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A simple and reliable assay to quantify host resistance and aggressiveness of the pathogen in the Fusarium head blight-barley pathosystem

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RESEARCH ARTICLE

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ABSTRACT

A simple and reliable method for quantifying Fusarium head blight (FHB), a widespread disease of barley, would enhance our capacity in identifying resistance sources and highly aggressive isolates. A detached head assay (DHA) was used to reliably assess: (i) resistance of two barley cultivars, Arabi Aswad (AS) and Arabi Abiad (AB) with different susceptibility to FHB and (ii) aggressiveness in a set of 16 fungal isolates of four *Fusarium* species. The two inoculated cultivars showed different responses in FHB incidence (DI) and severity (DS) using spray and point inoculation on detached barley heads, respectively. On AB, susceptible under several experimental conditions, inoculation with different *Fusarium* species resulted in significantly higher DI and DS, compared with AS, which showed *Fusarium* resistance. Furthermore, the values of DI and DS were significantly correlated with the previous findings generated under several experimental conditions. The use of this simple and reliable method in barley breeding programs can speed up the process of identification of sources of resistance to multiple FHB isolates. To our best knowledge, this is the first in-depth report investigating the usefulness of DHA for distinguishing susceptibility of barley plants and aggressiveness of diverse *Fusarium* species from a breeder's point of view.

KEYWORDS

detached head assay, *Fusarium* fungi, quantitative resistance, pathogenic variation, *in vitro* technique

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INTRODUCTION

Barley (*Hordeum vulgare* L.), which is ranked fourth among the most produced cereal crops worldwide providing well over 140 million tons per year (FAO, 2015), is susceptible to a diverse phytopathogenic group of harmful *Fusarium* fungi. Fusarium head blight (FHB) is a widespread disease that affects barley and other small-grain cereals and reduces crop productivity in humid and semi-humid areas (Parry et al., 1995). In addition, infection by certain *Fusarium* species causing FHB my impair grain quality particularly due to the accumulation of dangerous mycotoxins posing a significant threat to food and feed chains. The presence of mycotoxins in harvested grains may cause technical problems in malting and brewing industry (McMullen et al., 2012). Shortly after infection and under favorable conditions (warm, humid and wet), diseased spikelets display symptoms of premature bleaching. As the disease progresses, infected spikelets are shriveled and chalky white (Janssen et al., 2018).

At least seventeen *Fusarium* species are reported to cause FHB disease. *Fusarium graminearum* is known to be a major *Fusarium* species damaging barley in many countries in America, Europe and Asia. In addition to *F. graminearum*, causal agents of barley-FHB are *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium tricinctum*, *Fusarium langsethiae*, *Fusarium sporotrichioides* and *Fusarium poae* (Parry et al., 1995; Bottalico and Perrone, 2002; Bai and Shaner, 2004; Xue et al., 2006; McMullen et al., 2012; Becher et al., 2013; Dahl and Wilson, 2018). It is known that under diverse experimental conditions, *Fusarium* isolates show strong variability in aggressiveness (Xue et al., 2006; Hestbjerg et al., 2002; Opoku et al., 2011; Garmendia et al., 2018; Sakr, 2018a, 2019b, 2020a, b; Sakr and Shoaib, 2021), defined as the degree of damage caused by the pathogen to the host (Lannou, 2012). Diverse aggressiveness of several isolates of the same species might influence disease response (Janssen et al., 2018). However, the aggressiveness does not appear to be stable, as proven by the many significant 'isolate by environment' interactions in the FHB-barley pathosystem (Xu and Nicholson, 2009).

Over the last four decades, considerable research and resources have been devoted to improve the FHB resistance of barley. However, consistently efficient control measures against FHB are lacking (Dahl and Wilson, 2018). To date, there are no highly resistant barley cultivars and FHB control relies on integrated disease management that includes cultural practices, chemical control and the use of available resistant cultivars (Janssen et al., 2018). Two major types of FHB resistance are widely accepted: resistance to the initial infection (Type I), and resistance to the spread of infection in the spike (Type II), with Type I as the predominant type in barley (Zhu et al., 1999). Type I resistance is common in barley but rare in wheat, which is most likely contributed by spike morphology and by activation of systemic innate immune responses. In contrast, Type II resistance attributed to different resistant genes is more important in wheat (Bai and Shaner, 2004; Jansen et al., 2005).

Screening for aggressiveness of fungal isolates and FHB resistance in barley requires that plants are grown to anthesis, the most crucial time for the development of disease, prior to inoculation and left to grow for additional two to three weeks before they are rated for visual symptoms, under controlled and field conditions (Xu and Nicholson, 2009). In field assessments, it is generally accepted that FHB incidence should be quantified over several years to account for variability due to cultivar/year interactions (Janssen et al., 2018). Multiple sites for field assessments of FHB should also be considered (Dahl and Wilson, 2018). In order to

expedite this process, it would be useful to develop alternative approaches that are equally effective but less time-consuming and potentially less resource requiring.

In vitro methodologies have been evaluated to facilitate the analysis of multiple *Fusarium* isolates varying in aggressiveness and identify head blight resistant sources in barley plants. Viable tissue of young plant parts is planted onto culture media and then inoculated with FHB causing fungi. A small amount of plant material is required for each *in vitro* assay. However, some studies referenced herein did not investigate correlations with the head blight reaction in adult barley plant (Hestbjerg et al., 2002; Browne and Cooke, 2005; Kumar et al., 2011; Opoku et al., 2011; Bedawy et al., 2018). Sakr (2018a) observed a similar range of aggressiveness in FHB isolates recovered from diseased wheat heads on Arabi Aswad (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 Arabi Abiad (white seeded, AB) (Sakr, 2018b, 2019b). Recently, Sakr (2020b) noted that four *in vitro* components (seed germination, coleoptile length, coleoptile weight and root weight) in the coleoptile infection assay predicted resistance and aggressiveness occurring at the earliest and latest barley development stages during FHB infection. Under controlled and field conditions, there were significant differences in aggressiveness *intra*- and *inter*-species and in susceptibility between AS and AB; aggressiveness values over the two growing seasons in the field and in the growth chamber were significantly correlated with aggressiveness traits previously obtained *in vitro* (Sakr, 2020a; Sakr and Shoaib, 2021).

The detached head assay (DHA) is an *in vitro* tool which enables definite inoculation at the time of flowering (Takeda, 2004), under controlled conditions. Nevertheless, the information generated using this methodology was contradictory in barley. Han and Kim (2005) found statistically significant correlation between DHA and point inoculation (Type II resistance) in growth chambers where all biotic and abiotic conditions were strictly controlled. On the contrary, another research that was based on a set of five-year data, showed significant differences in the FHB severity of different plant materials by applying DHA and field inoculation methods (Usele et al., 2013). Despite the importance of DHA, there are no associated reports on the aggressiveness of diverse *Fusarium* isolates or species.

Barley breeding programs aiming to develop FHB resistant cultivars would benefit if a DHA is proven reliable with data obtained under different experimental conditions and especially in the field; where environmental conditions affect aggressiveness of fungi and plant resistance and complicate phenotyping and breeding efforts (Bai and Shaner, 2004). Therefore, the objective of the current study was to establish an improved protocol DHA to rapidly screen for barley resistance to FHB infection and evaluate aggressiveness of four *Fusarium* species. To assess the efficacy of the assay, DHA results were compared with those from *in vitro* detached leaf, Petri-dish and coleoptile infection tests and artificial inoculations under controlled and field experiments.

MATERIAL AND METHODS

Plant materials, fungal isolates and inoculum preparation

Experiments were conducted using two morphologically, physiologically and genetically different barley cultivars (Ceccarelli et al., 1987) with contrasting in susceptibility to FHB, including the susceptible cv. Arabi Abiad (AB) and the moderately resistant cv. Arabi Aswad (AS) as ranked from previous disease resistance assays (Sakr, 2018b, 2019b, 2020a, b; Sakr and Shoaib, 2021).

To date, the incidence of head blight pathogens on barley has not reported in Syria. But, FHB species are frequently recovered form infected wheat fields (Sakr, 2017). Sixteen single-spore derived cultures of four *Fusarium* species namely *F. culmorum* (F1, F2, F3, F28 and F30), *Fusarium solani* (F7, F20, F26, F29, F31 and F35), *Fusarium verticillioides* (synonym *Fusarium moniliforme*) (F15, F16, F21 and F27), and *Fusarium equiseti* (F43) were collected from FHB naturally wheat fields from Ghab Plain, one of the principal Syrian wheat production areas, during the 2015 growing season. They were selected for their contrasting aggressiveness based on previous experimental observations (Sakr, 2018a, 2019b, 2020a, b; Sakr and Shoaib, 2021). Although *F. graminearum* is considered the major causative of FHB complex worldwide (Parry et al., 1995), this species was not found in the surveyed region (Ghab Plain) as observed in other studies investigating the composition of FHB complex species in Ghab Plain during spring of three seasons (2008–2010) (Al-Chaabi et al., 2018). Thus, the selection of FHB species used in our study was reflective of other pathogen populations recovered from Ghab Plain and other principal Syrian wheat production areas (Alkadri et al., 2013; Al-Chaabi et al., 2018); *F. culmorum* was the most frequent causing agent in Syria. Isolates were identified morphologically according to keys described by Leslie and Summerell (2006). Recently, the 16 fungal isolates were molecularly analyzed using random amplified polymorphic DNA (Sakr and Shoaib, 2021). *Fusarium* cultures were stored in sterile distilled water at 4°C or at a freezer at −16°C until needed (Sakr, 2020c).

Fusarium inocula were prepared by independently growing each of the 16 fungal isolates on potato dextrose agar (PDA, HiMedia Laboratories) in 9 cm Petri dishes for ten days at 22ºC under continuous *darkness* in an incubator (JSPC, JS Research Inc). PDA is generally known as the most common media for growth and sporulation of fungi (Kavanagh, 2005). After incubation, cultures were flooded with 10 ml of sterile distilled water and spores were dislodged. Suspensions were filtered through two layers of sterile cheesecloth to remove mycelia and adjusted to a concentration of 5×10^4 spores/ml using a haemocytometer.

Aggressiveness tests under controlled and natural conditions

Pathogenic reactions of the 16 tested FHB isolates in these two barley cultivars, AS and AB, were determined using *in vitro* LP and AUDPC methodologies (Sakr, 2018a, b, 2019b) and DI detected using a head artificial inoculation generated under controlled and field conditions over the two growing seasons 2017/18 and 2018/19 (Sakr, 2020a; Sakr and Shoaib, 2021). In order to explorer the utility of detached head assay (DHA) to predict aggressiveness of various *Fusarium* isolates, DS for Type II adult plant resistance was detected using floret inoculation assay in the growth chamber and DI for Type I adult plant resistance were conducted in the field during the growing season 2019/20 according to methods described previously by Sakr (2019a, 2020a) in this current investigation. Therefore, we were able to examine the relationships between the current findings with the previous results of *in vitro* and artificial inoculations in the growth chamber and field.

Establishment of DHA assay using spray (DI, Type I) and point (DS, Type II) inoculation on detached barley heads

The DHA assay was conducted as described previously by Takeda (2004), with some modifications. Surface-sterilized barley seeds of AS and AB were sown in 20 × 15 cm pots filled with soil sterilized at 5 kGy of gamma irradiation with Cobalt 60 source (ROBO, Russia). The soil

used in this experiment was a clay soil (57% clay, 39% loam and 2% sand) collected from Sojji Agricultural Experiment Station (located east of the countryside of Damascus, Syria, 33°30 N, 36°07 E) with the following traits: pH = 7.8; phosphor = 13.4 ppm; potassium, sodium, calcium, magnesium = 1.81, 2.99, 33.1, 14 mg/100 g soil respectively, and organic matter = 1.25%. Each plastic pot contained 2 kg of air-dried, sieved (2 mm) soil. Barley plants were kept under chamber conditions (20°C at day/night temperature, and 16 h of light per day). Following emergence, plants were thinned and fertilized to avoid nitrogen deficiency by providing ammonium nitrate at two stages: thinning and tillering. The plants were watered when needed.

Ten spikes per barley cultivar were detached at the second internode from the top at mid-anthesis (growth stage 65 according to decimal code of growth stages of cereals) and were assumed as one replication. Ten detached spikes per replicate were left non-inoculated as control treatment. Three replicates of each isolate were set up in which the detached barley heads were arranged in a randomized block design, and the experiment was repeated twice. Detached spikes were put in containers of water inside a growth chambers set at the controlled conditions described above. Detached barley spikes were individually inoculated with a spore suspension for bleaching of spikes (DI, Type I) evaluations and injected into two adjacent florets (10 µL per floret) at the middle of each spike (without wounding) for bleaching of spikelets (DS, Type II) ratings of 16 *Fusarium* isolates or sterile distilled water (control). Moisture content inside the container was sufficient for primary disease infection for Type I and disease spread for Type II. Disease was evaluated at 3, 6 and 9 days after inoculation (DAI). Bleaching of spikes and spikelets was evaluated based on visual assessment of blighting 3, 6 and 9 days post inoculation (dpi). Disease incidence, DI was estimated as the percentage of spikes showing FHB symptoms at 9 dpi. Disease severity was assessed as the percentage of diseased spikelets per inoculated spike with visually detectable disease symptoms on a 0 (no visible FHB symptoms) to 9 (severely diseased, spike dead) scale described by Xue et al. (2006).

Statistical analyses

The experimental data were subjected to analysis of variance (ANOVA) using DSAASTAT add-in version 2011. To stabilize variances, the percentages of DI and DS were transformed using the angular transformation before statistical analysis. The differences were compared using Fisher's least significant difference (LSD) test at a probability level of $P = 0.05$ based on the analysis of transformed data. The Pearson correlation coefficient (Pearson r) were calculated using overall values of per isolates at $P = 0.05$.

RESULTS

Evaluation of *Fusarium* isolates aggressiveness and Type II barley resistance under controlled conditions

Both AS and AB cultivars showed FHB symptoms (Table 1). Distinct FHB symptoms generated by the 16 fungal isolates were visible and simple to record on the inoculated spikelets, whereas control plants were symptomless. On AS, the values of DS expressed as average percentage of affected spikelets per spike ranged from ~16% for the least pathogenic isolates F20 and F26 (*F. solani*), and F15 and F21 (*F. verticillioides*) to 66% for the most pathogenic isolate

F43 (*F. equiseti*). On AB, the values of DS ranged from 18% for the least pathogenic isolate F27 (*F. verticillioides*) to 79% for the most pathogenic isolate F30 (*F. culmorum*). Statistically significant difference in DS, after point inoculation of central spikelets carried out to quantify FHB resistance, was obtained between AS and AB cultivars (Table 1). The fraction of plants exhibiting FHB symptoms varied from 15% to 66% on AS and from 18% to 79% on AB. The fungus/host interaction in terms of DS was also significant. Although AS and AB were differently affected by all tested isolates except for F2 (*F. culmorum*), F31 (*F. solani*), and F15 and F27 (*F. verticillioides*); AB seemed to exhibit more DS (Type I resistance) scores than AS, which is not always significant. Thus, AS seemed to be less vulnerable than AB to the majority of isolates of *F. culmorum* and *F. solani*, but not to *F. equiseti*, while their susceptibility to *F. verticillioides* does not seem to differentiate significantly, as measured by DS.

Table 1. Disease severity (DS) after floret artificial inoculation under controlled conditions and disease incidence (DI) after head artificial inoculation under field conditions during the growing season 2019/20 for the two barley cultivars, Arabi Aswad (AS) and Arabi Abiad (AB), inoculated with a set of 16 isolates of four *Fusarium* species

Fungal isolates (identification)	DS (%)		DI (%)	
	AS	AB	AS	AB
F1(*F. culmorum*)	29cde B	42d A	33ef A	36ef A
F2(*F. culmorum*)	33cd A	25fg A	25f A	35ef A
F3(*F. culmorum*)	29cde B	58c A	33ef B	71ab A
F28(*F. culmorum*)	20ef B	44d A	37de A	36ef A
F30(*F. culmorum*)	28de B	79a A	43bcd B	66b A
F7(*F. solani*)	36bcd B	67bc A	55a A	66b A
F20(*F. solani*)	17f B	36de A	47abc B	72ab A
F26(*F. solani*)	16f B	28ef A	38cde A	48cd A
F29(*F. solani*)	29cde B	76ab A	47abc B	78a A
F31(*F. solani*)	42b A	36de A	35de A	31fg A
F35(*F. solani*)	58a A	26fg B	52ab A	33efg B
F15(*F. verticillioides*)	16f A	22fg A	31ef A	25g A
F16(*F. verticillioides*)	38bc A	23fg B	31ef B	55c A
F21(*F. verticillioides*)	15f B	32ef A	31ef B	47cd A
F27(*F. verticillioides*)	20ef A	18g A	37de A	31fg A
F43(*F. equiesti*)	66a A	36de B	43bcd A	41de A
	P (F) isolates = 3.34E-21		P (F) isolates = 2.46E-14	
	P (F) cultivars = 4.1E-11		P (F) cultivars = 3.86E-08	
	P (F) interactions = 3.59E-21		P (F) interactions = 3.13E-08	

According to Fisher's 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 cultivar and capital letters to quantitative resistance between the two cultivars within each *Fusarium* isolate, Probability (P (F)) ($P = 0.05$). In the current study, all fungal isolates were reanalyzed for DS on AS and AB; however, response of AS and AB to 16 *Fusarium* tested isolates was analyzed previously and presented by Sakr (2021).

Evaluation of *Fusarium* isolates aggressiveness and Type I adult plant resistance under field conditions in the growing season 2019/20

During the growing season 2019/20, all the 16 tested fungal isolates of four *Fusarium* species were pathogenic and induced typical disease symptoms in the inoculated barley heads (Table 1). Disease symptoms were clear and easy to score in the inoculated spikes, while no symptoms were present in the control treatments. The values of DI ranged from 25% to 55% on AS and from 25% to 78% on AB, compared to 0% of the control treatment. Significant differences were observed in the mean DI scores among the four *Fusarium* species and among isolates within each species on AS. The most aggressive isolate was F7 (*F. solani*) whereas the least aggressive isolates was F2 (*F. culmorum*). There were significant differences in *Fusarium* aggressiveness among the four species and among isolates within each species on AB. F20 and F29 isolates of *F. solani*, and F3 and F30 isolates of *F. culmorum* showed the greatest aggressiveness, while F15 (*F. verticillioides*) was the least aggressive one. Both barley cultivars were not differently affected by the tested isolates in 9 out of 16 cases except for F3 and F30 (*F. culmorum*), F20 , F29 and F35 (*F. solani*), and F16 and F21 (*F. verticillioides*). AB seemed to exhibit more FHB disease incidence (Type I resistance) than AS after inoculation with *F. culmorum* and *F. solani*, while disease incidence was not significantly different between the two cultivars after inoculation with *F. verticillioides* and *F. equiseti*.

Evaluation of *Fusarium* isolates aggressiveness and resistance of barley plants by applying DHA

Inoculated and control detached spikes and spikelets of AS and AB were significantly different for DI and DS (Table 2), suggesting a strong effect of the fungi on the growth of these two cultivars. FHB symptoms were obvious and simple to rate in the inoculated spikes and spikelets, while no symptoms were present in the control treatments (Fig. 1). The bleached spikes and spikelets appeared on the first evaluation at 3 DAI, and disease progressed with time reaching the maximum severity at 9 DAI (Fig. 2). Analysis of the relation between bleaching of spikes and spikelets based on the infection period ranged from 3 to 9 DAI showed that disease progressed slowly and less severely on AS compared to AB after infection with *F. culmorum* and *F. solani*. However, *F. equiseti*, showed much more bleaching of spikes on AS as much bleaching of spikes as compared with AB, and *F. verticillioides* did not exhibit no significantly different bleaching of spikes and spikelets comparing as the two cultivars.

Evaluation of aggressiveness of FHB isolates and resistance of barley plants for Type I adult plant resistance under DHA. The FHB DI assessed on AS and AB barley cultivars inoculated with each of the 16 *Fusarium* isolates at 9 DAI is presented in Table 2. The interaction of *Fusarium* isolates with cultivars for DI was significant. The values of DI caused by the 16 tested isolates ranged from 16% to 55%% and from 15% to 81% for AS and AB, respectively. Significant differences were observed in DI scores among the four *Fusarium* species and among the isolates within each species on AS and AB cultivars. Isolate F43 (*F. equiseti*) and F30 (*F. solani*) showed the highest aggressiveness, while F15 and F27 (*F. verticillioides*) were the least aggressive isolates on AS and AB, respectively. Although both barley cultivars were differently affected by all tested isolates except for F2 (*F. culmorum*) and F16 and F21

Table 2. Disease incidence (DI) observed using head artificial inoculation and disease severity (DS) detected using floret artificial inoculation following a detached head assay with two barley cultivars, Arabi Aswad (AS) and Arabi Abiad (AB), infected with a set of 16 isolates of four *Fusarium* species

Fungal isolates (identification)	DI (%)		DS (%)	
	AS	AB	AS	AB
F1(*F. culmorum*)	21gh B	39de A	19c B	35cd A
F2(*F. culmorum*)	24ghi A	31ef A	21c A	28de A
F3(*F. culmorum*)	35def B	61b A	31b B	55b A
F28(*F. culmorum*)	30fg B	42cd A	25bc B	39c A
F30(*F. culmorum*)	29fgh B	81a A	25bc B	75a A
F7(*F. solani*)	45bc B	58b A	41a A	51b A
F20(*F. solani*)	35def B	61b A	29b B	55b A
F26(*F. solani*)	29fgh B	49c A	25bc B	42c A
F29(*F. solani*)	49ab B	64b A	41a B	54b A
F31(*F. solani*)	40cd A	25f B	32b A	19f B
F35(*F. solani*)	49ab A	31ef B	41a A	25ef B
F15(*F. verticillioides*)	16i B	29f A	20c A	25ef A
F16(*F. verticillioides*)	30fg A	31ef A	25bc A	25ef A
F21(*F. verticillioides*)	31efg A	31ef A	25bc A	29de A
F27(*F. verticillioides*)	39cde A	15g B	29b A	18f B
F43(*F. equiesti*)	55a A	24f B	45a A	28de B
	P (F) isolates = 3.56E-15		P (F) isolates = 4.41E-15	
	P (F) cultivars = 2.54E-06		P (F) cultivars = 1.35E-08	
	P (F) interactions = 3.03E-15		P (F) interactions = 8.22E-14	

According to the Fisher's 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 cultivar and capital letters to quantitative resistance between the two cultivars within each *Fusarium* isolate, Probability (P (F)) ($P = 0.05$).

(*F. verticillioides*); AB seemed to exhibit more FHB disease incidence (Type I resistance) than AS. Thus, AS appeared to be more resistant than AB to *Fusarium* infection. Consequently, DI in AS was 17.1% less than AB.

Evaluation of aggressiveness of FHB isolates and resistance of barley plants for Type II adult plant resistance under DHA. The FHB disease severity (DS) quantified on AS and AB barley cultivars inoculated with each of the 16 Fusarium isolates 9 DAI is shown in Table 2. The interaction between fungus and host for FHB DS was significant. The values of FHB DI caused by the 16 analyzed isolates varied between 19% and 45% on AS and between 18% and 75% on AB. Significant differences were observed in FHB DS ratings among the four *Fusarium* species and among the isolates within each species on AS and AB cultivars. Isolates F43 (*F. equiseti*), F7, F29 and F35 (*F. solani*) and F30 (*F. culmorum*) showed the highest aggressiveness, while F1 and F2 (*F. culmorum*), F15 and F27 (*F. verticillioides*) were the least aggressive isolates on AS and AB, respectively. Although AS and AB cultivars were not differently affected by all tested isolates except for F2 (*F. culmorum*), F7 (*F. solani*), and F15, F16 and F21 (*F. verticillioides*); disease

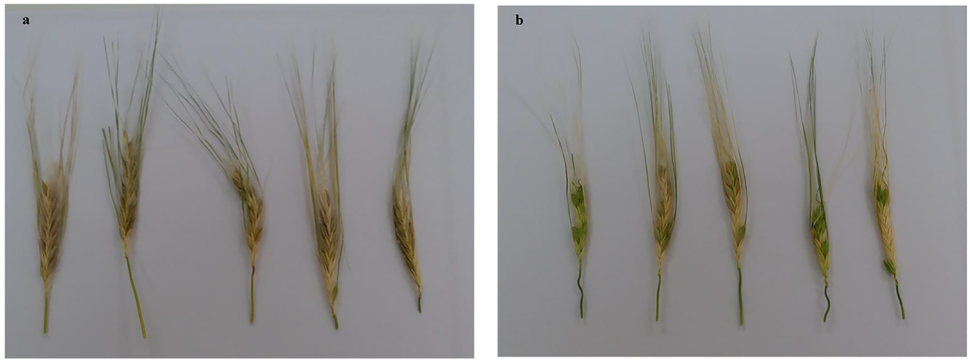

Fig. 1. Fusarium head blight symptoms 9 days after inoculation of spikes of two barley cultivars Arabi Aswad (a) and Arabi Abiad (b), inoculated with *Fusarium equiseti* (isolate F43) and *F. culmorum* (isolate F30), respectively

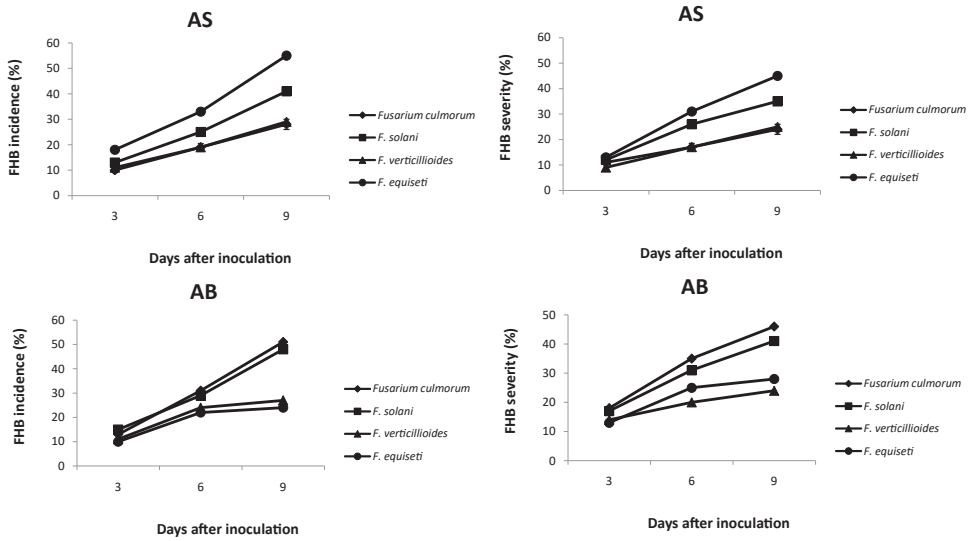

Fig. 2. Fusarium head blight progress in terms of disease incidence (FHB DI) and disease severity (FHB DS) obtained using a detached head assay with four *Fusarium* species tested in two barley cultivars, Arabi Aswad (AS) and Arabi Abiad (AB). Each point represents the mean value of DI or DS (expressed in %) of different isolates of *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti*

severity (DS) was higher in AB compared to AS cultivar, indicating that AS was more resistant than AB cultivar (21.5% difference).

Correlations between aggressiveness components generated under several experimental conditions. The values of DI and DS in the detached head assay (DHA) were significantly correlated in AS ($r = 0.970^{***}$) and AB ($r = 0.986^{***}$). Furthermore, the ratings of DI and DS in the

Table 3. Correlation coefficients among aggressiveness components generated under several experimental conditions on on two barley cultivars, Arabi Aswad (AS) and Arabi Abiad (AB) infected with 16 fungal isolates of four Fusarium head blight species determined by Pearson correlation coefficient

Aggressiveness components		DI	DS
LP	AS	0.501^{*}	0.497^{*}
	AB	0.632^{**}	0.646^{**}
AUDPC	AS	0.676^{**}	0.715^{**}
	AB	0.877^{***}	0.863^{***}
CL	AS	-0.709^{**}	-0.675^{**}
	AB	-0.818^{***}	-0.797^{***}
DI (CC)	AS	0.942^{***}	0.897^{***}
	AB	0.990^{***}	0.966^{***}
DS (CC)	AS	0.662^{**}	0.676^{**}
	AB	0.840^{***}	0.841^{***}
DI (FC, 17/18)	AS	0.753^{***}	0.773^{***}
	AB	0.835^{***}	0.809^{***}
DI (FC, 18/19)	AS	0.691^{**}	0.728^{**}
	AB	0.845^{***}	0.824^{***}
DI (FC, 19/20)	AS	0.703^{**}	0.759^{***}
	AB	0.837^{***}	0.819^{***}

Disease incidence (DI) and disease severity (DS) detected using a detached head assay 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) detected using a head artificial inoculation and disease severity (DS) detected using a floret artificial inoculation under controlled conditions in a growth chamber (CC) and disease incidence (DI) detected using a head artificial inoculation under field conditions (FC) during three growing seasons 2017/18, 2018/19 and 2019/20. Pathogenic reactions of the 16 tested FHB isolates in these two cultivars, AS and AB, were determined using *in vitro* LP and AUDPC methodologies (Sakr, 2018a, b, 2019b) and DI detected using a head artificial inoculation generated under controlled and field conditions over the two growing seasons 2017/18 and 2018/19 (Sakr, 2020a; Sakr and Shoaib, 2021). DI and DS detected using a detached head assay, and DS detected using a floret artificial inoculation under controlled conditions and DI detected using a head artificial inoculation under field conditions during the growing season 2019/20 were generated in the current research.
$(P = 0.05)^{*}$, $(P = 0.01)^{**}$, $(P = 0.001)^{***}$.

case of DHA were significantly correlated with previously obtained findings of *in vitro* latent period and area under disease progress curve and disease incidence and disease severity generated in a growth chamber under controlled and disease incidence obtained under field conditions during three growing seasons 2017/18, 2018/19 and 2019/20 on AS and AB (Table 3).

DISCUSSION

Monitoring of FHB disease incidence and severity in the growth chamber and field is a constant challenge for plant breeders, particularly for diseases like head blight on barley, which causes destructive economic damages (Janssen et al., 2018) due to variation in assessments

of the disease in the field caused by the interaction of phenotypic characteristics and environmental conditions (Dahl and Wilson, 2018). Developing *in vitro* methods that are simple to be conducted and less time consuming aimed to better monitor disease intensity is critical for the control of FHB (Hestbjerg et al., 2002; Browne and Cooke 2005; Kumar et al., 2011; Opoku et al., 2011; Bedawy et al., 2018; Sakr, 2018a, b, 2019b, 2020b; Sakr and Shoaib, 2021). Here, we report a simple, rapid and reliable, DHA (Takeda, 2004) to screen two morphologically, physiologically and genetically different barley cultivars (Ceccarelli et al., 1987) for resistance to FHB and to test aggressiveness of a collection of four Syrian *Fusarium* species: *F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti*. Differences in inoculated quantitative treatments were rated on detached heads relative to water controls, suggesting that these *Fusarium* species were suitable for the differential expression of barley cultivars' resistance in terms of DI and DS, detected using spray and point inoculations on detached barley heads.

During our investigation, DHA was found to be of particularly importance as a useful complement to growth chamber and field screening because of contradictory findings reported previously on barley plants (Han and Kim, 2005; Usele et al., 2013). DHA was selected on basis of its similarity to adult barley spike and spikelet inoculations using head and floret techniques for Type I and Type II resistance, respectively. In the case of DHA, *Fusarium* fungi overcame the morphology of the barley spike for spray inoculation and fungal inocula directly penetrated into the mature ovary for point inoculations (Takeda, 2004). Thus, FHB development is observed through the appearance of bleaching of spikes and spikeletes on detached head parts (Han and Kim, 2005; Usele et al., 2013) as observed for disease symptoms on adult plants in the growth chamber and field (Janssen et al., 2018).

The DHA used in the current study permits faster evaluation of barley cultivars and fungal isolates at a fraction of the cost of either the growth chamber and field evaluations. The DHA allows also evaluation of large numbers of cultivars and *Fusarium* isolates (Takeda, 2004). Furthermore, this *in vitro* technique provides a rapid assessment of disease reactions, comparison of the responses of AS and AB and 16 *Fusarium* isolates under DHA, growth chamber and field screens indicates that reliable resistance and aggressiveness can be confirmed in 9 days, whereas growth chamber and field evaluations required 21 days. In addition to providing a simple, rapid and reliable evaluation, using DHA has significantly less risk of obtaining ambiguous findings caused by co-infection of various pathogens, insect pests or abiotic stresses that occur in the field and may occur in growth chamber conditions (Han and Kim, 2005; Usele et al., 2013).

According to Takeda (2004), Han and Kim (2005) and Usele et al. (2013), quantitative resistant barley cultivars are identified by lower values of DI and DS compared to the susceptible ones. In our investigation, the use of detached heads has shown to be a reliable predictor of susceptibility/resistance cultivar response to *Fusarium* infection. FHB progressed slowly and less severely as generally observed on AS compared with AB. On AB, susceptible to the pathogen under several experimental conditions, inoculation with *Fusarium* species resulted in significantly higher levels of DI and DS, compared to AS. The resistance of AS was achieved by the measurement of DI quantitative resistance component reported in our investigation. When inoculated with *Fusarium* species, DI and DS of the susceptible cultivar AB, seemed to be 17.1% and 21.5% higher than those of the moderately resistant cultivar AS, respectively. Comparisons of disease intensity using DHA (DI and DS), seedlings, AUDPC, LP and CL (Sakr, 2018b,

2020b) and adult plants under controlled and field conditions over three growing seasons, DI and DS (Sakr, 2020a, b) showed completely convergence of these three methods. The data showed that AB was susceptible to FHB and AS was moderately resistant. The rating of the two tested barley cultivars shown to be either resistant or susceptible was completely consistent among the different tests.

The present study found that DI for Type I correlated with DS for Type II in AS and AB; it is of great importance to combine the two types in breeding programs to get FHB barley resistant plants. 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). Although infection response at the adult plant stage is usually the key indicator in resistance screening, the DHA has proven valuable for FHB studies in barley. Though several QTLs in host plant were shown to be effective to a broad range of pathogen isolates, some QTLs were shown to be isolate specific (Krenz et al., 2008). There were significant cultivar × isolate interactions observed in the present study which agree with previous report on barley conducted under controlled conditions (Xue et al., 2006; Sakr, 2020b). However, the statistically significant interaction between barley genotypes and *Fusarium* isolate used was not found (Takeda, 2004).

In this study, we evaluated the aggressiveness of 16 *Fusarium* isolates of four species by analyzing two components conventionally used to explain the disease response of the host (i.e., DI and DS). All analyzed *Fusarium* isolates generated FHB symptoms on barley spikes and spikelets under DHA, thus they were pathogenic. Significant differences in DS and DI were observed between fungal isolates. The wide range of variability of aggressiveness among the tested isolates in our investigation has been supported by other studies analyzing the damage of several FHB species on barley plants (Xue et al., 2006; Hestbjerg et al., 2002; Opoku et al., 2011; Garmendia et al., 2018; Sakr, 2018a, 2019b, 2020a, b; Sakr and Shoaib, 2021). Mutation, genetic recombination or selection in the 16 *Fusarium* isolates may play a crucial role in pathogenesis (Opoku et al., 2011). It is interesting, that the most aggressive isolate was *F. solani*, not the other *Fusarium* species. Although *F. solani* is known to cause rot diseases in many crops worldwide, these species were isolated from head blight infected wheat samples in Argentina (De Galich, 1997), India (Saharan et al., 2003) and Syria (Sakr, 2017). In barley, *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 (Sakr, 2020a). Re-isolation of the fungus is required from *F. solani* infected heads and grains after surface sterilization. Also, the content of mycotoxins in *F. solani* samples will be analyzed, that can be natural additional infection in *F. solani* isolates (Sakr, 2020a).

This work showed that the four *Fusarium* species were somewhat similar in the rate of FHB bleaching of spike and spikelets on AS and AB under DHA. Fernandez and Chen (2005) observed an apparent lack of difference in aggressiveness between *F. culmorum* and *F. graminearum* on wheat. In parallel, Sakr (2019b, 2020b) did not cluster the same fungal species on AS and AB using three *in vitro* criteria: AUDPC, LP and CL. Our results are not comparable with other reports showing that the four FHB species included in the present work varying in aggressiveness (Bottalico and Perrone, 2002; Xue et al., 2006). The differences in these data may be attributed to the contrasting isolates and host cultivars used in this study and previous work.

The origin of the tested *Fusarium* pathogens may play an important role in this aggressiveness similarity (Sakr, 2019b, 2020a, b; Sakr and Shoaib, 2021).

The two aggressiveness criteria detected under DHA were correlated, suggesting that these criteria are genetically indistinct, and also reflecting into complex polygenic nature of aggressiveness in the interaction in FHP-barley system. The two pathogenic indices, DI and DS, under DHA correlated with AUDPC, LP and CL, and DI and DS previously obtained under *in vitro*, controlled and field conditions (Sakr, 2019b, 2020a, b; Sakr and Shoaib 2021) with a large diversity depending on AS and AB. When considered together, these independent pathogenic researches indicate the usefulness of DI and DS for FHB evaluation concerning both the *Fusarium* and the barley. Our data are comparable with these obtained for pathogenic indices for *F. graminearum* and *Microdochium majus* on wheat (Browne, 2007; Purahong et al., 2012). Thus, the two *in vitro* components under DHA, DI and DS, predict aggressiveness occurring at the earliest and latest barley development stages during FHB infection.

CONCLUSION

To our best knowledge, this is the first in-depth report investigating the utility of DHA for distinguishing susceptibility of barley cultivars to *Fusarium* species and aggressiveness of diverse *Fusarium* species detected previously under different experimental conditions from a breeder's point of view. Here we quantified two disease components under DHA, DI and DS. However, in studies aimed to analyze the DHA (Han and Kim, 2005; Usele et al., 2013), only DS was applied. Furthermore, we compared our data with *in vitro* (AUDPC, LP and CL), growth chamber (DI for Type I and DS for Type II) and field evaluations (DI for Type I) over three growing seasons and found that barley resistance and aggressiveness in FHB fungi involves similar reactions to those controlling head blight in seedlings and adult plants, suggesting the potential of using the DHA for selection of FHB inocula with the proper and/or varying aggressiveness for breeding purposes. The DHA can be used to rapidly detect barley cultivars with superior FHB resistance and variation of diverse *Fusarium* isolates. The DHA is an efficient and reliable alternative approach for barley breeding programs. Further research efforts should consider increasing the number of barley cultivars screened for resistance to FHB.

ABBREVIATIONS

AB Arabi Abiad
AS Arabi Aswad
AUDPC area under disease progress curve
CL coleoptile length reduction
DHA detached head assay
DI Disease incidence
DS Disease severity
FHB Fusarium head blight
LP latent period

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