Research Article

Resistance to Fusarium Head Blight in Some Syrian Wheat and Barley Cultivars

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Abstract | Fusarium head blight (FHB) can have a destructive effect of barley and wheat along the whole world. To defeat it, sources of host resistance and pathogenic variation of FHB species need to be identified. Although barley and wheat are crucial crops in the dried Mediterranean area, there is insufficient information about their resistance to head blight infection and aggressiveness of *Fusarium* species. A 3-year (2019 to 2021) experiment was conducted under arid Mediterranean conditions to measure disease reactions, i.e., FHB incidence (DI, Type I resistance), FHB severity (DS, Type II) and FHB-damaged kernels (FDK, Type III), on barley, bread and durum wheat cultivars of varying susceptibility to four species of FHB. Although there were no marked differences in disease levels over the 3 years, a wide range of resistance was expressed by adult Type I and Type II to head blight among a group of eight cereal cultivars, indicating that host plays an important role in distinguishing head blight susceptibility. The differences of DI and DS among the cultivars tested with 16 FHB isolates revealed that the variation in pathogenicity among the FHB population is crucial for developing disease-resistant cultivars. FDK component did not differentiate the eight analyzed cereal genotypes and pathogen strains. Most significantly, stability of cultivars for head blight resistance was fulfilled during seasons as well as under several experimental conditions, suggesting that cultivars with stable and high disease resistance could be incorporated to crossbreeding programs to reinforce host resistance to fungal infection. This primarily study has recognized some favorable barley and wheat cultivars for selected breeding and commercialization aims in the arid Mediterranean area; Bohoth 10 (bread wheat) and Arabi Aswad (barley) ranking among the most head blight resistant cereal cultivars.

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Introduction

For 8000 years, barley (*Hordeum vulgare*) and bread wheat (*Triticum aestivum*) and durum wheat (*T. durum*) are currently the most widespread cereal crops in which they have been a participation of the history in Mediterranean agriculture (Haas *et al*., 2019). The barley- and wheat-growing area within the Mediterranean Basin represents ~ 25% of the wheat (219 million hectares) and barley (60 million hectares) global cultivated area. The Mediterranean basin occupies 60% of the earth's growing zone for durum wheat, and barley is the predominant crop of the driest Mediterranean areas (Mefleh, 2021). Syrian wheat and barley cultivars planted in the arid Mediterranean climate exhibit a predominately substantial collection of plant materials due to wide genetic diversity and their certificated tolerance to diseases and insect pests and resistance to non-biotic constraints (Bishaw *et al*., 2015). In Syria, bread wheat is cultivated mostly under irrigated cropping system. Durum wheat and barley are grown mainly in rain-fed environments, where their productivity are profoundly affected by rainfall and abiotic (i.e., drought, sunlight, cold, and salinity) stresses (Bishaw *et al*., 2011; Khan *et al*., 2021). Pathogens are among the main threats to high yield of barley and wheat and a threat into food security. Fusarium head blight (FHB) represents a great fungal defiance to the prosperous yielding of wheat, barley and other small-grain cereals in several states in Asia, Mediterranean basin, Europe and America (Parry *et al*., 1995).

Head blight is a much studied disease that can produce major decreases in output and contaminates grains with mycotoxins named deoxynivalenol (DON) produced from fungal infection. DON is of main interest to human and animal safety, its level within grain relay on the environmental conditions (Fernando *et al*., 2021). Flowering is the growing period most susceptible to fungal invasion. When humidity and temperature are convenient at time of flowering, water-soaked spots appearing on infected florets resulted in blighting the spikelet and production of deformed kernels usually mentioned to as FHB-damaged kernels, FDK (Dweba *et al*., 2017). More than 17 *Fusarium* species with various levels of pathogenicity favoured by different weather conditions have been sampled from naturally infected wheat and barley heads. While *F. graminearum* is the main causal pathogen and the maximum aggressive agent globally, the other *Fusarium* organisms were recovered extremely from small-grain crops (Bottalico and Perrone, 2002). Favorable conditions to FHB invasion and development are (a) the abundance and pathogenicity of different substances used for inoculation through the susceptive plant phase, which particularly spans several days around flowering, (b) the environmental factors over this critical stage and (c) the susceptibility or resistance level of the plant (Buerstmayr *et al*., 2020).

Recent epidemics of FHB worldwide have affirmed the necessity to breed novel commercial cultivars of wheat and barley with perfected levels of resistance to assist reduces the devastating impacts of FHB (Dweba *et al*., 2017). Plant resistance is the most environmentally sound and cost effective strategy of FHB management (Buerstmayr *et al*., 2020). Although breeding for head blight resistance has assured to be complicated because tolerance to *Fusarium* is under the inheritance of polygenic structure and the significant environment-by-cultivar interaction, resistant cereal cultivars exhibit harmonious resistance to nearly all strains of head blight pathogens worldwide (Xu and Nicholson, 2009). Up to six susceptibility classes to head blight have been identified: class I, susceptibility to premier fungal invasion; class II, susceptibility to the movement and development of pathogens in the spike; class III, susceptibility to grain invasion; class IV, the capability of plant to degrade toxins; class V, tolerance to toxins; and class VI, tolerance to FHB (Fernando *et al*., 2021). Under epidemic situations, *Fusarium* species causing FHB are agents of increased interest for the cereal cultivation due to their diverse pathogenicity and capacity to provoke serious damage (Xue *et al*., 2019). Variations in head blight incidence (DI) detected to evaluate class I and head blight severity (DS) detected to analyze class II have been observed for head blight strains sampled from several world provinces, states or countries and even separated fields (Xu and Nicholson, 2009), indicating that the maximum level of aggressive variability recorded in head blight isolates for barley and wheat must be considered in the progress of screening strategies (Xue *et al*., 2019).

Origins of reinforcing head blight susceptibility determined in barley and wheat genotypes, like Sumai 3 and Cheveron, have been incorporated extensively into crossing schemes to enhance FHB resistance worldwide (Xu and Nicholson, 2009), suggesting that stability of host resistance in these sources has been achieved, and they are still the chief sources for resistance to symptom development in the head (Buerstmayr *et al*., 2020). However, no total resistance against head blight has been recognized in Sumai 3 and Cheveron (Dweba *et al*., 2017). Almost all of the tested mediterranean commercial cultivars of wheat and barley were sensitive or very sensitive to head blight with the unique exception of some cultivars which assured to be moderately susceptible, indicating that no commercial cultivars exist with full resistance to head blight (Talas *et al*., 2011; Alkadri *et al*., 2015; Hadjout *et al*., 2017; Ogrodowicz *et al*., 2020).

Collecting sufficient phenotypic data to design trustworthy conclusions on the FHB resistance in wheat and barley and the pathogenic variation of FHB species requires a toolbox of different analyses under several experimental conditions which assess different resistance components (Fernando *et al*., 2021). However, the findings from trials conducted in the growth chamber may not regularly place in field environments. The correspondence of findings between growth chamber and non-controlling experiments has differed in trials measuring head blight susceptibility in transgenic wheat cultivars (Mackintosh *et al*., 2007). Further research is needed to best recognize how to use growth chambers and field study methodologies for evaluating wheat and barley susceptibility to head blight in the nearly all effective strategy.

Although barley and wheat are crucial cultivations in the arid Mediterranean region (Bishaw *et al*., 2011, 2015), there is insufficient information about their resistance to head blight infection and aggressiveness of *Fusarium* species (Talas *et al*., 2011; Alkadri *et al*., 2015; Sakr, 2019a, 2020a, d, 2021; Sakr and Al-Attar, 2021; Sakr and Shoaib, 2021). In this context, this three-year field experiment aimed to evaluate disease reactions, i.e., head blight incidence (DI, class I), head blight severity (DS, class II) and head blight-damaged kernels (FDK, class III), of diverse Mediterranean wheat and barley cultivars of Syrian origin of contrasting susceptibility to FHB inoculated with four head blight organisms under arid Mediterranean conditions. In addition, this field study combined with laboratory and growth chamber findings sought to explore a more definitive assessment of head blight resistance and pathogenicity of diverse fusaria under several experimental conditions.

Materials and Methods

Cereal resources

Eight Mediterranean cereal genotypes widely cultivated in Syrian field with agreeable quality and agronomic traits and resistance to fungal diseases (Bishaw *et al*., 2011, 2015) included six *T. aestivum* and *T. durum* cultivars and two *H. vulgare* cultivars: Arabi Abiad (AB) and Arabi Aswad (AS) was used. Field trials were conducted within the three successive growing periods 2018/19, 2019/20 and 2020/21 on these eight widely grown cultivars of different resistance levels to FHB (Figure 1). Wheat and barley were sown on November 4th in 2018, on November 11th in 2019, and on November 9th in 2020. Bohoth10 and AS (*T. aestivum* and *H. vulgare*) were moderately resistant to FHB and Acsad65 (*T. durum*) was consistently susceptible to FHB and as classified from laboratory and growth chamber findings (Sakr, 2018, 2019a, b, 2020b, c, 2021; Sakr and Al-Attar, 2021; Sakr and Shoaib, 2021). However, the five remaining lines were ranked as moderately susceptible to susceptible under laboratory conditions (Sakr, 2018, 2019a, b, 2020b, c, 2021; Sakr and Al-Attar, 2021). Nevertheless, spikelet and spike infection tests under controlled conditions (Sakr, 2019a, 2020b) permitted to divide this group into two distinct sub-groups as Cham 9 and Cham 7 (durum) ranked as sensitive to moderately sensitive, and Douma 4, Cham 4, and AB (bread and barley) recognized as moderately susceptible.

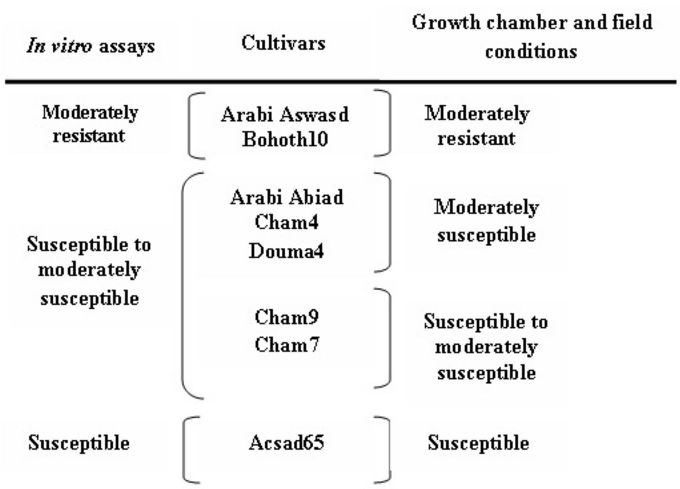

Figure 1: *Ranking of eight Mediterranean wheat and barley cultivars of Syrian origin infected with a set of 16 Fusarium head blight based on latent period of detached leaf inoculation, area under disease progress curve of Petri-dish inoculation and coleoptile length reduction of a coleoptile infection under in vitro conditions and on disease incidence and disease severity detected using a detached head test and disease incidence and disease severity following spike and spikelets in a growth chamber and field.*

Fungal isolates

Sixteen strains of four head blight pathogens, i.e. (*F. equiseti* (one strain), *F. verticillioides* (4 strains), *F. culmorum* (5 strains), and *F. solani* (6 strains) were used due to their different pathogenic behavior levels (built on earlier several experimental findings (Sakr, 2018, 2019a, b, 2020a, b, c, d, 2020; Sakr and Al-Attar, 2021; Sakr and Shoaib, 2021). At the 2015 growth season, strains were sampled from field infected *Triticum* heads over 9 locations in Ghab Plain with a FHB history, one of the major *Triticum* producing zones in Syria. On Petri-plates with potato dextrose agar (PDA), strains were classified morphologically to species level by utilizing the methods of Leslie and Summerell (2006). By using random amplified polymorphic DNA markers, the 16 strains were recently tested (Sakr and Shoaib, 2021). Fungal strains were preserved at-16°C by freezing or at 4°C in sterile distilled water (SDW) till use (Sakr, 2020e).

Head blight substance used for inoculation for the field trials was arranged as following: Preserved strains were installed at the surface of PDA plates and put in the incubator for 10 days at 22ºC in the dark climate to permit sporulation and fungal development. After fungal growth, isolates were dealt with 10ml of SDW and conidia were taken. By passage via 2 layers of sterilized cheesecloth, fungal suspensions were purified to take out mycelium portions and agar and instantly measured with a Neubauer chamber under an optical binocular and adjusted to 5 × 10^4 spores/ml.

Quantitative resistance and pathogenicity analyses in the arid Mediterranean climate

All head blight single-spore cultures were individually inoculated on Douma 4, Cham 4, Bohoth 10, Cham 9, Cham 7, Cham 4, Acsad 65, AS and AB to measure blight incidence (DI, class I), head blight severity (DS, class II) and head blight-damaged kernels (FDK, class III) as components of strain's pathogenicity and the genotype's susceptibility. Field trials were conducted on the eight tested wheat and barley cultivars over the 2019, 2020 and 2021 growing seasons at the Deir Al-Hajar Agricultural Experimentation Station (36°26′ E, 33°20′ N, over sea level by 600 m altitude), set south east in the countryside of Damascus, Syria. By utilizing the Allen's *et al*. (1998) presenting the FAO Penman-Monteith, the arid Mediterranean weather prevails the research zone, with yearly possible evapotranspiration (ET_0) of more than 2000 mm. The mean yearly precipitation built on 20 years' registration (2000–2019) is nearly 120 mm. Table 1 presents some meteorology findings of the research zone, accumulated in three analyzed growth seasons.

Surface-sterilized wheat and barley seeds were planted in clay soil in plastic pots with 15-cm. The pot/soil encompassed of ~ 40% loam and 60% clay and less than 2% sand, gathered from the Sojji Agricultural Experimentation Station (36°07′ E, 33°30′ N, over sea level by 700 m altitude), set east in the countryside of Damascus, Syria with organic matter= 1.25%; Mg, Ca, K, Na= 14, 33.1, 1.81, 2.99 mg/100 g soil respectively; P= 13.4 mM and pH=7.8 was air dehydrated, screened to exceed into a sieve with a 3 mm, and sterilized in the gamma irradiator (ROBO, Russia) at 5 k Gy of Gamma Ray with a cobalt material. A completely randomized design represented the experimental layout; and each cultivar/isolate included 3 replications. The non-inoculated treatment included 3 pots per replication which were left without fungal inoculation. After appearance, thinning of plants was applied and N fertilization was conducted to prevent any nitrogen deficiency: emergence at December, and tillering at March.

Table 1: *Some climatic data collected over the three growing seasons 2018/19, 2019/20, 2020/21 at the experimental station.*

Growing season	Variable	November	December	January	February	March	April	May
2018/19	T_{min} (°C)	8.7	5.6	2.6	4.8	5.5	8.1	15.0
	T_{max} (°C)	20.2	14.9	12.6	15.1	18.3	22.2	34.1
	RH (%)	73	65	79	68	63	56	33
	Rainfall (mm)	31.9	35.8	48.0	32.5	17.4	11.6	0.0
2019/20	T_{min} (°C)	7.0	3.8	3.8	4.1	7.5	9.7	13.4
	T_{max} (°C)	21.6	15.5	12.5	15.1	19.6	24.3	31.6
	RH (%)	71	76	85	80	73	63	57
	Rainfall (mm)	25.0	75.9	31.4	24.5	44.6	6.7	3.0

2020/21	T_{min} (°C)	9.8	5.6	3.7	3.7	6.9	10.1	13.0
	T_{max} (°C)	20.4	16.6	15.8	17.8	21.0	36.0	31.0
	RH (%)	73	72	82	71	74	46	50
	Rainfall (mm)	70	8.1	22.3	11.7	2.8	6.0	5.0

T_{min}: minimum temperature; T_{max}: maximum temperature; RH: relative air humidity.

A subset of 8 (3 durum, 3 bread and 2 barley) cultivars was tested to clarify stability of cultivars for head blight susceptibility through growing seasons. Artificial inoculation of cereal heads with a set of 16 head blight single-spore cultures was established at the stage of complete flowering. The spraying of FHB inoculum (spore suspension 5 × 10^4 conidia/ml) onto wheat and barley plants of a pot was achieved on one time at April. In clear plastic bags for one day, infected heads were put to five continual elevated moisture to stimulate initial invasion. The trial was repeated two times on Douma4, Cham4, Bohoth10, Cham9, Cham7, Cham4, Acsad65, AB and AS in each growing season. To minimize growing season impacts on data, it appeared required in this arid environment to assist head blight progress at organized periods subsequently by watering of cereal pots (Sakr, 2020a, d).

FDK, DS and DI and were estimated to detect the rate of head blight invasion built on apparent disease damages. Analyzing of FHB disease progress levels (DPLs) was carried out at the starting of spikes with discolored spikelets that are distinctive of head blight around 7 days following infection. Afterwards, the continuous blighting of spikes at the soft dough stage was rated at 2 weeks, 3 weeks and 4 weeks after inoculation (wai). For each FHB isolate/cereal cultivar, Type I susceptibility (DI) was evaluated by rating the amount of symptomatic heads at 3 wai visually *in situ*. The values of DPLs estimated at 1, 2, 3 and 4 wai during the analyzing period were seen as a component to define Type II susceptibility (DS) as reported earlier by Sakr (2020d). Before harvesting, barley and wheat plants were permitted to mature. Mature spikes from each replicate were collected, and then the kernels were accurately gathered, maintaining that both diseased (pinkish or discolored) and damaged kernels from each head were sampled. On one hundred grains for each replication, Type III susceptibility (FDK) was visually evaluated from the amount of damaged infected garins and registered as proportion of FDK (Mesterhazy *et al*., 1999).

Statistical analysis

A DSAASTAT add-in version 2011 was used to analyze the phenotypic data. A combined analysis of data over the three growing seasons was achieved to prove if resistance level and pathogenicity may have a significant and constant impact during year. Environments being years (the three growing seasons 2018/19, 2019/20 and 2020/21) are recognized as random effects, while treatments (cultivars and fungi) are recognized as fixed effects (Gomez and Gomez, 1984). At the 5% level of significance, ANOVA incorporating the Fisher's LSD test was utilized to distinguish pathogenicity of 16 head blight cultures and the eight analyzed wheat and barley cultivars. The significant cereal cultivar × FHB isolate interactions were evaluated for all assessed criteria: DI, DS and FDK. The homogeneity of variance was evaluated using Levene's test among the six replications (2 for 2018/19, 2 for 2019/20 and 2 for 2020/21 for growing seasons) for all measured quantitative criteria under field conditions. Utilizing overall average estimates per isolates at $P \leq 0.05$, the sample correlation coefficients (Pearson r) were evaluated.

Results and Discussion

Not at all of the eight analyzed barley, durum and bread wheat and escaped from FHB infection. In comparing to non-inoculated fungal treatments, cereals growing in the existence of 16 FHB cultures in the field exhibited representative FHB symptoms. Clear symptoms of diseased infected heads were recorded from the primarily rating (1 wai) onwards, while in the non-inoculated water treatments no symptoms were existent.

Quantitative resistance and pathogenicity analyses in the arid Mediterranean climate

Table 2 summarized Fisher-analyze estimates from tests of variance for FDK, DS and DI during the three years. Whilst no remarkable interaction treatment × year was recorded (weather findings for the station were quite identical over the three years (Table 1), results are presented as the means of the three years (Tables 3, 4 and 5). Remarkable cultivar × *Fusarium* interaction were observed for FDK, DS and DI, however, there were no pronounced cultivar × *Fusarium* interactions for FDK.

The reliability and constancy of field experiments did not reveal pronounced variations within the 3 criteria: FDK, DS and DI for the 16 fungal strains estimated on the eight barley and wheat genotypes from six replicates (2 for 2018/19, 2 for 2019/20 and 2 for 2020/21 years) ($p>0.05$ on Douma 4, Cham 4, Bohoth 10, Cham 9, Cham 7, Cham 4, Acsad 65, AB and AS for FDK, DS and DI, respectively).

Table 2: *Analyses of variance for disease incidence (DI) for type I, disease severity (DS) for type II and Fusarium-damaged kernels (FDK) for type III over the three growing seasons 2018/19, 2019/20, 2020/21 at the experimental station (F-test values).*

Source of variation	df	DI	DS	FDK
2018/19				
Cultivar (C)	7	++	++	ns
Isolate (I)	15	++	++	ns
C × I	105	++	++	ns
Error	256			
2019/20				
Cultivar (C)	7	++	++	ns
Isolate (I)	15	++	++	ns
C × I	105	++	++	ns
Error	256			
2020/21				
Cultivar (C)	7	++	++	ns
Isolate (I)	15	++	++	ns
C × I	105	++	++	ns
Error	256			
Combined analysis 2018/19, 2019/20 and 2020/21				
Year (Y)	2	ns	ns	ns
C	7	++	++	ns
I	15	++	++	ns
Y × C	14	ns	ns	ns
Y × I	30	ns	ns	ns
C × I	105	++	++	ns
Y × C × I	210	ns	ns	ns
Error	768			
CV (%)		16.2	16.3	9.9

++: significant at 1% level; ns: non-significant at 5% level; df: degree of freedom. DI: disease incidence for type I resistance; DS: disease severity for type II resistance; FDK: Fusarium-damaged kernels for type III resistance.

Quantitative resistance in cereal cultivars

The findings of the head infection assay revealed a range of host responses as detected by type I susceptibility (DI) (Table 3) and type II susceptibility (DS) (Table 4). For type I, the average proportion of cereals showing head blight damage varied from less than 40% to 54% and for type II from 29% to 41%. AS and Bohoth 10 displayed the least inoculation scores, with average incidence and severity values below 39% and 29%, respectively, while Acsad 65 was the most influenced genotype, with average incidence and severity estimate of 54% and 41%, respectively. Overall, AS, AB and *T. aestivum* cultivars showed lower DI and DS scores than the *T. durum* ones. A ranking of cultivars in terms of these two disease estimations (DI and DS) is shown in Figure 1 over three growing seasons. The four statistically defined (Fisher's LSD test) groups of cultivars based on DI and DS with contrasting responses as termed moderately resistant including AS and Bohoth10, moderately susceptible containing AB, Cham4 and Douma4, sensitive to moderately sensitive involving Cham7 and Cham9 and sensitive comprising Acsad65. No remarkable variations were shown in the susceptibility analyzed by the damaged kernels across the eight wheat and barley cultivars, but the average portion of cereals exhibiting whitened kernel symptoms ranged from 38% to 39% (Table 5).

There were significant correlations among the resistances assessed by incidence and severity determined utilizing an artificial spraying infection in the field over the 3 years 19, 20 and 21 (Pearson $r=0.954^{***}$, Pearson $r=0.983^{***}$ and Pearson $r=0.976^{***}$, respectively). Furthermore, correlation coefficients between the resistances assessed by incidence and severity under field conditions and the resistance measured by several aggressive criteria under diverse analyzed conditions were significant (Table 6).

Pathogenicity of fungal isolates

Over the three growth seasons, all the analyzed *Fusarium* isolates causing FHB were aggressive and produced ideal head blight damages in the infected barley and wheat heads (Tables 3, 4 and 5). Sixteen isolates exhibited a great diversity in pathogenicity as distinguished by DI (Table 3) and DS (Table 4). The average DI values of FHB isolates varied one and a half fold from 37% for the least aggressive strain of *F. verticillioides*, F27 to 53% for the most aggressive strain of *F. solani*, F29 on the analyzed cereals in comparing to 0% for the non-infected fungal controls. Regarding DS evaluations, F20 (*F. solani*) was the most pathogenic isolate at 44% and a strain of *F. verticillioides*, F27 was the least aggressive one at 28% as compared with 0% for the water controls, with mean DS values of FHB isolates varied one and a half fold. The differences in FDK of the 16 isolates were not significant, but, FDK varied from 37% to 40% on eight genotypes. Correlation coefficients between the data for DI and DS during the three growing seasons and other aggressive criteria observed under different tested conditions were significant (Table 7).

Table 3: *Mean values for disease incidence (DI) for type I (%) under field conditions over the three growing seasons 2018/19, 2019/20 and 2020/21 in eight Mediterranean wheat and barley cultivars of Syrian origin inoculated with a set of 16 fungal isolates of four Fusarium head blight species.*

Fungal isolates (identification)	DI								
	Acsad 65	Cham 4	Cham 7	Douma 4	Cham 9	Bohoth 10	Arabi Abiad	Arabi Aswad	Mean
F1 (*F. culmorum*)	65	53	55	42	39	38	33	31	45cde
F2 (*F. culmorum*)	43	57	40	60	34	50	53	24	45cde
F3 (*F. culmorum*)	58	32	56	42	68	42	56	32	48b
F28 (*F. culmorum*)	72	30	35	27	42	53	63	36	44cde
F30 (*F. culmorum*)	70	30	37	40	63	30	40	44	44cde
F7 (*F. solani*)	53	30	46	36	67	37	48	58	47bc
F20 (*F. solani*)	69	53	53	54	59	40	63	46	54a
F26 (*F. solani*)	49	49	42	42	55	43	56	40	47bc
F29 (*F. solani*)	70	38	63	40	79	43	52	43	53a
F31 (*F. solani*)	33	43	59	56	31	33	33	39	41fg
F35 (*F. solani*)	41	53	64	71	32	29	42	49	47bc
F15 (*F. verticillioides*)	40	37	55	22	28	29	43	28	35gh
F16 (*F. verticillioides*)	53	30	36	49	52	40	39	31	41fg
F21 (*F. verticillioides*)	56	40	65	49	46	40	48	31	47bc
F27 (*F. verticillioides*)	49	38	32	36	31	34	37	36	37h
F43 (*F. equiesti*)	48	34	54	47	40	39	40	47	43ef
Mean	54a	40de	49b	45d	48b	38e	46cd	39e	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at P ≤ 0.05. In the current study, all isolates were reanalyzed for DI on Acsad 65, Cham 7, Cham 9, Cham 4, Douma4 , Bohoth 10, Arabi Abiad and Arabi Aswad over the three growing seasons 2018/19, 2019/20 and 2020/21; however, pathogenic reaction for all isolates was analyzed previously and presented by (Sakr, 2020a, b, d, 2022).

Table 4: *Mean values for disease severity (DS) for type II (%) under field conditions over the three growing seasons 2018/19, 2019/20 and 2020/21 in eight Mediterranean wheat and barley cultivars of Syrian origin inoculated with a set of 16 fungal isolates of four Fusarium head blight species.*

Fungal isolates (identification)	DS								
	Acsad 65	Cham 4	Cham 7	Douma 4	Cham 9	Bohoth 10	Arabi Abiad	Arabi Aswad	Mean
F1 (*F. culmorum*)	47	39	46	32	31	27	25	25	34cde
F2 (*F. culmorum*)	31	41	32	44	27	36	41	19	34cde
F3 (*F. culmorum*)	41	24	45	31	55	30	42	26	37bc
F28 (*F. culmorum*)	52	22	27	19	33	38	48	29	34cde
F30 (*F. culmorum*)	51	22	29	29	51	21	31	36	34cde
F7 (*F. solani*)	43	22	36	27	50	28	39	41	36cd
F20 (*F. solani*)	56	40	45	40	52	31	51	33	44a
F26 (*F. solani*)	40	36	35	32	38	33	45	29	36cd
F29 (*F. solani*)	57	28	49	30	61	33	42	31	41ab
F31 (*F. solani*)	27	32	46	42	24	25	27	27	31ef
F35 (*F. solani*)	34	39	51	54	25	22	34	35	37bc
F15 (*F. verticillioides*)	30	30	45	17	24	21	36	23	28f
F16 (*F. verticillioides*)	40	24	30	37	44	29	33	26	33de

F21 (*F. verticillioides*)	42	32	54	37	39	29	40	26	37bc
F27 (*F. verticillioides*)	29	31	25	28	27	25	31	30	28f
F43 (*F. equiesti*)	36	28	43	37	36	27	33	34	34cde
Mean	41a	31cd	40ab	34c	39ab	29d	37cb	29d	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at P ≤ 0.05. In the current study, all isolates were reanalyzed for DS on Douma4 and Cham7 over the growing season 2018/19; however, pathogenic reaction for all isolates was analyzed previously and presented by Sakr (2020d).

Table 5: *Mean values for Fusarium-damaged kernels (FDK) for type III (%) under field conditions over the three growing seasons 2018/19, 2019/20 and 2020/21 in eight Mediterranean wheat and barley cultivars of Syrian origin inoculated with a set of 16 fungal isolates of four Fusarium head blight species.*

Fungal isolates (identification)	FDK								
	Acsad 65	Cham 4	Cham 7	Douma 4	Cham 9	Bohoth 10	Arabi Abiad	Arabi Aswad	Mean
F1 (*F. culmorum*)	37	35	37	38	37	37	36	38	37a
F2 (*F. culmorum*)	36	36	35	37	36	35	37	37	36a
F3 (*F. culmorum*)	38	37	37	39	37	37	40	40	38a
F28 (*F. culmorum*)	40	40	40	39	40	41	40	41	40a
F30 (*F. culmorum*)	37	36	35	37	37	36	37	39	37a
F7 (*F. solani*)	37	36	36	37	37	36	39	38	37a
F20 (*F. solani*)	39	37	36	39	38	38	38	39	38a
F26 (*F. solani*)	36	41	41	38	37	37	40	40	39a
F29 (*F. solani*)	38	39	39	37	37	39	38	38	38a
F31 (*F. solani*)	38	40	38	37	39	38	37	39	38a
F35 (*F. solani*)	39	39	40	38	39	39	38	39	39a
F15 (*F. verticillioides*)	38	38	38	39	40	38	37	37	38a
F16 (*F. verticillioides*)	40	39	40	38	38	41	37	38	39a
F21 (*F. verticillioides*)	38	38	38	37	37	37	38	40	38a
F27 (*F. verticillioides*)	38	36	37	38	37	37	38	41	38a
F43 (*F. equiesti*)	38	36	37	38	39	36	37	38	37a
Mean	38a	38a	38a	38a	38a	38a	38a	39a	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at P ≤ 0.05. In the current study, all isolates were reanalyzed for FDK on Douma 4, Cham 7, Arabi Abiad and Arabi Aswad over the growing season 2018/19; however, pathogenic reaction for all isolates was analyzed previously and presented by (Sakr, 2020a, d).

Table 6: *Correlation coefficients between the resistance measured by disease incidence (DI, Type I) and disease severity (DS, type II) detected using a head artificial inoculation under field conditions (FC) over the three growing seasons 2018/19, 2019/20 and 2020/21 and the resistance measured by latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile length reduction (CL) of a coleoptile infection detected in vitro, disease incidence (DI, type I) and disease severity (DS, type II) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI^{CC}, type I) detected using a head artificial inoculation and disease severity (DS^{CC}, type II) detected using a floret artificial inoculation under controlled conditions in a growth chamber on eight Mediterranean wheat and barley cultivars of Syrian origin infected with a set of 16 fungal isolates of four Fusarium head blight species.*

Resistance component	$DI^{FC2018/19}$, Type I	$DI^{FC2019/20}$, Type I	$DI^{FC2020/21}$, Type I	$DS^{FC2018/19}$, Type II	$DS^{FC2019/20}$, Type II	$DS^{FC2020/21}$, Type II
LP	0.806*	0.708*	0.711*	0.765*	0.732*	0.726*
AUDPC	0.826*	0.785*	0.810*	0.741*	0.768*	0.759*
CL	-0.786*	-0.748*	-0.772*	-0.740*	-0.745*	-0.747*
DI^{DHT}, Type I	0.799*	0.751*	0.781*	0.709*	0.712*	0.759*
DS^{DHT}, Type II	0.876**	0.839**	0.862**	0.785*	0.818*	0.806*

DI^{CC}, Type I	0.811*	0.759**	0.790*	0.709*	0.741*	0.729*
DS^{CC}, Type II	0.836**	0.798*	0.822*	0.730*	0.765*	0.752*

($P \leq 0.05$), ($P \leq 0.01$)**, ($P \leq 0.001$)***.*

Since the threat of FHB disease is gradually augmenting in the arid Mediterranean region (Parry *et al*., 1995), this study reports resistance to FHB in Mediterranean wheat and barley cultivars of Syrian origin and pathogenicity of *Fusarium* species across 3 years (2019 to 2021). To our best knowledge, this is

Table 7: *Correlation coefficients between two pathogenicity components (disease incidence (DI) and disease severity (DS)) detected using a head artificial inoculation under field conditions over the three growing seasons 2018/19, 2019/20 and 2020/21 and latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile length reduction (CL) of a coleoptile infection detected in vitro, disease incidence (DI) and disease severity (DS) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI) and disease severity (DS) detected using a head and floret artificial inoculation, respectively under controlled conditions (CC) for a set of 16 fungal isolates of four Fusarium head blight species on eight Mediterranean wheat and barley cultivars of Syrian origin.*

Pathogenicity components		$DI^{2018/19}$	$DI^{2019/20}$	$DI^{2020/21}$	$DS^{2018/19}$	$DS^{2019/20}$	$DS^{2020/2021}$
LP	Acsad65	0.510*	0.532*	0.529*	0.511*	0.585*	0.556*
	Cham7	0.534*	0.512*	0.537*	0.519*	0.528*	0.535*
	Cham9	0.667**	0.661*	0.670**	0.710**	0.656**	0.689**
	Cham4	0.564*	0.634**	0.607*	0.580*	0.650**	0.625**
	Douma4	0.721**	0.699**	0.708**	0.663**	0.713**	0.692**
	Bohoth10	0.623**	0.672**	0.664**	0.574*	0.613*	0.610*
	Arabi Abiad	0.618*	0.516*	0.567*	0.539*	0.557*	0.499*
	Arabi Aswad	0.762***	0.731**	0.805***	0.720**	0.640**	0.727**
AUDPC	Acsad65	0.752**	0.759**	0.767***	0.705**	0.759**	0.742**
	Cham7	0.510*	0.520*	0.535*	0.529*	0.513*	0.533*
	Cham9	0.640**	0.551*	0.597*	0.610*	0.551*	0.530*
	Cham4	0.534*	0.628**	0.588*	0.557*	0.635**	0.605*
	Douma4	0.538*	0.554*	0.537*	0.498*	0.559*	0.529*
	Bohoth10	0.682**	0.604*	0.662*	0.633**	0.577*	0.623**
	Arabi Abiad	0.869***	0.852***	0.871***	0.820***	0.808***	0.822***
	Arabi Aswad	0.750**	0.675**	0.755**	0.694**	0.542*	0.661**
CL	Acsad65	-0.686**	-0.686**	-0.697**	-0.653**	-0.687**	-0.679**
	Cham7	-0.579*	-0.499*	-0.549*	-0.570*	-0.497*	-0.534*
	Cham9	-0.682**	-0.543*	-0.611*	-0.654**	-0.508*	-0.582*
	Cham4	-0.515*	-0.559*	-0.593*	-0.521*	-0.564*	-0.501*
	Douma4	-0.643**	-0.741**	-0.688**	-0.666**	-0.737**	-0.705**
	Bohoth10	-0.548*	-0.563*	-0.568*	-0.616*	-0.630**	-0.635**
	Arabi Abiad	-0.635**	-0.568*	-0.662*	-0.603*	-0.554*	-0.575*
	Arabi Aswad	-0.783***	-0.721**	-0.771**	-0.720**	-0.677**	-0.708**
DI (DHT)	Acsad65	0.835***	0.779***	0.819***	0.857***	0.853***	0.865***
	Cham7	0.641**	0.628**	0.693**	0.724**	0.620*	0.696**
	Cham9	0.788***	0.742**	0.770***	0.810***	0.776***	0.801***
	Cham4	0.878***	0.882***	0.892***	0.832***	0.839***	0.848***
	Douma4	0.853***	0.847***	0.860***	0.849***	0.843**	0.855***
	Bohoth10	0.800***	0.678**	0.763***	0.828***	0.692**	0.782***
	Arabi Abiad	0.845***	0.837***	0.847***	0.806***	0.819***	0.821***
	Arabi Aswad	0.691**	0.703**	0.737**	0.631**	0.630**	0.664**

DS (DHT)	Acsad65	0.789***	0.820***	0.817***	0.681**	0.732**	0.716**
	Cham7	0.742**	0.694**	0.725**	0.705**	0.700**	0.714**
	Cham9	0.720**	0.711**	0.722**	0.683**	0.625**	0.660**
	Cham4	0.582*	0.641**	0.620*	0.548*	0.632**	0.599*
	Douma4	0.725**	0.709**	0.739**	0.744**	0.710**	0.740**
	Bohoth10	0.779***	0.552*	0.673**	0.526*	0.508*	0.523*
	Arabi Abiad	0.824***	0.819***	0.825***	0.804***	0.813***	0.816***
	Arabi Aswad	0.728**	0.759**	0.775**	0.660**	0.678**	0.699**

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Pathogenicity components		**DI2018/19**	**DI2019/20**	**DI2020/21**	**DS2018/19**	**DS2019/20**	**DS2020/2021**
DI (CC)	Acsad65	0.838***	0.761***	0.811***	0.846***	0.828***	0.847***
	Cham7	0.686**	0.684**	0.735**	0.758**	0.678**	0.741**
	Cham9	0.847***	0.815***	0.837***	0.856***	0.824***	0.849***
	Cham4	0.821***	0.816***	0.830***	0.766***	0.764***	0.777***
	Douma4	0.864***	0.856***	0.874***	0.871***	0.857***	0.874***
	Bohoth10	0.762***	0.696**	0.751**	0.795***	0.720**	0.780***
	Arabi Abiad	0.850***	0.852***	0.856***	0.802***	0.824***	0.821***
	Arabi Aswad	0.559*	0.535*	0.579*	0.517*	0.502*	0.537*
DS (CC)	Acsad65	0.721**	0.735**	0.739**	0.655**	0.701**	0.687**
	Cham7	0.731**	0.705**	0.716**	0.690**	0.715**	0.712**
	Cham9	0.679**	0.679**	0.686**	0.641**	0.596*	0.624**
	Cham4	0.564*	0.610*	0.595*	0.516*	0.591*	0.562*
	Douma4	0.757**	0.723**	0.761***	0.773**	0.725**	0.762***
	Bohoth10	0.796***	0.689**	0.701**	0.519*	0.617*	0.624**
	Arabi Abiad	0.807***	0.750**	0.785***	0.767***	0.720**	0.749**
	Arabi Aswad	0.502*	0.536*	0.599*	0.534*	0.602*	0.640**

($P \leq 0.05$), ($P \leq 0.01$)**, ($P \leq 0.001$)***.*

the first study based on analyses of multiple *in vitro*, growth chamber and field conditions to see how FHB resistance and pathogenicity of fungi can be evaluated. The current preliminary investigation has recognized some favorable wheat and barley cultivars like Bohoth10 and AS for selected breeding and commercialization aims in the arid Mediterranean area. The stability and repeatability of findings under field conditions were achieved in respect to none significant differences among the replicates (2 for 2018/19, 2 for 2019/20 and 2 for 2020/21 years) in the three analyzed quantitative criteria i.e. DI, DS and FDK, suggesting the similarity of variation over location over the three years.

It well reported that invasion situations of head blight species causing FHB in barley are genotype specific and diverse from growing conditions in wheat (Schoneberg *et al*., 2018); however, the favorable factors for wheat and barley inoculation at flowering were fulfilled under arid Mediterranean field conditions tested herein. Our field observations support pervious findings (Fernando *et al*., 2021) in which the manifestation of FHB in barley and wheat is completely dissimilar. The invasion of barley grains is much distinguishable and is remarked by the browning of spikelets. In wheat, head blight generally seems as the blanching of considerable parts of the head and produces chalk-like tombstone grains in more extreme invasions. At maturity, head blight-damaged grains can be more complicate to characterize in barley, which can be distinguished as marginally stained and shrivelled.

Our data showed that there were no marked differences in disease levels among the 3 years. Three major factors, may have participated to this, involving environmental condition, FHB recording method and inoculum concentration. While weather findings for the Deir Al-Hajar Agricultural Experimentation Station were quite identical during the three years, our findings suggest that environmental conditions that contribute to susceptibility to primary invasion and susceptibility to the spread and movement of

Fusarium pathogens in the head do not vary across 3 years (2019 to 2021) during infection. However, this was not in accordance with other data highlighted by other reports (Buerstmayr *et al*., 2020; Dweba *et al*., 2017), who found that the environment has a strong influence on FHB. When joining the findings in different conditions (Khatibi *et al*., 2012) and weak head blight severity (Choo *et al*., 2004), second and third possible reasons on FHB scoring method and inoculum concentration should consider the general great variations under field conditions in the domain of head blight scores to make correct conclusions. In our investigation, wheat and barley cultivars were sprayed at full maturity with and heterogeneous and aqueous fungal mixtures of macrocondia (50,000 spores/ml) with a view to acquire adequate disease in the field required to assess head blight pathogenic reactions. Spraying at flowering time is not infrequent and has been utilized earlier to evaluate resistance in wheat and barley (Xu and Nicholson, 2009). Our data highlighted that the cereal cultivar played a notable function in defining the level of FHB susceptibility in all seasons of the research, showing the effect of individual wheat and barley genotypes and their particular genes on the levels of head blight progression. As stated earlier, resistant cultivars of *T. aestivum* and *H. vulgare* were exhibited to have weaker levels of head blight in comparing to with susceptible *T. durum* cultivars (Khatibi *et al*., 2012; Mesterhazy *et al*., 1999). In spite of that *H. vulgare* has common susceptibility of type II to head blight (Fernando *et al*., 2021), exciting diversities in DS between AS and AB is probably because of variances in type II.

The extreme level of pathogenic variability that is found in the head blight species complex for barley and wheat should be taken into account in management of phenotyping strategies (Xu and Nicholson, 2009). In our research, substantial differences in the percentage of symptomatic spikes, DI, and spikelets, DS, of barley and wheat spikes grown under the arid Mediterranean conditions were found among 16 fungal strains causing head blight collected from one of the main *Triticum* production areas of Syria, Ghab Plain, proposing a strong influence of the fungi on the growth of wheat and barley plants. Similar to our findings, high pathogenic variability in FHB populations has been found earlier in Ghab Plain (Al-Chaabi *et al*., 2018) and across several wheat and barley regions worldwide (Xue *et al*., 2019). The cell wall degradation enzymes (Phalip *et al*., 2009) and DON (Dweba *et al*., 2017) released by *Fusarium* species during FHB infection to enter and colonize cereal hosts may play a basic role in pathogenesis. According to the severity of FHB symptoms, DI and DS, the isolates were arranged as low, medium and highly pathogenic, concluding that the variation found across nine localities in the surveyed region was not geographically structured as reported for FHB pathogens (Xu and Nicholson, 2009; Xue *et al*., 2019). The current variation in pathogenicity among the disease population grown in the arid Mediterranean climate is important in the progress of head blight resistant cultivars by considering the most pathogenic isolates for successful phenotyping of wheat and barley cultivars for disease resistance. More importantly, the results of aggressive indices generated under the arid Mediterranean field conditions were very identical to those from the *in vitro* and growth chamber observations (Sakr, 2018, 2019a, b, 2020b, c, 2021; Sakr and Al-Attar, 2021), suggesting that field indices like DI and DS can predict aggressive characteristics obtained under diverse tested situations.

FHB infection can be evaluated after harvest as fraction of FDK; whitened kernel scores (Mesterhazy *et al*., 1999). In wheat and barley, it is well accepted that FDK permitted a more accurate and effective distinguishing of resistant cultivars (He *et al*., 2015; Jin *et al*., 2014). However, *Fusarium*-damaged kernels trait did not differ the eight analyzed cereal genotypes varying in their resistance to *Fusarium* pathogens and 16 head blight single-spore cultures of diverse pathogenicity. In line to our findings, FDK did not differentiate Syrian *T. durum* wheat cultivars infected with Italian and Syrian *F. culmorum* isolates (Alkadri *et al*., 2015). Furthermore, no effect of head blight pathogens (*F. meridionale and F. graminearum*) was observed on *Fusarium*-damaged kernels estiamtes analyzed wheat cultivars of Brazilian origin (Mendes *et al*., 2018). It is remarkable that the complication of the head blight resistance and pathogenicity of different fungal species in wheat and barley needs a better understanding as stressed by Ma *et al*. (2020).

Although significant interactions were observed between cereal cultivars and isolates of four head blight pathogens in our investigation, there is no confirmation for constant races such as are observed in cereal powdery mildew pathogens, rust pathogens, and some other specialized pathogens as reported earlier by Xu and Nicholson (2009). Based on the current tests of wheat and barely cultivar to different species of *Fusarium* causing head blight, resistance to 16 FHB isolates and other head blight pathogens was not isolate- or species-specific in barley and wheat genotypes as notified in previous study conducted by Mesterhazy *et al.* (1999). In our research, *Fusarium* organisms that provoke FHB on wheat can infect other cereals, i.e., barley without exhibiting specialization for any one host, and a host-specific has not been reported till now (Fernando *et al.*, 2021).

Susceptibility and resistance of eight wheat and barley cultivars of contrasting susceptibility to disease to FHB were determined by evaluating of DI and DS as described previously (Buerstmayr *et al.*, 2020). Under the tested arid Mediterranean field conditions, the different wheat and barley cultivars were proven to have highly varying reactions accounting on the resistance types evaluated, Type I and Type II, which may be an indication of diverse gene interaction and genes influencing the susceptibility. However, stable genotypes were identified across years since variable weather conditions do not contribute to fluctuations of head blight susceptibility. In general, the level of susceptibility to FHB infection reduces from durum wheat to bread wheat to barley (Buerstmayr *et al.*, 2020; Dweba *et al.*, 2017). Overall, AS, AB and *T. aestivum* cultivars, Bohoth 10, Cham 4, Douma 4, exhibited lower invasion spikelet and spike estimations than did *T. durum* cultivars, Acsad 65, Cham 7 and Cham 9, indicating that barley and bread wheat provided universal, although not total, susceptibility to the four *Fusarium* species evaluated in comparing to durum wheat. As expected, our data proven earlier laboratory and growth chamber results (Sakr, 2018, 2019a, b, 2020b, c, 2021; Sakr and Al-Attar, 2021) that Acsad 65 was susceptible and AS and Bohoth 10 were moderately resistant. The reliability of this genotype ranking was fulfilled by the considerable linkage among both head blight type I and type II susceptibility over three growing seasons and damage responses in the seedlings and adult plant testes obtained under controlled conditions. Most significantly, stability of cultivars for head blight resistance was fulfilled during seasons as well as under several experimental conditions, suggesting that cultivars with stable and high disease resistance could be incorporated to crossbreeding programs to reinforce host resistance to fungal infection. Similar to our findings, wheat and barley cultivars of Mediterranean origin were incorporated into breeding programs (Talas *et al.*, 2011; Alkadri *et al.*, 2015; Hadjout *et al.*, 2017; Ogrodowicz *et al.*, 2020) because of deficiency of 100% resistance to head blight in the present commercial genotypes (Mesterhazy *et al.*, 1999).

Conclusion and Recommendations

These findings certificate the potential of evaluating wheat and barley resistance to head blight and pathogenicity of *Fusarium* pathogens in arid Mediterranean field conditions on the basis of visual symptom rating, DI and DS. Although the FHB infection level on wheat and barley is influenced by meteorological factors such as precipitation, temperature and humidity, no marked differences in FHB levels were detected among the 3 growing seasons, suggesting that different years of testing and locations are requested to recognize resistant wheat and barley cultivars. The presence of more pathogenic forms of fungi requires the need to choose the isolates that best represent the pathogen population, also necessities continual updating when screening breeding cultivars for resistance. More importantly, stability of cultivars for head blight resistance was fulfilled across years as well as under several experimental conditions, suggesting that cultivars with elevated and stable FHB resistance like Bohoth10 and AS could be utilized as novel resistance sources or released as cultivars provided they have good grain quality and acceptable resistance to other diseases.

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Novelty Statement

This is the first study based on analyses of multiple seedlings, controlled and arid Mediterranean field conditions to see how head blight resistance and pathogenicity of *Fusarium* fungi can be eavaluated based on such different findings. This primarily study has recognized some favorable wheat and barley cultivars for selected breeding and commercialization aims in the arid Mediterranean area; Bohoth10 (bread wheat) and Arabi Aswad (barley) classified among the most head blight resistant cereal cultivars.

Conflict of interest
The author has declared no conflict of interest.

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