability=0.1171) criteria among wheat cultivars (Table 1). The mean values of DHS evaluations ranged from 29.62 to 42.22% among tested cultivars. The mean DD rating varied from 25.33 to 34.01% among tested cultivars. Quantitative resistant wheat cultivars are identified by low DHS and DD values of the fungus compared with the susceptible one (Parry *et al.,* 1995). Results in the current research highlighted that the level of resistance in Syrian cultivars to Fusarium species is characterized with low to moderate DHS and DD evaluations (%). These results are in accordance with previous analysis on the comportment of local wheat cultivars in which differential reactions on cultivars were detected (Alazem, 2007; Bakri *et al.,* 2012; Talas *et al.,* 2011). However, Fusarium resistance scores ranged one fold and half between resistant and susceptible cultivars for the two tested parameters (Table 1). Thus our observation suggests that in resistant wheat cultivars, the development of the pathogen was slowed, and may be due to resistance mechanisms expressed by accumulation of QTL in host cultivars (Alazem, 2007; Talas *et al.,* 2011). Results in the current study showed that the level of quantitative resistance in the six wheat cultivars made it possible to detect significant differences between isolates of four *Fusarium* species. These results are in accordance with our previous analysis on the behavior of theses cultivars *in vitro* (Sakr, 2017). The variability of resistance for the Syrian cultivars is interesting and promising for ecological framing/breeding and also for improving resistance of wheat cultivars.

For *F. graminearum*, Purahong *et al.,* (2012) validated the modified Petri-dish method (used by Sakr, 2017) by highly significant correlation with the data from floret inoculation in adult plants in a growth chamber. Results indicted that the Petri-dish aggressiveness test conducted on other Fusarium species (*F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiesti*) is repeatable and stable with the six wheat cultivars in a growth chamber (Table 1). It will be necessary to analyze the pathogenic variation in a large number of Fusarium isolates on several wheat cultivars under controlled and field conditions to screen Fusarium resistance in Syrian wheat cultivars.

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