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## AGGRESSIVENESS OF *FUSARIUM* SPECIES CAUSING HEAD BLIGHT IN BARLEY LANDRACES GROWN UNDER FERTILE CRESCENT CONDITIONS

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### ABSTRACT

Aggressiveness assessment of *Fusarium* head blight (FHB) is crucial for understating the interaction between host and pathogen in the *Hordeum vulgare*-FHB system. More effective and accurate disease evaluation methods should be sought for successful identification of pathogenic variation in FHB species. In order to achieve this objective, a pot experiment under natural climatic conditions over two growing seasons 2017/18 and 2018/19 was conducted to evaluate aggressiveness on a set of 16 fungal isolates of four Syrian FHB species. Then, the current findings were compared with previous analyzed *in vitro* and growth chamber data. Pathogenic variation was quantified as disease development rates, disease incidence (DI) and *Fusarium*-damaged kernels (FDK) on two barley landraces widely cultivated along the Fertile Crescent: Arabi Aswad (AS) and Arabi Abiad (AB) varying in resistance to FHB causing agents. All tested FHB species caused disease symptoms on the two barley landraces; and FHB disease progressed slowly and less severally as generally observed on AS compared with AB. Regarding DI, there were significant differences in aggressiveness *intra*- and *inter*-species and in susceptibility between AS and AB. FDK criterion did not differentiate FHB isolates and the two tested landraces. The values of DI were significantly correlated with pathogenicity traits previously obtained *in vitro* and under controlled conditions over the two growing seasons. Our current findings confirmed previous *in vitro* and growth chamber data that AS exhibits higher resistance levels to initial fungal infection compared to AB. AS may be a new source for livestock and AB for malting and brewing resistance breeding programs because of their resistance to FHB. To our best knowledge, this is the first report investigating both: pathogenicity on AS and AB grown under Fertile Crescent conditions and potential use of *in vitro* and growth chamber aggressiveness indices in predicting FHB data generated under natural climatic conditions in barley.

**Keywords:** AUDPC<sub>standard</sub>, controlled conditions, FHB species, *Hordeum vulgare*, latent period.

### INTRODUCTION

*Fusarium* head blight (FHB) is a globally important disease on barley (*Hordeum vulgare* L.). When rainfall and warm weather are prevalent around the time of flowering (Landschoot *et al.*, 2012), the disease causes premature bleaching of spikelets resulting in sterility or production of deformed, shrunken and pale, rose colored kernels commonly referred to as *Fusarium*-damaged kernels (Jin *et al.*, 2014). The result is a reducing in grain

yield and quality by producing mycotoxins (especially deoxynivalenol (DON)) that make barley unfit for food and feed; and causing technological problems of malt production and brewing quality (Parry *et al.*, 1995; He *et al.*, 2015).

FHB is caused by a complex of at least seventeen *Fusarium* species, but just four of them are the main FHB agents, such as *F. graminearum*, *F. poae*, *F. avenaceum* and *F. culmorum*. Other fungi including *F. crookwellense*, *F. equiseti* and *F. sporotrichioides* could be present with a low incidence (Parry *et al.*, 1995; Xue *et al.*, 2006). Although *F. verticillioides* and *F. solani* are known to cause rot diseases in many crops worldwide, these species were isolated from head blight infected wheat samples in Argentina (De Galich,

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1997), India (Saharan *et al.*, 2003) and Syria (Sakr, 2017). Barley resistance to FHB is partial trait and conditioned by a polygenic system (quantitative trait loci detected on chromosome 2H bin8 (2Hb8) and 6H bin7 (6Hb7) in the six-rowed cultivar Chevron), with no strong evidence for cultivar-specific aggressiveness in the FHB complex (Parry *et al.*, 1995; Chrpova *et al.*, 2011).

Aggressiveness assessment of FHB is crucial for understating the interaction between host and pathogen in the *H. vulgare*-FHB system (Xue *et al.*, 2006). Some papers focusing on aggressiveness, as defined by the extent to which it can attack a susceptible host, of FHB complex have been published. Highly significant variations in aggressiveness, as measured *in vitro* and under controlled and field conditions of different isolates within the same species, are reported (Hestbjerg *et al.*, 2002; Xue *et al.*, 2006; Garmendia *et al.*, 2018; Sakr, 2018b, 2019b, 2020a). Sakr (2018a) reported a similar range of pathogenicity in FHB isolates recovered from diseased wheat heads on winter AS barley and durum wheat (*Triticum durum*) plants *in vitro*. Area under disease progress curve and latent period, out of nine tested components, differentiated FHB isolates and barley cultivars, AS and AB (Sakr, 2019b). Recently, Sakr (2020a) noted that four *in vitro* components (seed germination, coleoptile length, coleoptile weight and root weight (RW) in the coleoptile infection assay predicted resistance and pathogenicity occurring at the earliest and latest barley development stages during FHB infection. However, little information is available on the comparative aggressiveness of species associated with FHB on barley (Xue *et al.*, 2006). Comparing with some reports on wheat plants (Purahong *et al.*, 2011; Sakr, 2017, 2020b), few studies that use more than one assay to assess aggressiveness of the FHB isolates on barley plants have been reported until now (Sakr, 2019b).

Barley was originated and domesticated from *H. spontaneum* prior to 7000 B.C. in the Fertile Crescent (F. C.) which encompasses parts of seven countries including Syria (Ceccarelli and Grando, 2000). It is still one of the main Syrian cereal crops with a cultivated surface of one million hectares (approximately 20% of barley cultivated area in the F. C.) with more than one million tons in 2011. Barley

production in Syria is entirely based on two old cultivars: Arabi Aswad (black seeded, AS) used mainly for livestock feed and Arabi Abiad (white seeded, AB) for malting and brewing industry. Moreover, these cereal plants have been widely cultivated along the F. C. in Iraq, Jordan and Lebanon. To date, the incidence of FHB on barley has not reported in Syria. But, FHB species are frequently recovered from infected wheat fields (Sakr, 2017). Furthermore, FHB agents are recovered from F. C. barley kernels, i.e., Iran and Iraq (Matny *et al.*, 2012; Chehri and Godini, 2017).

The agricultural practices and environmental conditions might induce FHB spread in barley disease-free areas, being FHB agents already present in F. C. barley and wheat kernels, i.e., Iran, Iraq and Syria (Matny *et al.*, 2012; Chehri and Godini, 2017; Sakr, 2017). Although AS and AB were collected by the beginning of the twentieth century in Syria, pathogenicity of different FHB species on these important cereal crops was not analyzed under climatic natural conditions. Moreover, there is still a need to compare the aggressiveness of FHB species among different assays (e.g. controlled, field, etc) to check whether the ranking in aggressiveness is stable. In this context, the aims of this research were: 1) to evaluate aggressiveness generated under F. C. conditions of four FHB species (*F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti*) on AS and AB over the two growing seasons 2017/18 and 2018/19; and 2) to compare the current findings with previous analyzed *in vitro* and growth chamber data.

## MATERIALS AND METHODS

### Fungal isolates, inoculum preparation and barley landraces

: Sixteen fungal isolates of four *Fusarium* 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 recovered from wheat spikes exhibiting FHB symptoms originating from Ghab Plain, one of the principal Syrian wheat production areas, during the 2015 growing season. On potato dextrose agar (PDA), single spore pure isolates have been identified to species on the basis of macroscopic observation of cultural features such as pigmentations and growth rates of cultures on PDA in 9-cm Petri dishes, as well as of the microscopic observation of morphological characteristics

involving size of macroconidia, presence of microconidia and chlamydospores (Leslie and Summerell, 2006). Single spore isolates (conidia) are separated by dilution plating and the spores allowed to germinate overnight (Leslie and Summerell, 2006). Recently, the 16 fungal isolates were molecularly analyzed by random amplified polymorphic DNA (Sakr). For long term preservation, fungal cultures were maintained in sterile distilled water at 4 °C and freezing at -16 °C (Sakr, 2019a).

For inoculum preparation, four to six agar plugs out of each stored isolate were put over the surface of PDA in 9-cm Petri dishes and incubated for ten days at 22 °C in the dark to allow mycelial growth and sporulation. Following growth, ten ml of sterile distilled water were added to each dish, and the resulting spore suspensions were adjusted to  $5 \times 10^4$  spores/ml for inoculation following a count in a hemocytometer.

Aggressiveness testing was performed using two barley landraces: Arabi Aswad (AS) and Arabi Abiad (AB) with highest agronomic characteristics and resistance to powdery mildew diseases. AS is adapted to drier areas and popular in the northeast Syria. AB is adapted and primarily planted in the wetter areas in western and northwestern. Both genetically different landraces are two-rowed, with thin stems and high tillering ability (Ceccarelli and Grando, 2000). AB is more susceptible to FHB infection than AS in the resistance as measured by latent period (LP) of detached leaf inoculation and standardized area under disease progress curve (AUDPC<sub>standard</sub>) of Petri-dish inoculation detected *in vitro* (Sakr 2018b) and in the adult FHB resistance type I (resistance to initial penetration of the pathogen) under controlled conditions (Sakr).

**Aggressiveness tests under natural climatic conditions:** The 16 FHB isolates were individually inoculated on AS and AB to measure disease development rates, disease incidence (DI) and Fusarium-damaged kernels (FDK) as an indicators of the isolate's aggressiveness. AS and AB were cultivated at the Deir Al-Hajar Agricultural Experiment Station, located south east of Damascus, Syria (33°20' N, 36°26' E) at 617 m above sea level over the two growing seasons 2017/18 and 2018/19. Some climatic data for the station during the two growing seasons are given in Table 2.

Barley plants were grown in plastic 15-cm pots containing soil pasteurized at 5 k Gray of Gamma Ray (GR) with 60Co source using a gamma irradiator (ROBO, Russia). The soil used was a clay soil (57% clay, 39% loam and 2% sand) collected from Sojji research station (located east of Damascus, Syria, 33°30' N, 36°07' E) with the following characteristics: pH =7.8; P = 13.4 ppm; K, Na, Ca, Mg = 1.81, 2.99, 33.1, 14 mg/100 g soil respectively, and organic matter = 1.25%. Each plastic pot was filled with 2 kg of air-dried, sieved (2 mm) soil. The experimental layout was a randomized complete design with three replicates (pots). 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: *thinning* and *tillering*. The plants were watered when needed. Plants of a pot were sprayed with a spore suspension at  $5 \times 10^4$  spores/ml of 16 FHB isolates or sterile distilled water (control) when each spike reached 50% anthesis. The inoculated and control pots were covered for 48 h using polythene bags to ensure 100% RH to promote primary infection. The experiment was repeated twice on AS and AB.

Probability (P (F)) at  $P > 0.05$ . According to the Fisher's LSD test, values followed by the same letter are not significantly different at  $P > 0.05$ ; lowercase letters refer to aggressiveness among fungal isolates within each barley landrace and capital letters to quantitative resistance between the two landraces within each *Fusarium* spp. isolate. Response measured by LP and AUDPC<sub>standard</sub> for AS and AB to 16 tested FHB isolates was presented by Sakr (2018b). Response measured by DI under controlled conditions for AS and AB to 16 tested FHB isolates was presented by Sakr). When a comparison of 16 fungal isolates among themselves for each barley landrace was conducted in this study, fungal isolates with lower values of LP were considered as more pathogenic isolates and *vice versa* for fungal isolates with higher values of AUDPC standard and DI generated under controlled conditions. When a comparison of two barley landraces among themselves for each isolate was conducted in this study, a barley landrace with higher values of LP was considered as resistant and *vice versa* for a barley landrace with lower values of AUDPC<sub>standard</sub> and DI generated under controlled conditions.

Table 1. Disease responses measured by latent period (LP) of detached leaf inoculation and standardized area under disease progress curve (AUDPC<sub>standard</sub>) of Petri-dish inoculation detected *in vitro* and disease incidence (DI) detected using a head artificial inoculation generated under controlled conditions (CC) for the two Syrian barley landraces, Arabi Aswad (AS) and Arabi Abiad (AB), infected with a set of 16 fungal isolates of four *Fusarium* head blight species

Fungal isolates (identification)	LP		AUDPC <sub>standard</sub>		DI % (CC)	
	AS	AB	AS	AB	AS	AB
F1( <i>F. culmorum</i> )	7.7 ef A	8.1 f A	0.22 f A	0.35 ef B	24 fg A	42 de B
F2( <i>F. culmorum</i> )	5.8 bc A	3.6 ab B	0.29 def A	0.26 gh A	26 efg A	33 ef A
F3( <i>F. culmorum</i> )	4.4 a A	4.9 cd A	0.39 abc A	0.58 c B	35 bcdef A	64 bc A
F28( <i>F. culmorum</i> )	5.8 bc A	6.3 e A	0.29 def A	0.45 d B	32 cdef A	40 e A
F30( <i>F. culmorum</i> )	7.5 ef A	8.4 fg A	0.34 bcd A	0.70 a B	31 defg A	85 a B
F7( <i>F. solani</i> )	9.0 g A	9.4 g A	0.45 a A	0.67 ab B	40 abcd A	61 bc B
F20( <i>F. solani</i> )	8.0 fg B	5.6 de A	0.40 ab A	0.40 de A	32 cdef A	60 bc B
F26( <i>F. solani</i> )	7.9 fg B	5.6 de A	0.39 abc A	0.40 de A	24 fg A	52 cd B
F29( <i>F. solani</i> )	7.5 ef A	8.4 fg A	0.38 abc A	0.60 bc B	45 ab A	66 b B
F31( <i>F. solani</i> )	6.5 cde B	4.2 abc A	0.33 bcde A	0.30 fgh A	42 abc B	27 fg A
F35( <i>F. solani</i> )	7.7 ef A	5.3 cde B	0.39 abc A	0.38 def A	43 abc A	34 ef A
F15( <i>F. verticillioides</i> )	4.4 a A	3.5 ab A	0.22 f A	0.25 h A	20 g A	27 fg A
F16( <i>F. verticillioides</i> )	5.0 ab A	3.1 a B	0.31 cde A	0.41 de A	38 bcde A	37 ef A
F21( <i>F. verticillioides</i> )	7.1 def A	5.4 cde B	0.35 bcd A	0.38 de A	32 cdef A	35 ef A
F27( <i>F. verticillioides</i> )	6.3 cd A	5.8 de A	0.25 ef A	0.22 h A	37 bcde B	18 g A
F43( <i>F. equiseti</i> )	8.0 fg A	4.7 bcd B	0.40 ab A	0.33 efg A	52 a B	20 g A
	P (F) isolates= 2.4E-14		P (F) isolates= 6.24E-14		P (F) isolates= 5.89E-13	
	P (F) cultivars= 7.38E-06		P (F) cultivars= 1.13E-07		P (F) cultivars= 2.03E-08	
	P (F) interactions= 0.000977		P (F) interactions= 2.37E-06		P (F) interactions= 1.15E-14	

Table 2. Some climatic data collected over the two growing seasons 2017/18 and 2018/19 at the experimental station

Growing season	Variable	November	December	January	February	March	April	May
2017/18	Minimum temperature (°C)	8.2	3.6	3.3	5.7	7.9	10.0	15.6
	Maximum temperature (°C)	22.4	18.9	14.2	18.8	24.3	27.2	31.5
	Relative air humidity (%)	69	65	79	20	75	54	15
	Precipitation (mm)	0.0	0.0	34.0	30.3	1.0	14.0	9.9
2018/19	Minimum temperature (°C)	8.7	5.6	2.6	4.8	5.5	8.1	15.0
	Maximum temperature (°C)	20.2	14.9	12.6	15.1	18.3	22.2	34.1
	Relative air humidity (%)	66	65	79	68	63	56	33
	Precipitation (mm)	0.0	35.8	48.0	32.5	0.6	0.4	0.0

Disease development rates started with the observation of FHB symptoms at 7 days after the inoculation. Subsequently, the progressive blighting of spikes was scored at 14, 21 and 28 days, when plants were at the soft dough stage. Disease incidence (% of symptomatic spikes) was estimated visually as the percentage of spikes in a plant with visible FHB symptoms. Barley plants were allowed to mature prior to harvest. Mature

spikes from each replicate were pooled, and the grain was carefully harvested, ensuring that all of the tombstone infected kernels from each spike were collected. Fusarium damaged kernels (FDK) was determined on one hundred seeds for each replicate, estimating visually the number of scabby infected kernels and recorded as percentage of FDK (Mesterhazy *et al.*, 1999).

Table 3: Analyses of variance for disease incidence and Fusarium-damaged kernels over the two growing seasons 2017/18 and 2018/19 at the experimental station (F-test values)

Source of variation	df	DI	FDK
2017/18			
Isolate (I)	15	13.9**	<1 ns
Cultivar (C)	1	39.1**	1.4 ns
I × C	15	8.6**	<1 ns
Error	64		
CV (%)		17.2	13.8
2018/19			
I	15	11.2**	<1 ns
C	1	35.6**	<1 ns
I × C	15	5.8**	<1 ns
Error	64		
CV (%)		17.1	13.3
2017/18 and 2018/19			
Year (Y)	1	<1 ns	<1 ns
I	15	24.7**	<1 ns
C	1	74.6**	<1 ns
Y × I	15	<1 ns	<1 ns
Y × C	1	<1 ns	1.1 ns
I × C	15	14.1**	1.0 ns
Y × I × C	15	<1 ns	<1 ns
Pooled error	128		
CV (%)		17.2	13.5

\*\* = significant at P>0.01, ns = non significant at P>0.05. df = degree of freedom, DI = disease incidence, and FDK = Fusarium-damaged kernels .

**STATISTICAL ANALYSES**

Data were performed using DSAASTAT add-in version 2011. Before statistical analysis, the percentages were transformed using the angular transformation to stabilize variances. ANOVA incorporating the Fisher’s LSD test at P>0.05 was used to differentiate aggressiveness of 16 FHB isolates and the two tested barley landraces. The sample correlation coefficients (Pearson r) were calculated using overall mean values per isolates at P>0.05, P>0.01 and P>0.001.

Table 4. Mean values for disease incidence and Fusarium-damaged kernels (%) of four Fusarium head blight species measured over the two growing seasons 2017/18 and 2018/19 on two Syrian barley landraces, Arabi Aswad (AS) and Arabi Abiad (AB) under natural climatic conditions

Fungal isolates (identification)	Disease incidence (%)		Fusarium-damaged kernels (%)	
	AS	AB	AS	AB
F1 ( <i>F. culmorum</i> )	30 gh A	40 efg A	37 a A	37 a A
F2 ( <i>F. culmorum</i> )	25 h A	32 g A	37 a A	36 a A
F3 ( <i>F. culmorum</i> )	31 fgh A	67 b B	38 a A	41 a A
F28 ( <i>F. culmorum</i> )	34 defgh A	48 def B	41 a A	38 a A
F30 ( <i>F. culmorum</i> )	45 bcd A	61 bc B	38 a A	35 a A
F7 ( <i>F. solani</i> )	60 a A	66 b A	35 a A	40 a A
F20 ( <i>F. solani</i> )	45 bcde A	57 bcd B	36 a A	37 a A
F26 ( <i>F. solani</i> )	43 bcdef A	54 cd A	40 a A	39 a A
F29 ( <i>F. solani</i> )	40 bcdefg A	81 a B	37 a A	38 a A
F31 ( <i>F. solani</i> )	41 bcdefg A	29 g A	39 a A	36 a A
F35 ( <i>F. solani</i> )	47 bc B	31 g A	39 a A	39 a A
F15 ( <i>F. verticillioides</i> )	25 gh A	29 g A	34 a A	39 a A
F16 ( <i>F. verticillioides</i> )	29 gh A	51 cde B	40 a A	35 a A
F21 ( <i>F. verticillioides</i> )	33 efgh A	48 def B	39 a A	36 a A
F27 ( <i>F. verticillioides</i> )	36 cdefgh A	29 g A	40 a A	36 a A
F43 ( <i>F. equiseti</i> )	50 ab B	38 fg A	36 a A	38 a A

According to the Fisher’s LSD test, values followed by the same letter are not significantly different at P>0.05; lowercase letters refer to aggressiveness among fungal isolates within each barley landrace and capital letters to quantitative resistance between the two landraces within each *Fusarium* spp. isolate. When a comparison of 16 fungal isolates among

**RESULTS**

F-test values from analyses of variance for disease incidence (DI) and Fusarium-damaged kernels (FDK) over the two growing seasons were summarized in Table 3. Since no significant interaction year × aggressiveness treatments was observed (climatic data for the station were somewhat similar during the two growing seasons (Table 2)), data are shown as the averages of the two growing seasons (Table 4 and Figures 1 and 2).

themselves for each barley landrace was conducted in this study, fungal isolates with higher values of DI were considered as more pathogenic isolates. When a comparison of two barley landraces among themselves for each isolate was conducted in this study, a barley landrace with lower values of DI was considered as resistant.

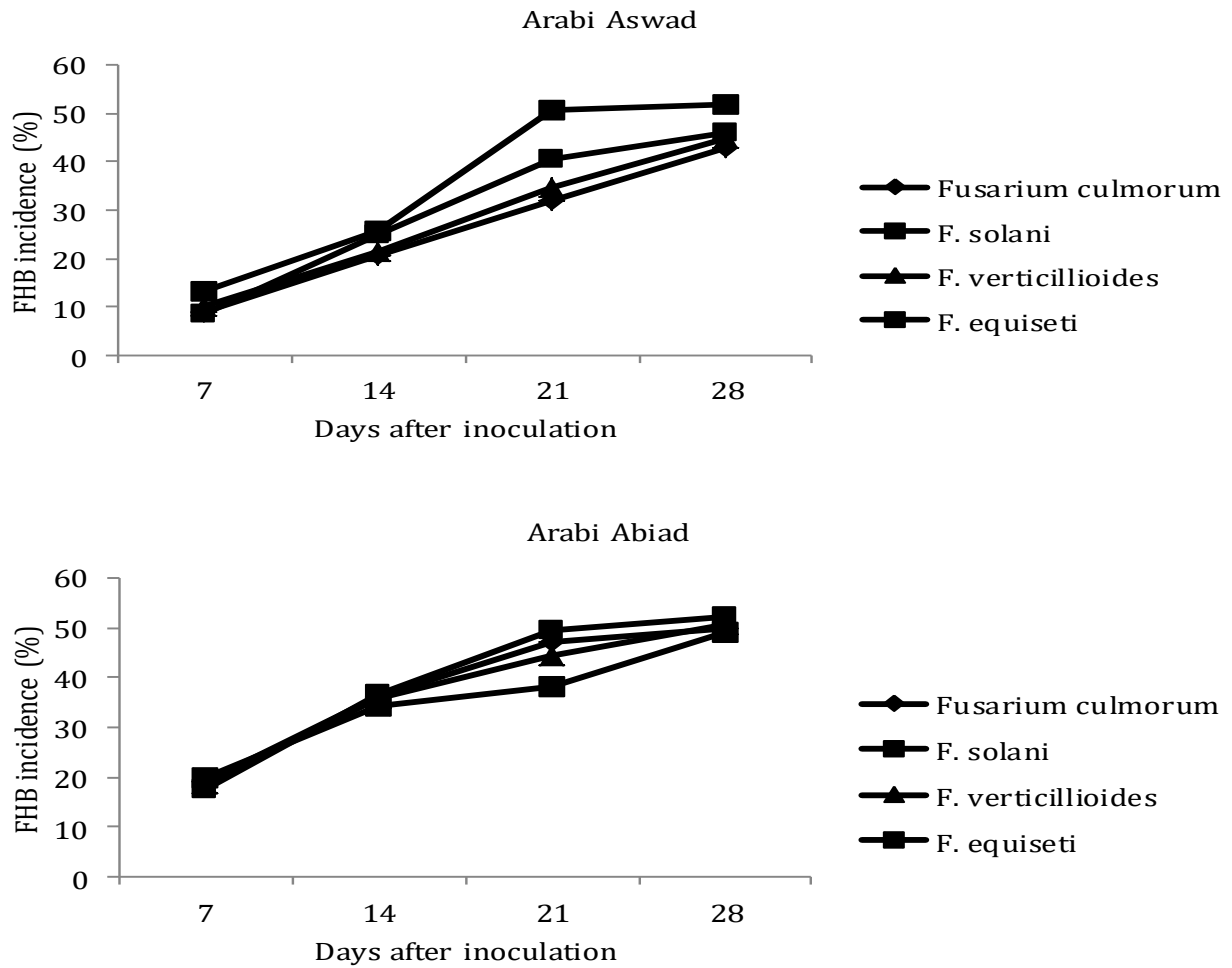


Figure 1. Mean values of Fusarium head blight progress curves for four *Fusarium* sp. on two Syrian barley landraces, Arabi Aswad and Arabi Abiad over the two growing seasons 2017/18 and 2018/19 under natural climatic conditions. Each point is the mean of isolates each for *F. culmorum*, *F. solani*, *F. verticillioides*, and *F. equiseti*

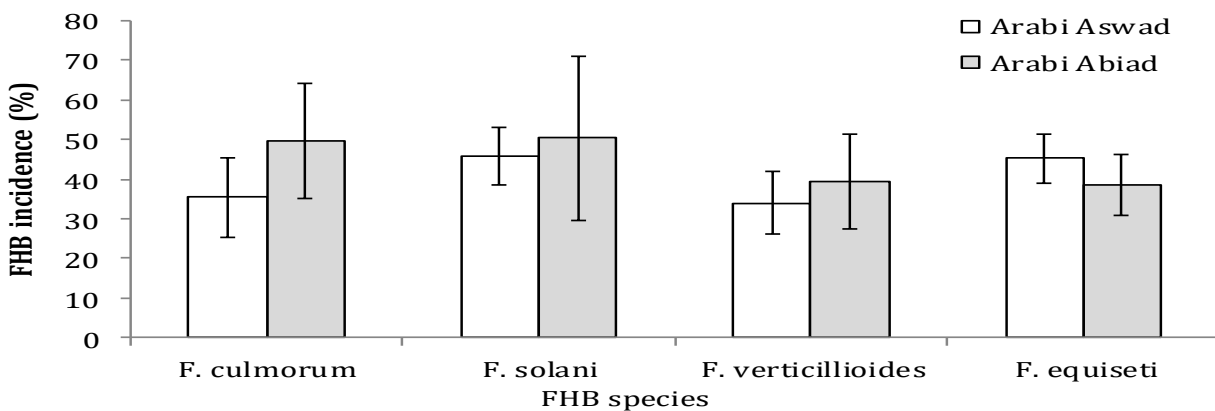


Figure 2. Mean disease incidence (%) of four Fusarium head blight species on two Syrian barley landraces, Arabi Aswad and Arabi Abiad over the two growing seasons 2017/18 and 2018/19 under natural climatic conditions. Bars represent the standard errors of means

During the course of the two growing seasons, all the 16 tested fungal isolates of four species were pathogenic and induced typical FHB symptoms in the inoculated barley plants. Disease symptoms were clear and easy to score in the inoculated spikes, while no symptoms were present in the control treatments. Symptoms were rated 7 days after inoculation (DAI) with FHB isolates on AS and AB, and disease reached maximum severity 28 DAI (Figure 1). Analysis of the relation between sporulation percentages based on the infection period ranged from 7 to 28 days showed that disease progressed slowly and less severely as generally observed on AS compared with AB (Figure 1). *F. culmorum*, *F. verticillioides* and *F. solani* showed nearly twice as much sporulation comparing with AS after 7 and 14 DAI, and more than one-fold sporulation after 21 and 28 DAI. However, *F. equiseti*, represented only by one isolate, showed much more sporulation on AS as much sporulation as compared with AB. Generally, the four *Fusarium* sp. were somewhat close in the rate of FHB symptom development on any of the two tested landraces (Figure 1).

The mean values of DI ranged two-fold from 25 to 60% on AS and varied three-fold from 29 to 81% on AB 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 on AS. The most aggressive isolate was F7 (*F. solani*) whereas the two least aggressive isolates were F2 (*F. culmorum*) and F15 (*F. verticillioides*). There were significant differences in FHB aggressiveness among the four FHB species and among isolates within each species on AB. F29 (*F. solani*) showed the greatest aggressiveness, while F2 (*F. culmorum*), F35 (*F. solani*), F31 (*F. solani*), F15 (*F. verticillioides*) and F27 (*F. verticillioides*) were the least aggressive isolates. However, it was not possible to distinguish the four FHB species on the two tested landraces (Figure 2). No significant correlation was detected between the values of DI for AS and AB over 2017/18 and 2018/19 ( $r=0.280$  not significant and  $r=0.351$  not significant, respectively). The values of DI were significantly correlated with standardized area under disease progress curve and latent period values previously obtained *in vitro* (Sakr, 2019b), ( $r=0.769^{***}$  and  $r=0.848^{***}$  for AS, and  $r=0.865^{***}$  and  $r=0.559^*$  for AB over 2017/18) and ( $r=0.750^{***}$  and  $r=0.762^{***}$  for AS, and  $r=0.869^{***}$  and  $r=0.618^*$  for AB over 2018/19). Also, a significant correlation was detected between the values of DI

generated under controlled (Sakr) and natural climatic conditions for AS and AB, ( $r=0.591^*$  and  $r=0.841^{***}$  during 2017/18, and ( $r=0.559^*$  and  $r=0.850^{***}$  during 2018/19, respectively).

Although the both barley landraces were differently affected by all tested isolates except for F1 and F2 (*F. culmorum*), F7, F26 and F31 (*F. solani*), and F15 and F27 (*F. verticillioides*); AB seemed to exhibit more FHB disease incidence (type I) than AS. Thus, AS appeared to be more resistant than AB to FHB infection.

There were no significant differences among all the 16 FHB isolates and the two tested landraces for FDK, however, FDK ranged from 34 to 41% on AS and varied from 35 to 41% on AB. No significant correlation was assigned between the mean values of DI and FDK (Type III, resistance to kernel infection) for the two tested landraces over the two growing seasons ( $r=0.164$ , not significant).

#### DISCUSSION

The present study is the first report on aggressiveness levels of Syrian FHB species under natural climatic conditions on two barley landraces widely planted along the Fertile Crescent (F. C.), a high biodiversity region where most temperate-zone cereal agricultural species originated and were first domesticated (Ceccarelli and Grando, 2000). Thereby, these landraces may constitute an important group of genetic resources since they possess high agronomic characteristics, including acceptable levels of resistance to FHB (Sakr, 2018b, unpublished data). Furthermore, the current research investigates the potential use of *in vitro* and growth chamber aggressiveness indices in predicting FHB data generated under natural climatic conditions in barley. While the disease has been present in the *barley* cultivated area in the F. C., i.e., Iran (Chehri and Godini, 2017), the preliminary data in this report gain knowledge on four FHB species aggressiveness on a Syrian scale, where the environment is quite similar to some F. C. barley growing areas, and highlight that AS and AB could be new resistant donors with favorable agronomical characteristics in FHB-barley breeding programs.

Compared to the water control, barley plants growing in the presence of 16 tested isolates showed typical FHB symptoms (Xu and Nicholson, 2009; Chrpova *et al.*, 2011). During our investigation, addition applied irrigation ensuring high humidity and warm weather around the time of flowering on the inoculation day over



the two growing seasons created suitable conditions for infection of barley plants, which agrees with a previous report (Landschoot *et al.*, 2012). Visual disease development and disease incidence (DI) rating is done directly on growing plants; therefore, it is quicker and easier than Fusarium-damaged kernels (FDK) estimation that needs to be done on harvested grain; thus, disease development and DI evaluations may be a more useful estimate of FHB damage than FDK experiments (Jin *et al.*, 2014). Additional research using the electron microscope and other *in vitro* and *in vivo* techniques are required to know, whether *F. culmorum* and the three other species caused similar or different symptoms or symptom development.

The variation in pathogenicity of four FHB species (*F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti*) on Arabi Aswad (AS) and Arabi Abiad (AB) has not been previously investigated under natural climatic conditions. The four FHB species fulfilled the requirement to stimulate FHB typical symptoms, thus they are pathogenic. This research demonstrated that the rate of FHB symptom development was almost similar of four *Fusarium* sp. on any of the two tested landraces. Also, results shown in the current research indicated an overall similar comparative aggressiveness in the four FHB species because of similarity in spike damage among the 16 fungal isolates. In parallel, Sakr (2018a, 2019b) did not cluster the same fungal species on AS and AB using an *in vitro* criterion: standardized area under disease progress curve (AUDPC<sub>standard</sub>) and latent period. Fernandez and Chen (2005) observed an apparent lack of difference in pathogenicity between *F. culmorum* and *F. graminearum* on wheat. Our results did not support previous reports showing that FHB species were classified as highly, moderately and weakly pathogenic on barley and wheat plants (Xue *et al.*, 2004; Xue *et al.*, 2006; Malhipour *et al.*, 2012). *F. culmorum* and *F. equiseti*, included in the present research, were recognized to be highly and weakly pathogenic, respectively, among several examined FHB species (Xue *et al.*, 2004; Xue *et al.*, 2006; Malhipour *et al.*, 2012). The differences in these data may be attributable to the contrasting isolates and host cultivars used in this study and previous work. Origin of FHB cultures may play a crucial role in this pathogenic similarity (Sakr, 2018a). In wheat, *F. solani* is a rare pathogen. Among more than 600 isolates coming from scabby grains were found only four *F. solani*. So, the research will be continued to see

the real significance of *F. solani* in FHB development. Re-isolation of the fungus is required from *F. solani* infected heads, grains after surface sterilization. Also, the content of DON in *F. solani* samples will be analyzed, that can be natural additional infection in *F. solani* isolates.

Although FDK decreased significantly in *Fusarium* sp. treatments by a third compared with the control on AS and AB; this criterion did not differentiate FHB isolates and the two tested landraces, Type III. Our data agree with a field study investigating resistance of Syrian durum wheat cultivars using Syrian and Italian *F. culmorum* strains and Syrian durum and bread wheat cultivars infected with the same tested 16 FHB isolates grown under the above experimental conditions mentioned in this research (Alkadri *et al.*, 2015; Sakr). FDK is a method commonly used for assessment of resistant barley and wheat cultivars, and particularly in estimating DON in breeding programs (Jin *et al.*, 2014; He *et al.*, 2015). Regarding DI, there were significant differences in aggressiveness *intra*- and *inter*-species and in susceptibility between the two barley landraces. Inter and intraspecific differences were observed in aggressiveness of several FHB species toward barley genotypes (Xue *et al.*, 2006; Garmendia *et al.*, 2018). Mutation, genetic recombination or selection may play crucial roles in *pathogenesis*. In barley, DI criterion, measured to initial infection, is of great importance in quantifying traits related to pathogenicity and resistance since barley plants exhibit a natural level to fungal spread within plant tissue (Xu and Nicholson, 2009; Chrпова *et al.*, 2011). Our results showed that no significant correlation has been detected between the values of DI and FDK. Our results did not agree with He *et al.*, (2015), who found higher correlation coefficients for DI/FDK in Canadian barley cultivars. It seems that mechanisms underlying these two aggressiveness criteria did not share the same genetic background in Syrian barley cultivars. It could be that dichotomy among Syrian, European and U.S. cereal cultivars reflects the different genetic control of the highly effective FHB resistance. However, further investigation is required in order to draw any final conclusions.

Results shown in this research indicated that a complex genotype interaction may exist among hosts and pathogens, suggesting that aggressiveness mechanisms and resistance genes maybe different to disease caused by individual FHB species. No significant correlation was detected between the values of DI for AS and AB infected

with 16 fungal isolates of four species over 2017/18 and 2018/19. Our results agree with previous data showing a possibility of a cultivar-specific aggressiveness between the values of DI for AS and AB under growth chamber conditions ( $r=-0.115$ , not significant) (Sakr). This type of interaction has previously been reported by Foroud *et al.*, (2012), who noted that *F. graminearum* aggressiveness is host-dependent. Strong evidence was reported for specific aggressiveness interactions among fungal species implicated in the FHB complex and barley plants (Parry *et al.*, 1995; Sakr, 2019b). But this specificity should be stable; otherwise the specificity cannot be proven. Thus, it seems that a minor gene-for-minor gene interaction may exist between the two barley landraces and 16 fungal isolates, suggesting that the isolate-specific effectiveness may lead to erosion of barley quantitative resistance to FHB invasion. However, further investigation is required in order to draw any final conclusions.

This research supports the view that the correlations of different aggressiveness indices exist and are stable with *in vitro*, under controlled and natural climatic conditions pathogenicity assays over the two growing seasons, suggesting that natural climatic conditions indices can predict pathogenic traits generated under several experimental conditions. Results indicated that the *in vitro* and growth chamber aggressiveness tests conducted on FHB species are repeatable and stable with AS and AB barley plants under natural climatic conditions. AUDPC<sub>standard</sub> and latent period could reflect aspects of pathogen development at early stages of plant growth by promoting the interaction between barley tissues and fungi. The situation in an *in vitro* assay was similar to spray inoculation under controlled and field conditions because FHB species need to overcome the morphology of the head spike and they could directly penetrate and infect germinating seeds (Sakr, 2018c, 2019b). AUDPC<sub>standard</sub> and latent period are indicators of aggressiveness occurring in the whole plant during FHB infection (Sakr, 2019b).

Our findings show that disease progressed slowly and less severely as generally observed on AS compared with AB over the two growing seasons. The two tested landraces can resist high pathogenic isolates from a certain species can also resist another pathogenic isolates from another species, the results here are consummative to the ideas of Xue *et al.*, (2006). Thus, the difference between high (AS) and low (AB) resistant

cultivar is coming from the resistance variation. Furthermore, AS and AB were shown to exhibit moderately susceptible to moderately resistant levels to initial fungal infection (Type I) depending on fungal isolates. Our data support the view that Syrian barley materials can be promising sources of resistance to FHB under F. C. conditions because of lack of 100% resistance to FHB in the current commercial varieties (Chrpova *et al.*, 2011).

This work supports the view that AS shows to exhibit higher resistance levels to initial fungal infection compared to AB under natural climatic conditions, indicating that AS provided broad, though incomplete, resistance to the four *Fusarium* sp. examined. As expected, these results confirmed previous *in vitro* and growth chamber findings that AB was more susceptible to FHB infection than AS (Sakr 2018c), suggesting 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* and growth chamber observations of AS and AB in FHB resistance (Sakr 2018b), there were significant cultivar × isolate interactions observed in the present study, which agree with previous reports on barley and wheat (Xue *et al.*, 2004; Xue *et al.*, 2006). In spite of the most FHB resistant barley landraces exhibit poor agronomical characteristics (Chrpova *et al.*, 2011), the variability of resistance for AS and AB, with the highest agronomic traits, is promising resistance sources to FHB into specific barley breeding programs in stress environments and for poor farmers not only in F. C. but also in regions with similar climatic conditions. AS may be a new source for livestock and AB for malting and brewing resistance breeding. Since only two barley landraces were analyzed here, further research using a large sample of available Syrian barley cultivars is needed to validate our aggressiveness data generated under *in vitro*, controlled and natural climatic conditions. Furthermore, it is important to measure directly DON or other toxins characteristics to the given fungal species in Syrian food safety programs.

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