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ORIGINAL ARTICLE

Pathogenicity and quantitative resistance in Mediterranean durum and bread wheat cultivars of Syrian origin towards Fusarium head blight agents under controlled conditions

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Abstract

Although Syrian high-yielding wheat cultivars grown under Mediterranean conditions include acceptable levels of resistance to biotic constraints, little is known about their susceptibility to Fusarium head blight (FHB), a harmful disease of wheat cultivation worldwide. The capacity of 16 fungal isolates of four FHB species to confer the disease on spikes and spikelets of six widely grown old and modern Syrian durum and bread wheat cultivars with known *in vitro* quantitative resistance to FHB was evaluated. Quantitative traits were visually assessed using spray and point inoculations for determining disease development rates, disease incidence (DI) and disease severity (DS) under controlled conditions. Differences in pathogenicity and susceptibility among wheat cultivars were observed, emphasizing the need for breeders to include aggressive isolates or a mixture of isolates representative of the FHB diversity in their screenings for selection of disease resistant cultivars. Bread wheat cultivars showed lower levels of spike and spikelet damage than durum cultivars regardless of the date of cultivar release. Overall, the six wheat cultivars expressed acceptable resistance levels to initial fungal infection and fungal spread. Quantitative traits showed significant correlation with previous standardized area under disease progress curve ($AUDPC_{standard}$) data generated *in vitro*. Thus, the predictive ability of $AUDPC_{standard}$ appears to be crucial in assessing pathogenicity and resistance in adult wheat plants under controlled conditions. While in the Mediterranean countries the risk of disease is progressively increasing, the preliminary data in this report adds to our knowledge about four FHB species pathogenicity on a Syrian scale, where the environment is quite similar to some Mediterranean wheat growing areas, and show that Syrian cultivars could be new resistant donors with favorable agronomical characteristics in FHB-wheat breeding programs.

Keywords: Fusarium head blight (FHB), pathogenic variation, *Triticum aestivum*, *Triticum durum*, wheat resistance

Introduction

Wheat is the major strategically important crop occupying up to 24% of the cultivated Syrian area, with an annual total production of 3.8 million tons in 2011 (FAO/WFP 2015). Bread wheat (*Triticum aestivum*) is cultivated primarily in irrigated areas, and durum wheat (*T. durum*) in rainfed areas. Wheat production is entirely based on several old and modern wheat cultivars released for commercial production. Syrian genetically different cultivars may harbor gene complexes for quality characteristics and tolerance to biotic and abiotic constraints in the Mediterranean region (FWD 2007; Achtar *et al.* 2010; Bishaw *et al.* 2011, 2015).

Wheat, along with other small-grain cereals, can be heavily damaged by pathogenic *Fusarium* fungi causing Fusarium head blight (FHB). The disease is a major devastating disease of wheat cultivation recorded in many countries in America, Europe, the Mediterranean basin and Asia (Parry *et al.* 1995). Outbreaks of FHB occurring in seasons with frequent rainfall and high humidity during flowering, and lasting until soft dough and maturation stages, reduce yield and contaminate grain with dangerous mycotoxins (McMullen *et al.* 2012). A complex of 17 *Fusarium* species has been isolated from wheat heads with FHB symptoms. Globally, *F. graminearum* and *F. culmorum* have been found to be the main agents causing disease. In addition, other causal agents are less frequently encountered such as species *F. poae*, *F. cerealis* and *F. equiseti*, and, to a lesser extent, *F. solani*, *F. oxysporum* and *F. verticillioides* (Bottalico and Perrone 2002). In Syria, FHB species *F. culmorum* was the most frequent (43.8%), followed by *F. equiseti* (23.3%), *F. moniliforme* (14.6%), *F. proliferatum* (7.1%), *F. sambucinum* (2.9%), *F. compactum* (2.1%), *F. solani* (1.7%), *F. crookwellense* (0.8%), *F. avenaceum* (0.8%), and *F. semitectum* (0.4%). These were recovered during the spring of three seasons (2008–2010) from wheat seeds showing FHB across 20 locations/fields of the Ghab Plain, one of the principal Syrian wheat production areas (Al-Chaabi *et al.* 2018). The most frequent FHB species were *F. tricinctum* (30% of all *Fusarium* isolates), *F. culmorum* (18%), *F. equiseti* (14%) and *F. graminearum* (13%) from wheat spikes with FHB symptoms across five different Syrian provinces, except for the Ghab Plain (Alkadri *et al.* 2013).

Pathogenicity is the most important fungal trait affecting disease invasion and stability of host resistance. However, the expression of pathogenicity and quantitative resistance is largely influenced by the environment (Mesterhazy 1995). There have been some reports focusing on pathogenicity, defined as a disease induced by a pathogenic isolate on a susceptible host in a non-race-specific pathosystem, of FHB complex. The high degree of pathogenic variation, as detected *in vitro* and under controlled and field conditions of different isolates within and among the species sampled across a definite geographical scale, has been highlighted (Bottalico and Perrone 2002; Xue *et al.* 2004; Fernandez and Chen 2005; Xu *et al.* 2008; Foroud *et al.* 2012; Purahong *et al.* 2012; Sakr 2017, 2018a, c, d). However, little information is available on the comparative pathogenicity of other species associated with FHB on wheat as compared to the *F. graminearum* species complex (Xu *et al.* 2008; Sakr 2017). Parry *et al.* (1995) showed no strong evidence for cultivar-specific pathogenicity in the FHB complex.

Several control strategies used to manage FHB are difficult and expensive (McMullen *et al.* 2012). Development of disease resistant cultivars has been considered of high priority since it seems to be the most effective, economic, and environmentally safe way to control FHB disease (Lenc 2015; Lenc *et al.* 2015; Khaledi *et al.* 2018). Resistance in wheat to disease invasion is not race specific, i.e. the same plant cultivars display an equivalent ranking against all pathogen isolates. Two primary kinds of quantitative resistance to FHB are recognized as type I (resistance to initial infection after spray inoculation) and type II (resistance to fungal spread within the head after point inoculation) resistance (Mesterhazy 1995). Durum wheat is exposed to higher FHB infection levels than bread. However, no wheat cultivars are immune to FHB invasion. Most of them are susceptible and only a few are moderately resistant (Parry *et al.* 1995; Cai *et al.* 2005). Few Mediterranean wheat cultivars with improved levels of resistance have been recognized (Talas *et al.* 2011; Purahong *et al.* 2012; Alkadri *et al.* 2015; Hadjout *et al.* 2017). Till now, the Chinese cultivar Sumai 3 and its derivatives harboring major quantitative trait loci (*Fhb1*) for Type II resistance have been extensively used in wheat breeding, however, these valuable materials lack complete resistance to FHB (McMullen *et al.* 2012).

Although Syrian genetically different high-yielding wheat varieties grown under Mediterranean climatic conditions represent a particularly important group of genetic resources (FWD 2007; Achtar *et al.* 2010; Bishaw *et al.* 2011, 2015), little is known about their susceptibility to FHB agents (Talas *et al.* 2011; Alkadri *et al.* 2015). Indeed, there is still a need to fully investigate pathogenic and varietal differences in Syrian wheat plants tested in growth chambers where all biotic and abiotic factors are strictly controlled (Sakr 2017). Furthermore, comparing quantitative traits in wheat cultivars towards FHB agents among different experimental assays is of great importance to check whether their rankings are consistent (Purahong *et al.* 2012; Sakr 2017, 2018c, d, 2019). In this context, this study was undertaken to: (1) evaluate pathogenicity and quantitative resistance generated under controlled conditions on six old and modern Syrian durum and bread wheat cultivars infected with four FHB species and (2) compare the current findings with previous analyzed *in vitro* data.

Materials and Methods

Fungal isolates and inoculum preparation

Sixteen fungal isolates belonging to four FHB species: *F. culmorum* (F1, F2, F3, F28 and F30), *F. verticillioides* (synonym *F. moniliforme*) (F15, F16, F21 and F27), *F. solani* (F7, F20, F26, F29, F31 and F35), and *F. equiseti* (F43), were obtained from heads displaying observable disease symptoms collected during the 2015 growing season in several localities of the Ghab Plain. All isolates were morphologically identified on the basis of macroscopic features such as pigmentation and growth rates on the surface of potato dextrose agar (PDA, HiMedia, HiMedia Lab.) in 9-cm Petri dishes. Their microscopic characteristics involving size of macroconidia, and the presence of microconidia and chlamydospores were also identified (Leslie and Summerell 2006). For long term storage, fungal cultures were preserved in sterile distilled water at 4°C and frozen at –16°C (Sakr 2018b).

Prior to quantitative trait tests, the isolates were placed on PDA (Potato Dextrose Agar) Petri dishes and incubated for 10 days in an incubator (JSPC, JS Research Inc.) at 22°C in the dark to allow mycelial growth and sporulation. Following growth, 10 ml of sterile distilled water were added to each dish, and the resulting spore suspensions were diluted to desirable concentrations with a hemacytometer.

Wheat cultivars

Pathogenicity and quantitative resistance evaluations were performed using six widely grown high-yielding Syrian durum (Acsad65 released in 1984, Cham7 in 2004 and Cham9 in 2010) and bread (Cham4 released in 1986, Douma4 in 2007 and Bohoth10 in 2014) wheat cultivars with the most desirable agronomic characteristics and greatest resistance to biotic and abiotic stresses (FWD 2007; Bishaw *et al.* 2011, 2015). The six tested cultivars (four of these cultivars with differential resistance reactions to four FHB isolates (Sakr 2017) were classified using *in vitro* standardized area under disease progress curve ($AUDPC_{standard}$) of Petri-dish inoculation methodology (Sakr 2018a, c, d) as follows: Acsad65 classified as susceptible, Cham4, Cham7, Douma4 and Cham9 classified as susceptible to moderately susceptible, and Bohoth10 as moderately resistant. Regardless of the botanical origin, wheat cultivars, i.e., Acsad65 and Cham4 released before the year 2000 were considered as old materials, and the four remaining cultivars as modern wheats. Therefore, we were able to investigate the resistance reaction between the durums and breads as well as the new and old bread wheat cultivars.

Quantitative trait tests under controlled conditions

The 16 FHB isolates were individually inoculated on six wheat cultivars in a growth chamber at 20°C day/night temperature, and 16/8 h light/dark cycle to measure disease development rates, disease incidence (DI) and disease severity (DS) on discrete heads of the same cultivar as indicators of the pathogenicity and quantitative resistance. Wheat seeds were surface-sterilized with 5% sodium hypochlorite solution for 8 min and then washed six times in sterile distilled water. They were sown into plastic 15-cm pots containing sterilized clay soil. The potting soil consisted of 57% clay, 39% loam and 2% sand. The experimental design was a completely randomized design, comprising three replicates for each isolate. Three pots per replicate were left non-inoculated as control treatment. Following emergence, plants were thinned and nitrogen fertilizer was applied twice at two dates: emergence and tillering. Both types of inoculations, head and floret, were made on the discrete heads of the same cultivar in two separate experiments. When each spike reached 50% anthesis, the plants in a pot were sprayed with a spore suspension at 5×10^4 spores · ml^{-1} for DI evaluations and injected into two adjacent florets (10 spores · ml^{-1} per floret) at the middle of each spike (without wounding) for DS ratings of 16 FHB isolates or sterile distilled water (control). After inoculation, plants were individually covered with polythene bags for 48 h to create a high level of humidity to promote FHB infection. The two separate experiments were repeated twice on the six tested wheat cultivars.

Assessment of disease development rates was made with the initiation of symptoms about 1 week after inoculation. Subsequently, the progressive blighting of spikes was scored at 14, 21 and 28 days after inoculation (DAI), when plants were at the soft dough stage. Disease incidence was estimated as the percentage of spikes showing pathogenic symptoms at 21 DAI visually *in situ* for each inoculated spike on a 0 – no visible FHB symptoms to 9 – severely diseased, spike dead scale described by Xue *et al.* (2004). Disease severity was assessed as the percentage of spikelets on the inoculated spikes with visually detectable disease symptoms using Xue's *et al.* (2004) scale at 21 DAI. According to Bai *et al.* (2001), highly resistant cultivars may have DI and/or DS values as low as 5% while highly susceptible cultivars can reach 100% DI and/or DS; moderately resistant and susceptible cultivars have DI and/or DS values between these two extremes.

Statistical analysis

Data were performed using StatView, 4.57® Abacus Concepts, Berkley, Canada. Before statistical analysis, the percentages were transformed using angular transformation to stabilize variances. ANOVA incorporating Fisher's LSD test at $p \leq 0.05$ was used to differentiate pathogenicity of the 16 FHB isolates and quantitative resistance among the six tested wheat cultivars. The sample correlation coefficients (Pearson's r) were calculated using overall mean values per isolates at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

Results

Disease development rates

All 16 fungal isolates of four species tested with head and floret inoculations were capable of inducing FHB on wheat spikes and spikelets, suggesting a strong effect of different Fusarium isolates on the growth of the six wheat cultivars. Typical FHB symptoms were clear and easy to score in the inoculated spikes and spikelets, while no disease symptoms of FHB infection were detected in the negative control. The bleached spikelets and spikes appeared on the first evaluation at 7 DAI, and disease increased with time reaching the maximum severity 28 DAI (Figs. 1 and 2). Analysis of the relation between sporulation percentage based on the infection period ranged from 7 to 28 DAI showed that the four FHB species were somewhat close in the rate of FHB symptom development on any of the six wheat cultivars (Figs. 1 and 2). However, differential progression of disease and varying severities depending on species were observed on Cham4 (Figs. 1 and 2). Also, *F. equiseti*, represented only by one isolate, showed much more sporulation than the remaining species on Bohoth10 (Fig. 2).

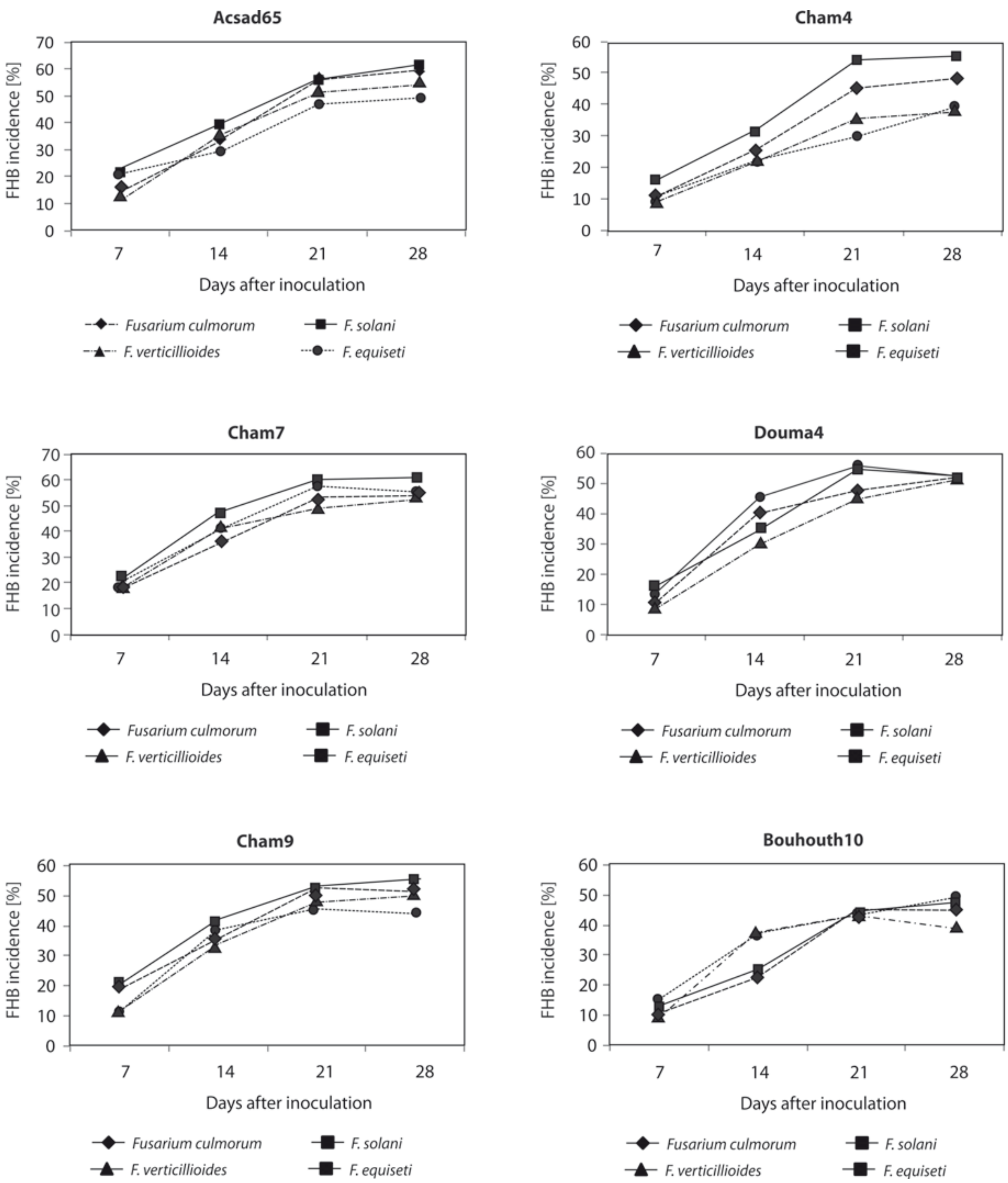

Fig. 1. Fusarium head blight progress curves based on disease incidence evaluations (%) for four *Fusarium* sp. on six Syrian wheat cultivars under controlled conditions. Each point is the mean of isolates each for *F. culmorum*, *F. solani*, *F. verticillioides*, and *F. equiseti*

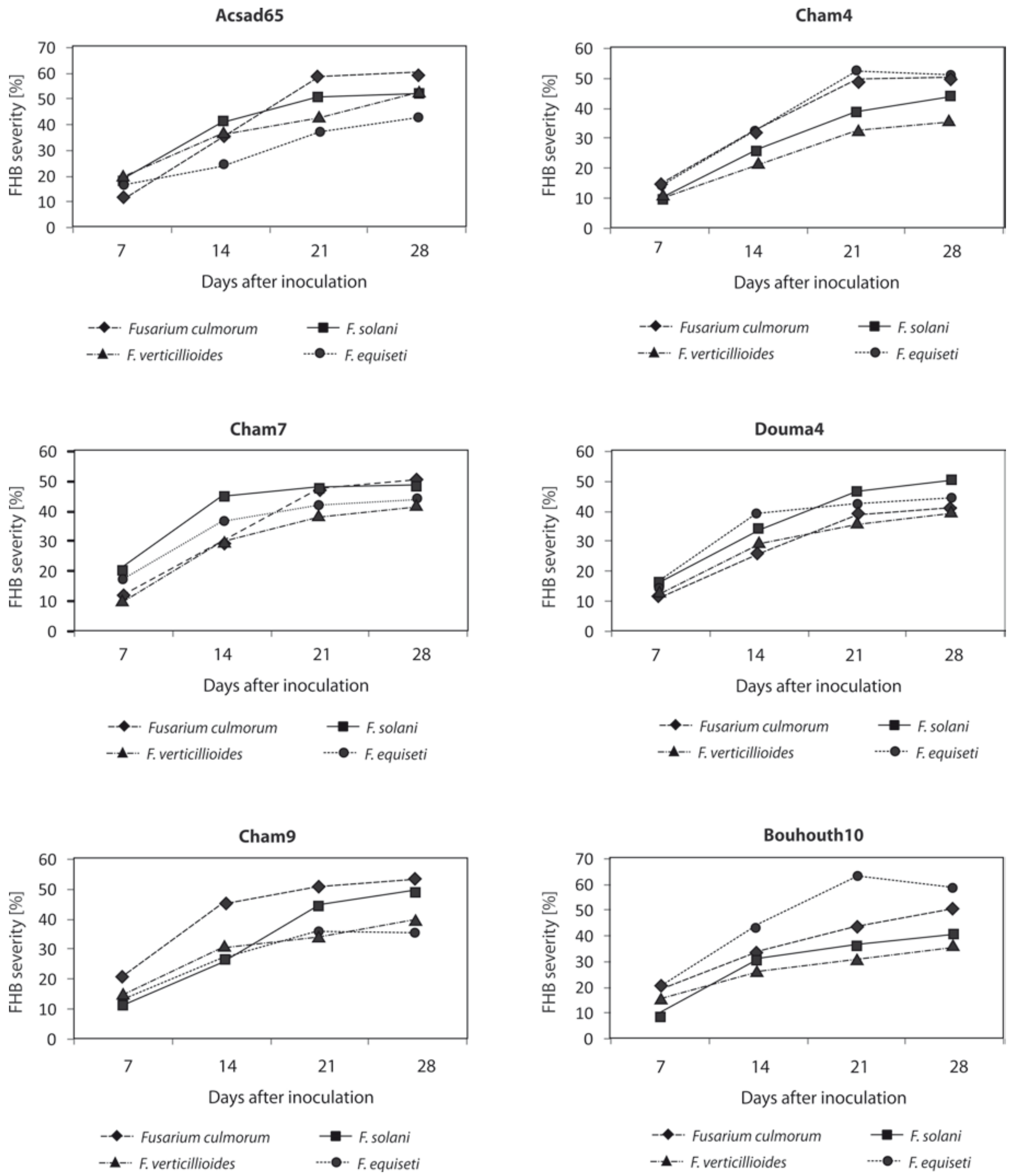

Fig. 2. Fusarium head blight progress curves based on disease severity evaluations (%) for four *Fusarium* sp. on six Syrian wheat cultivars under controlled conditions. Each point is the mean of isolates each for *F. culmorum*, *F. solani*, *F. verticillioides*, and *F. equiseti*

Disease incidence (DI) of head inoculation

The values (%) of DI estimations for all 16 FHB isolates on the six wheat tested cultivars, 21 DAI, are presented in Table 1. The mean DI values of fungi ranged two-fold from 38.4 to 66.8% on wheat cultivars as compared with 0% for the control treatment. Significant differences were observed in the mean DI scores among the four FHB species and among isolates within each species ($p \leq 0.0001$). The most pathogenic isolate was F20 (*F. solani*) whereas the two least pathogenic isolates were F15 (*F. verticillioides*) and F30 (*F. culmorum*). However, it was not possible to distinguish the four FHB species on wheat cultivars (Fig. 3). Correlation values of DI criterion among the six wheat cultivars showed that six of the 15 possible comparisons were significantly correlated (Table 2). Significant correlation coefficients were obtained between the data of DI and standardized area under disease progress curve ($AUDPC_{standard}$) previously generated *in vitro* for the six tested wheat cultivars (Sakr 2018a, c, d unpublished

Table 1. Disease incidence within and among four Fusarium head blight species measured on six Syrian wheat cultivars under controlled conditions

Fungal isolates (identification)	*Disease incidence [%]						
	Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10	mean
F1 (*F. culmorum*)	68.2	50.4	68.8	49.9	42.9	40.7	53.5 cd
F2 (*F. culmorum*)	49.2	61.1	48.2	67.5	58.8	54.0	56.5 bc
F3 (*F. culmorum*)	60.0	46.2	64.5	49.7	62.4	45.6	54.7 bcd
F28 (*F. culmorum*)	63.8	33.3	37.4	28.9	53.8	50.7	44.7 efg
F30 (*F. culmorum*)	51.0	33.6	35.5	39.1	37.8	33.3	38.4 h
F7 (*F. solani*)	52.0	41.4	48.0	38.8	48.0	40.9	44.9 efg
F20 (*F. solani*)	78.0	78.0	54.3	49.6	78.1	63.0	66.8 a
F26 (*F. solani*)	61.1	59.8	61.9	61.4	53.3	50.8	58.1 b
F29 (*F. solani*)	72.8	36.3	50.7	34.1	54.6	54.2	50.5 de
F31 (*F. solani*)	37.8	46.8	61.8	61.8	38.3	33.3	46.6 ef
F35 (*F. solani*)	41.6	59.4	65.7	77.6	40.8	31.8	52.8 cd
F15 (*F. verticillioides*)	44.0	39.6	43.5	24.7	49.5	38.3	39.9 h
F16 (*F. verticillioides*)	61.1	32.4	41.1	57.1	47.1	49.3	48.0 ef
F21 (*F. verticillioides*)	57.2	36.0	61.4	45.2	53.3	47.6	50.1 de
F27 (*F. verticillioides*)	47.3	36.0	42.8	52.6	39.8	39.0	42.9 gh
F43 (*F. equiseti*)	45.1	29.4	55.1	46.5	45.1	49.4	45.1 efg
Mean	55.6 a	45.0 d	52.5 ab	49.0 c	50.2 bc	45.1 d	

F isolates = 14.991; *p* = 0.0001

F cultivars = 12.342; *p* = 0.0001

F interactions = 4.876; *p* = 0.0001

*disease incidence values were evaluated as percentage of spikes showing FHB symptoms using Xue's *et al.* (2004) scale. According to Fisher's LSD test, means followed by the same letter are not significantly different at $p \leq 0.05$, *F* tests ($p \leq 0.05$) (*F*), probability (*p*). In the current study, isolates F2, F35, F27 and F43 were reanalyzed for disease incidence on Acsad65, Cham4, Cham7 and Douma4, however, pathogenic reaction for the four isolates was analyzed previously and presented by Sakr (2017). Also, pathogenic responses for the 16 isolates on Cham7 and Douma4 were analyzed previously and presented by Sakr (unpublished data)

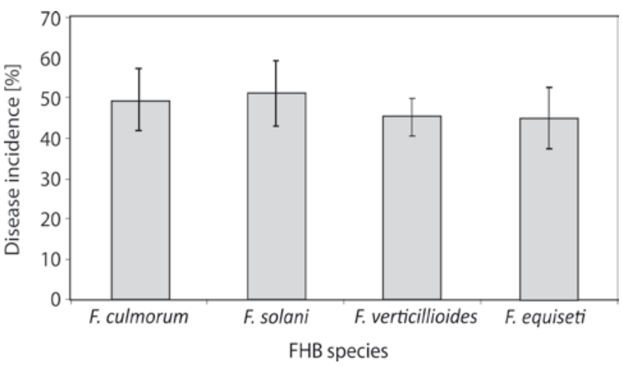

Fig. 3. Mean disease incidence of four Fusarium head blight species on six Syrian wheat cultivars under controlled conditions. Bars represent the standard errors of means

data): $r = 0.551$, $p \leq 0.05$ (Acsad65), $r = 0.600$, $p \leq 0.05$ (Cham4), $r = 0.627$, $p \leq 0.01$ (Cham7), $r = 0.652$, $p \leq 0.01$ (Douma4), $r = 0.531$, $p \leq 0.05$ (Cham9) and $r = 0.519$, $p \leq 0.05$ (Bohoth10) (Fig. 4).

Head inoculation of spikes conducted to asses Type I FHB resistance revealed statistically significant differences ($p \leq 0.0001$) in the resistance of wheat cultivars. The mean fraction of plants showing disease symptoms ranged from 45.0 to 55.6%. The two bread cultivars Bohoth10 (modern) and Cham4 (old) had the lowest mean DI values, while the durum cultivar Acsad65 (old) showed the highest mean DI score. Based on Type I resistance, Bohoth10 and Cham4 were moderately resistant cultivars, Douma4 (bread, modern) was moderately susceptible, the two durums, modern Syrian cultivars Cham7 and Cham9, were susceptible to moderately susceptible, and Acsad65 was susceptible.

Disease severity (DS) of point inoculation

Table 3 shows the scores (%) of DS evaluations for all 16 fungal isolates on the six wheat tested cultivars, at 21 DAI. The mean DS ratings of fungi varied two-fold from 30.7 to 54.2% on wheat cultivars as compared with

Table 2. Correlation coefficients of disease incidence criterion among six Syrian wheat cultivars infected with 16 isolates of four Fusarium head blight species

	Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10
Acsad65	1.000					
Cham4	0.253 ns	1.000				
Cham7	0.041 ns	0.460 ns	1.000			
Douma4	–0.274 ns	0.532*	0.509*	1.000		
Cham9	0.660**	0.556*	0.090 ns	–0.108 ns	1.000	
Bohoth10	0.720**	0.298 ns	–0.057 ns	–0.117 ns	0.834***	1.000

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ns = not significant

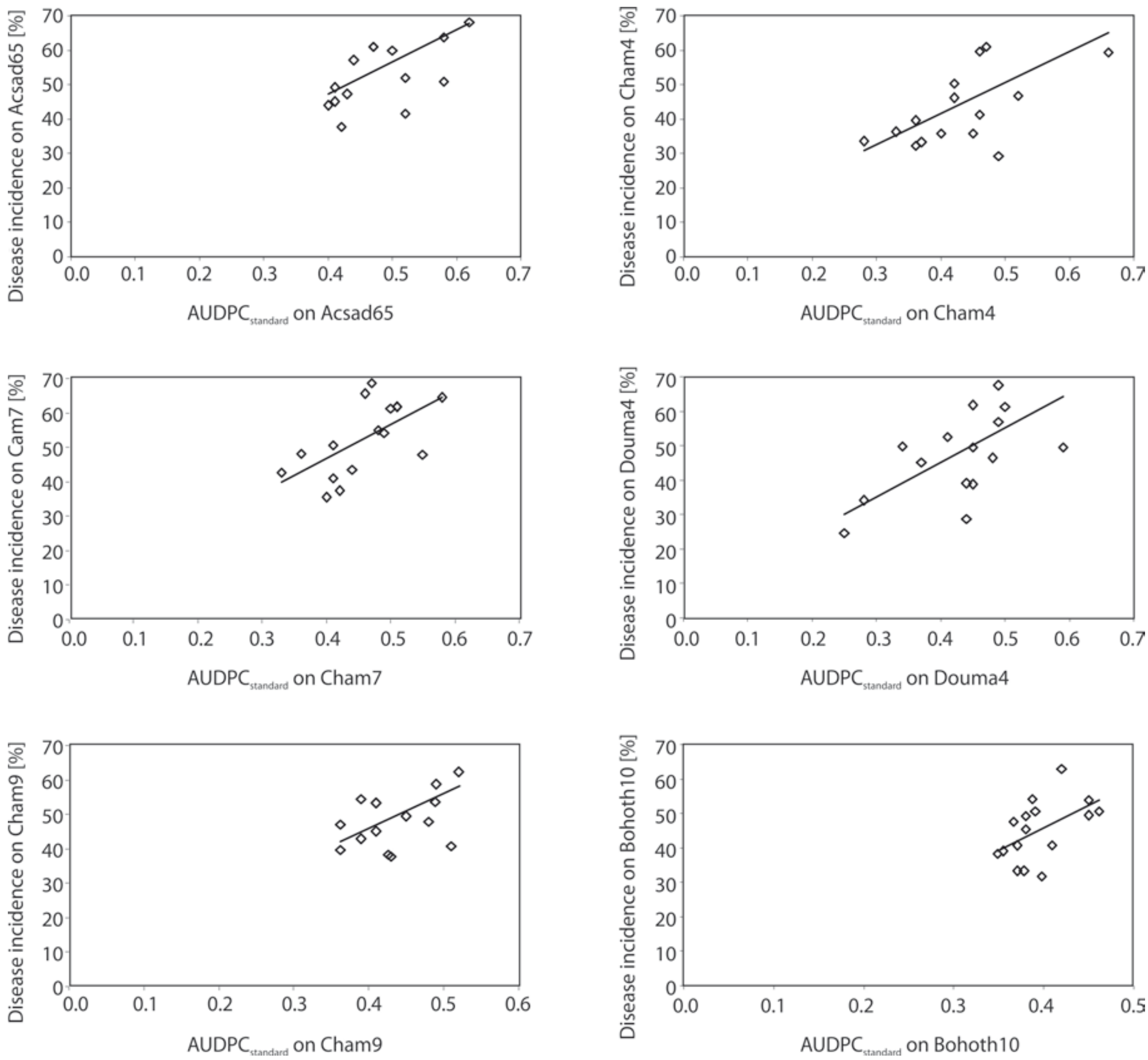

Fig. 4. Correlation coefficients between standardized area under disease progress curve ($AUDPC_{standard}$) and disease incidence in head inoculation in growth chamber on six Syrian wheat cultivars infected with 16 fungal isolates of four Fusarium head blight species determined by Pearson correlation coefficient, $r = 0.551$, $p \leq 0.05$ (Acsad65), $r = 0.600$, $p \leq 0.05$ (Cham4), $r = 0.627$, $p \leq 0.01$ (Cham7), $r = 0.652$, $p \leq 0.01$ (Douma4), $r = 0.531$, $p \leq 0.05$ (Cham9) and $r = 0.519$, $p \leq 0.05$ (Bohoth10). Data on Cham7 and Douma4 were previously presented by Sakr (unpublished data)

0% for the negative control. There were significant differences in FHB severity among the four FHB species and among isolates within each species ($p \leq 0.0001$). F3 (*F. culmorum*) showed the greatest pathogenicity, while F15 (*F. verticillioides*) was the least pathogenic isolate. As shown in Figure 5, the four FHB species were not different at the level of pathogenicity. Table 4 shows the correlation values of DS criterion among

Table 3. Disease severity within and among four Fusarium head blight species measured on six Syrian wheat cultivars under controlled conditions

Fungal isolates (identification)	*Disease severity [%]						
	Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10	Mean
F1 (*F. culmorum*)	68.2	63.0	62.6	45.3	31.2	48.1	53.1 b
F2 (*F. culmorum*)	36.9	75.2	40.1	56.3	39.2	67.5	52.5 ab
F3 (*F. culmorum*)	55.0	54.6	71.7	33.1	72.8	38.0	54.2 a
F28 (*F. culmorum*)	69.6	29.6	31.2	20.6	63.5	32.3	41.1 defg
F30 (*F. culmorum*)	63.8	25.2	32.3	35.5	47.3	30.3	39.1 efg
F7 (*F. solani*)	57.2	27.6	32.0	55.4	52.8	36.8	43.6 cdef
F20 (*F. solani*)	46.8	41.6	38.8	41.4	57.3	33.6	43.3 cdef
F26 (*F. solani*)	32.9	41.4	41.3	47.3	36.9	35.2	39.2 efg
F29 (*F. solani*)	83.2	23.1	46.1	31.0	54.6	34.8	45.5 cde
F31 (*F. solani*)	50.4	31.2	68.7	44.1	42.6	33.3	45.1 cde
F35 (*F. solani*)	31.2	70.3	59.7	59.7	25.5	43.7	48.4 bc
F15 (*F. verticillioides*)	36.0	32.4	36.3	16.5	31.5	31.4	30.7 h
F16 (*F. verticillioides*)	37.6	46.8	29.4	51.9	29.0	49.3	40.7 efg
F21 (*F. verticillioides*)	52.8	24.0	55.8	37.7	49.2	22.0	40.3 efg
F27 (*F. verticillioides*)	47.3	27.0	32.9	40.5	29.0	21.3	33.0 g
F43 (*F. equiseti*)	36.2	52.7	42.4	51.7	36.2	62.9	47.0 bcd
Mean	50.3 a	41.6 c	45.1 b	41.7 c	43.7 bc	38.8 d	

F isolates = 15.838; p = 0.0001

F cultivars = 13.991; p = 0.0001

F interactions = 10.589; p = 0.0001

*disease severity values were evaluated as percentage of spikes showing FHB symptoms using Xue's *et al.* (2004) scale. According to Fisher's LSD test, means followed by the same letter are not significantly different at $p \leq 0.05$, F tests ($p \leq 0.05$) (F), probability (p). Pathogenic responses for the 16 isolates on Cham7 and Douma4 were analyzed previously and presented by Sakr (unpublished data)

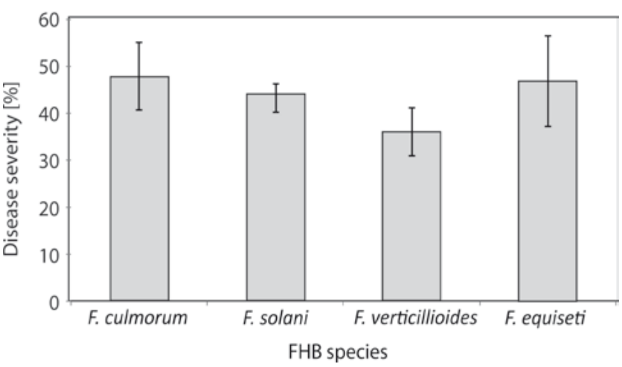

Fig. 5. Mean disease severity (%) of four Fusarium head blight (FHB) species on six Syrian wheat cultivars under controlled conditions. Bars represent the standard errors of means

the six wheat cultivars indicating that four of the 15 possible comparisons were significantly correlated. There were significant correlations between the data of DS and AUDPC$_{standard}$ previously obtained *in vitro* for all wheat cultivars (Sakr 2018a, c, d): $r = 0.551$, $p \leq 0.05$ (Acsad65), $r = 0.600$, $p \leq 0.05$ (Cham4), $r = 0.627$, $p \leq 0.01$ (Cham7), $r = 0.652$, $p \leq 0.01$ (Douma4), $r = 0.531$, $p \leq 0.05$ (Cham9) and $r = 0.519$, $p \leq 0.05$(Bohoth10) (Fig. 6).

The point-inoculated spikelet to test Type II highlighted significant differences ($p \leq 0.0001$) in the susceptibility of wheat cultivars. The mean FHB severity scores varied from 38.8 to 50.3%. Bohoth10 showed the lowest infection levels and Acsad65 was the most affected cultivar. Regarding Type II resistance, Bohoth10 was a moderately resistant cultivar, Douma4 and Cham4 were moderately susceptible, Cham7 and Cham9 were susceptible to moderately susceptible, and Acsad65 was susceptible. Overall, bread wheat cultivars showed lower levels of spike and spikelet damage than durum cultivars regardless of the date of cultivar release.

A significant correlation was seen between the mean values of DI and DS of the 16 fungal isolates measured on all tested wheat cultivars ($r = 0.499$, $p \leq 0.05$). Also, correlation coefficients between the resistance measured by AUDPC$_{standard}$ of Petri-dish inoculation and both FHB Type I and Type II resistance were

Table 4. Correlation coefficients of disease severity criterion among six Syrian wheat cultivars infected with 16 isolates of four Fusarium head blight species

	Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10
Acsad65	1.000					
Cham4	–0.461ns	1.000				
Cham7	0.076 ns	0.339 ns	1.000			
Douma4	–0.450 ns	0.562*	0.097 ns	1.000		
Cham9	0.562*	–0.312 ns	0.134 ns	–0.423 ns	1.000	
Bohoth10	–0.333 ns	0.792***	0.028 ns	0.563*	–0.263 ns	1.000

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ns = not significant

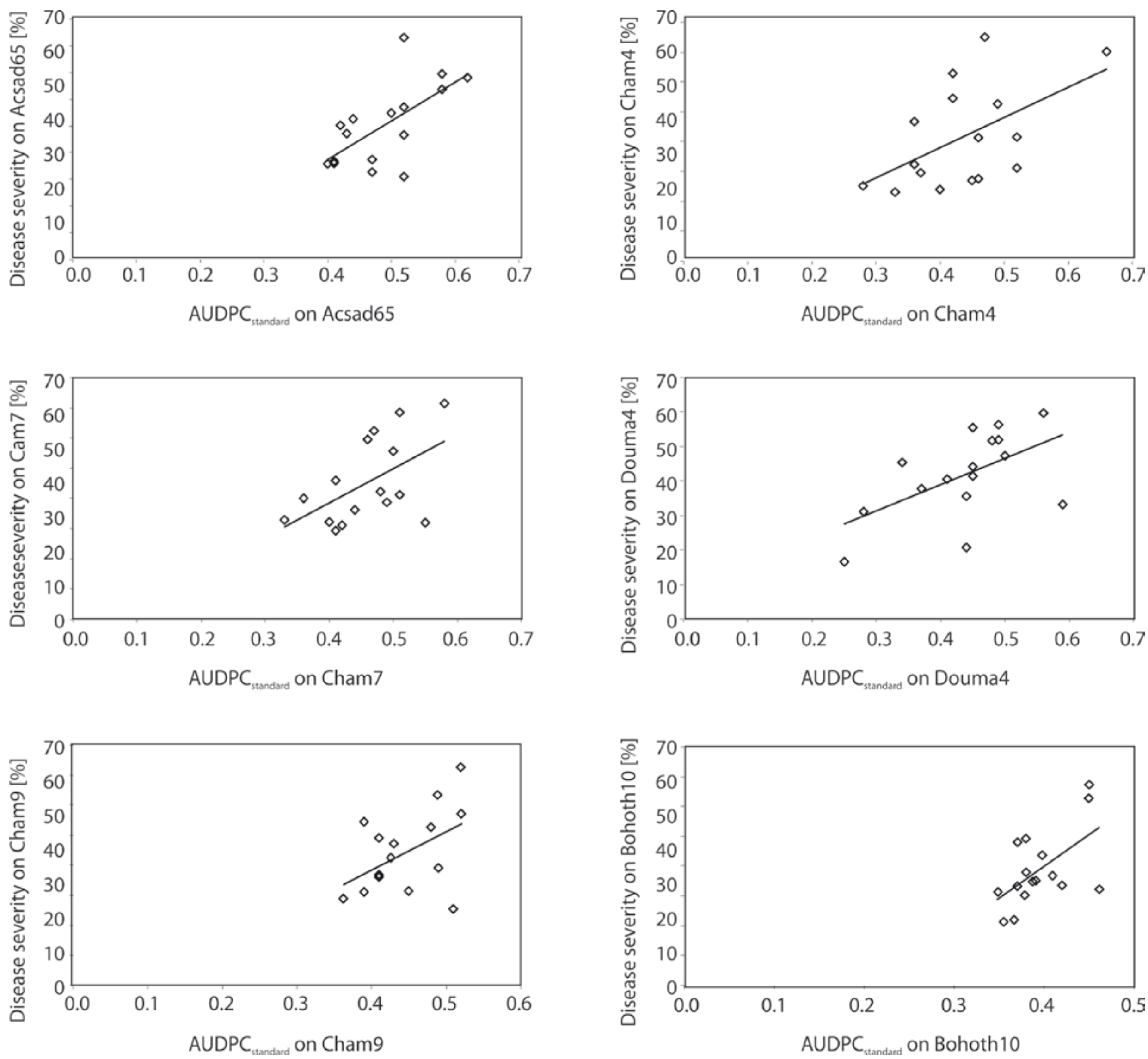

Fig. 6. Correlation coefficients between standardized area under disease progress curve ($AUDPC_{standard}$) and disease severity in floret inoculation in growth chamber on six Syrian wheat cultivars infected with 16 fungal isolates of four Fusarium head blight species determined by Pearson correlation coefficient, $r = 0.551$, $p \leq 0.05$ (Acsad65), $r = 0.600$, $p \leq 0.05$ (Cham4), $r = 0.627$, $p \leq 0.01$ (Cham7), $r = 0.652$, $p \leq 0.01$ (Douma4), $r = 0.531$, $p \leq 0.05$ (Cham9) and $r = 0.519$, $p \leq 0.05$ (Bohoth10). Data on Cham7 and Douma4 were previously presented by Sakr (unpublished data)

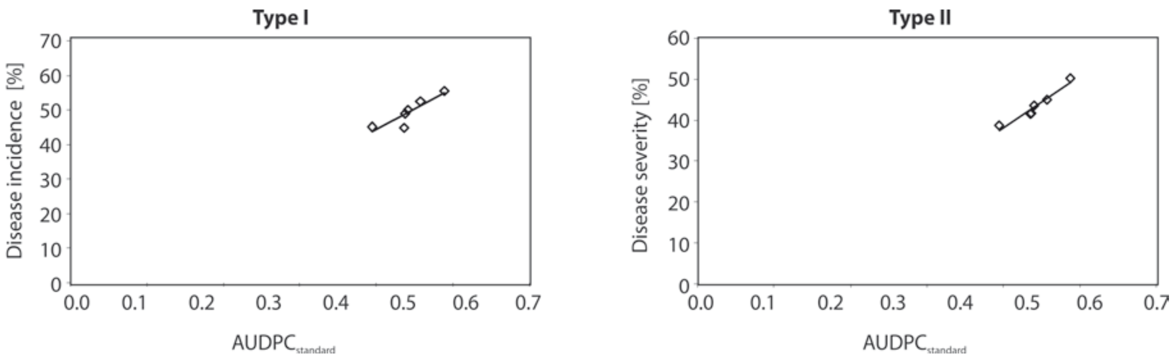

Fig. 7. Correlation between the resistance measured by standardized area under disease progress curve (AUDPC$_{standard}$) of Petri-dish inoculation assay and spraying inoculation (Type I) and point inoculation (Type II) on six Syrian wheat cultivars infected with 16 fungal isolates of four Fusarium head blight species determined by Pearson correlation coefficient, $r = 0.965$, $p \leq 0.001$ (Type I) and $r = 0.888$, $p \leq 0.05$ (Type II)

significant ($r = 0.965$, $p \leq 0.001$ and $r = 0.888$, $p \leq 0.05$, respectively) (Fig. 7). Moreover, correlation between Type I resistance and Type II resistance was significant ($r = 0.931$, $p \leq 0.01$). The interactions of fungal isolates × wheat cultivars for any of the measurements taken were significant ($p \leq 0.0001$ for DI, $p \leq 0.0001$ for DS, respectively).

Discussion

Knowledge of quantitative traits in terms of pathogenicity and resistance in any environment is crucial for predicting the pathogenic potential of FHB agents and for the deployment of resistant wheat cultivars in a given location (Lenc 2015; Lenc *et al.* 2015; Khaledi *et al.* 2018). This necessitates that their measurements should be taken in a growth chamber ensuring control of fungal spore concentration and quantity, mode of infection, heading date and environment after inoculation. While in the Mediterranean countries the risk of disease is progressively increasing (Parry *et al.* 1995; McMullen *et al.* 2012), the preliminary data in this report add to our knowledge about four FHB species pathogenicity on a Syrian scale, where the environment is quite similar to some Mediterranean wheat growing areas, and show that Syrian cultivars could be new resistant donors with favorable agronomical characteristics in FHB-wheat breeding programs. In addition, the current research investigated the potential use of *in vitro* quantitative trait indices in predicting FHB data generated under controlled conditions in wheat.

The selection of FHB species used in our study: *F. culmorum* (31.3%), *F. solani* (37.5%), *F. verticillioides* (25.0%) and *F. equiseti* (6.3%), was reflective of other populations recovered from the Ghab Plain and other principal Syrian wheat production areas (Alkadri *et al.* 2015; Al-Chaabi *et al.* 2018); *F. culmorum* was the most frequent causing agent in Syria. In comparison to other Mediterranean regions, i.e., Algeria, Tunisia and other European countries, *F. culmorum* was found to be the major fungal pathogen associated with FHB (Gargouri-Kammoun *et al.* 2009; Pasquali *et al.* 2016; Touati-Hattab *et al.* 2016). This observation is of great importance since wheat materials in Mediterranean countries may be exposed to the same FHB causing agent, *F. culmorum*. So, the occurrence of *F. culmorum* appears to be increasing in warmer regions like the Mediterranean basin (Parry *et al.* 1995). In addition, Syrian wheat cultivars were usefully deployed in a Mediterranean breeding program involving a crossing of cultivars from Europe and Syria, Waha from ICARDA (Hadjout *et al.* 2017). By using a field experiment with four different *F. culmorum* strains, two potential wheat lines were shown to exhibit a higher resistance to both initial fungal infection and disease spread and to mycotoxin contamination than a set of commercial cultivars (Hadjout *et al.* 2017).

The variability in pathogenicity of four FHB species on Syrian wheat cultivars was not fully reported under controlled conditions (Sakr 2017). All analyzed species generated FHB symptoms on wheat spikes and spikelets, thus they are pathogenic. This study showed that the four *Fusarium* sp. were somewhat similar in the rate of FHB symptom development on any of the six wheat cultivars (Figs. 1 and 2). Also, results shown in Figures 3 and 5 indicated an overall similar comparative pathogenicity in the four Syrian FHB species because of similarity in spike and spikelet damage among the 16 fungal isolates. Fernandez and Chen (2005) observed an apparent lack of difference in pathogenicity between *F. culmorum* and *F. graminearum* on wheat. Similarly, Sakr (2018a, c, d) did not cluster the same fungal species on all tested wheat cultivars using an *in vitro* criterion: standardized area under disease progress curve ($AUDPC_{standard}$). Our results are not comparable with other reports showing that the four FHB species included in the present research were classified as highly, moderately and weakly pathogenic on wheat plants. *Fusarium culmorum* was characterized by high pathogenicity, *F. equiseti* was moderate, and *F. verticillioides*, *F. solani* were weakly pathogenic (Bottalico and Perrone 2002; Xue *et al.* 2004). The differences in these data may be attributable to the contrasting isolates and host cultivars used in this study and previous work. The origin of FHB cultures may play a crucial role in this pathogenic similarity (Sakr 2018a).

Pathogenicity (measured as FHB disease incidence and severity) differed significantly among the 16 fungal isolates inoculated onto spikes and spikelets of six wheat cultivars in the growth chamber (Tables 1 and 3). The values of DI and DS underlined a variation in pathogenicity within and among four tested FHB species. Inter and intraspecific differences were observed in the pathogenicity of several FHB species toward wheat genotypes (Xue *et al.* 2004). Differences in pathogenicity among 16 FHB isolates of four species may be due to mutation, genetic recombination or selection. Our results showed that significant correlation ($r = 0.499$, $p \leq 0.05$) was detected between the mean values of DI and DS of fungi on all tested cultivars; it seems that mechanisms underlying these two pathogenicity criteria share the same genetic background. This research supports the view that correlations of different pathogenicity indices exist and are stable with *in vitro* and under controlled assays (Figs. 4 and 6), suggesting that seedling stage indices can predict pathogenic traits obtained in the growth chamber. $AUDPC_{standard}$ could reflect aspects of pathogen development at early stages of plant growth by promoting the interaction between wheat tissues and fungi (Purahong *et al.* 2012; Sakr 2019). The situation in an *in vitro* assay was similar to artificial inoculation because FHB species need to overcome the morphology of the spike and spikelet and they could directly penetrate and infect germinating seeds (Purahong *et al.* 2012; Sakr 2019).

More interestingly, results shown in this research indicated that a complex genotype interaction may or may not exist among bread and durum old and modern cultivars and pathogens for DI and DS criteria (Tables 2 and 4). Our results agree with previous *in vitro* $AUDPC_{standard}$ data showing the presence or absence of a cultivar-specific pathogenicity in the same fungal isolates and wheat cultivars (Sakr 2018a, c, d). This type of specific interaction was previously reported by Foroud *et al.* (2012), who noted that *F. graminearum* pathogenicity is host-dependent in wheat. Parry *et al.* (1995) showed no strong evidence for specific pathogenicity interactions among fungal species implicated in the FHB complex and wheat plants. In our investigation, a differential interaction between quantitative resistance genes in wheat and FHB isolates was based on pathogenic responses registered on plant materials. This method allowed for detecting the isolate specificity which was already shown to occur as a significant cultivar-isolate interaction in barley-*Puccinia hordei* by Gonzalez *et al.* (2012) and -*Blumeria graminis* by Romero *et al.* (2018). Thus, it seems that a minor gene-for-minor gene interaction may exist between six wheat cultivars and 16 fungal isolates, suggesting that the isolate-specific effectiveness may lead to erosion of wheat quantitative resistance to FHB invasion. However, further investigation is required in order to draw any final conclusions.

The data obtained show that all tested cultivars differed in their FHB resistance and susceptibility behavior (Tables 1 and 2), in which initial fungal infection (Type I) and the spread of the pathogen within the head (type II resistance) were related to cultivar resistance (van Ginkel *et al.* 1996), emphasizing the need for breeders to include aggressive isolates or a mixture of isolates representative of the FHB diversity in their screenings for selection of disease resistant cultivars. Resistance of a given tested cultivar is not related to a certain FHB species. Also, the six wheat cultivars which can resist highly pathogenic isolates of a certain species can also resist other pathogenic isolates from another species (Tables 1 and 2). The results here are consistent with the ideas of Xue *et al.* (2004). Overall, the six wheat cultivars expressed acceptable resistance levels to initial fungal infection and fungal spread (Tables 1 and 2). Our data support the view that Syrian wheat cultivars can be promising sources of resistance to FHB under Mediterranean conditions as observed for other wheat cultivars (Talas *et al.* 2011; Purahong *et al.* 2012; Alkadri *et al.* 2015; Hadjout *et al.* 2017) because of lack of 100% resistance to FHB in the current commercial varieties (Mesterhazy *et al.* 2011).

Bread wheat is more resistant to FHB infection than durum, since there is no high disease resistance (Mesterhazy 1995). Overall, bread wheat cultivars showed lower infection spike and spikelet levels than durum cultivars regardless of the date of cultivar release, indicating that old and modern breads provided broad, though incomplete, resistance to the four *Fusarium* sp. species examined compared to old and modern durums (Tables 1 and 2, Fig. 8). As expected, our data confirmed previous *in vitro* findings that `Acsad65, old durum` was susceptible and `Bohoth10, modern bread` was moderately resistant (Sakr 2018a) (Fig. 8). The reliability of this cultivar order was validated by the significant correlation between the $AUDPC_{standard}$ of Petri-dish inoculation and both FHB

Petri-dish assay	Cultivars	Head and point inoculations
Moderately resistant	Bohoth10	Moderately resistant
Susceptible to moderately susceptible	Cham4 Douma4	Moderately susceptible
	Cham9 Cham7	Susceptible to moderately susceptible
Susceptible	Acsad65	Susceptible

Fig. 8. Ranking of six Syrian wheat cultivars based on area under disease progress curve in 16 Fusarium head blight-mediated *in vitro* Petri-dish assay and on FHB incidence and severity following head and point inoculations, respectively, of spikes and spikelets in a growth chamber

Type I and Type II resistance (Fig. 7). More importantly, head and spikelet inoculation tests made it possible to divide the group which included four remaining cultivars classified as susceptible to moderately susceptible *in vitro* into two distinct sub-groups as the two modern durums `Cham7 and Cham9` classified as susceptible to moderately susceptible and the two breads `Cham4, old and Douma4, modern` recognized as moderately susceptible (Fig. 8). In general, wheat plants with the lowest values for $AUDPC_{standard}$ were those having the highest levels of FHB Type I and Type II resistance scores (Fig. 7). The biological clarification for an association between *in vitro* and adult plant responses to FHB infection remains largely speculative, but it can be hypothesized that similar genetic pathways become activated at both developmental stages. Our data suggest that the assessment of resistance level is repeatable and stable under several experimental conditions.

Although the differences in reaction to the four Fusarium sp. were generally similar to *in vitro* observations of the six wheat cultivars in FHB resistance (Sakr 2018a, c, d), there were significant cultivar × isolate interactions observed in the present study, which agree with a previous report on wheat (Xue *et al.* 2004). Taking into consideration that there were wide genetic variations among some of the tested wheat cultivars, i.e., Cham4 and Cham7 (Achtar *et al.* 2010), selection and development of FHB resistant cultivars must be carried out by phenotypic selection and under epidemic conditions as recommended by Cai *et al.* (2005) that selection should be independent of plant height, flowering date and maternal genotype within a cross.

While the relationship between the mean values for Type I and Type II resistance was found to be significant ($r = 0.931$, $p \leq 0.01$), it is of great importance to combine the two types in breeding programs to get FHB Syrian resistant plants under Mediterranean conditions, where the climatic conditions are quite similar to some Syrian wheat growing areas. Our results are in accordance with those found by Browne *et al.* (2005) on 30 soft red winter wheat cultivars for *F. graminearum* under field conditions ($r = 0.87$, $p \leq 0.01$). However, there was no significant correlation between both types on 29 winter Korean wheat cultivars for *F. graminearum* under controlled conditions (Shin *et al.* 2014). It has been hypothesized that the genetic background of initial fungal infection differs from that of fungal spread (van Ginkel *et al.* 1996); however, the relationship between FHB damage described as Type I and Type II resistance is not fully understood (Browne *et al.* 2005). It would be helpful to have included a cultivar with well-characterized resistance (like Sumai) or susceptibility for a frame of reference. Further work would be strengthened by the inclusion of isolates from additional Mediterranean wheat-growing regions. Additional research using a broad range of available Syrian old and modern wheat cultivars would provide more choices in FHB-wheat breading programs to get resistant plants under Mediterranean conditions.

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