Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Components of quantitative resistance in barley plants to Fusarium head blight infection determined using three *in vitro* assays

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Vol. 58, No. 2: 176–183, 2018

DOI: 10.24425/122933

Received: October 26, 2017
Accepted: June 14, 2018

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Abstract

Quantitative resistance in barley to four Fusarium head blight (FHB) species was investigated *in vitro*. Nine components involved in three assays (detached leaf, modified Petridish and seedling tests) were compared on two widely grown Syrian barley cultivars: Arabi Aswad (AS) and Arabi Abiad (AB). On AB, inoculation with FHB species resulted in a significantly shorter latent period and larger lesion length of detached leaf inoculation, more standardized area under disease progress curve ($AUDPC_{standard}$) of modified Petridish inoculation and a higher percentage of infected seedlings of pin-point inoculation than on AS. The latent period of AB was 14.89% less than AS, lesion length of AS was 6.01% less than AB, $AUDPC_{standard}$ of AS was 17.07% less than AB and the percentage of infected seedlings of AS was 4.87% less than AB. Inoculation with FHB species resulted in no significant differences in the other five components measured: incubation period of detached leaf inoculation, germination rate reduction and coleoptile length reduction of modified Petridish inoculation, percentage of infected seedlings of foliar-spraying inoculation and lesion length of clip-dipping inoculation. AS was more resistant to *in vitro* FHB infection than AB. The latent period and $AUDPC_{standard}$ recorded the highest values compared with the lowest values for lesion length and percentage of infected seedlings. It seems that measurement of the latent period and $AUDPC_{standard}$ may be useful in identifying barley cultivars which are highly susceptible or resistant to FHB at early stages.

Key words: barley, detached leaf assay, FHB, *in vitro*

Introduction

Globally, Fusarium head blight (FHB) is a widespread disease of barley (*Hordeum vulgare* L.). The infestation of barley is caused by 17 *Fusarium* species, mainly by *F. graminearum*. Also, other FHB causal agents are isolated frequently from FHB infected kernels (Parry *et al.* 1995; Xue *et al.* 2006). FHB infects barley after anthesis and invades the developing caryopsis, resulting in varying degrees of deformed, shrunken and pale, rose colored grains. Substantial yield and quality losses are associated with FHB incidence (Parry *et al.* 1995). The potential accumulation of mycotoxins in the harvest can be toxic to humans and animals and causes technological problems in malt production and brewing quality (Chehri and Godini 2017).

The deployment of resistant cultivars is the most practical, cost-effective and environmentally friendly way of controlling FHB (Chrpova *et al.* 2011; He *et al.* 2015; Lenc 2015; Lenc *et al.* 2015; Khaledi *et al.* 2018). To date, conventional breeding programs have been limited by a lack of known immunity and quantitative inheritance (Capettini *et al.* 2003). Similar to wheat, two primary forms of quantitative resistance to FHB, termed type I (resistance to initial infection) and type II (resistance to fungal spread within plant

tissue), have also been reported in barley, although the former is regarded to be more important since barley exhibits a natural level of type II resistance (Geddes *et al.* 2008). Resistance of barley to FHB is controlled by a polygenic system [quantitative trait loci detected on chromosomes 2H bin8 (2Hb8) and 6H bin7 (6Hb7) in the six-rowed cultivar Chevron] (Canci *et al.* 2004).

Artificial inoculation in whole plants under controlled and field conditions represents the traditional evaluation of FHB resistance on barley plants (Chrpova *et al.* 2011; He *et al.* 2015). However, it is difficult, expensive, and needs a large area with appropriate equipment over several years. Furthermore, environmental factors are even more complicated than in wheat (Chrpova *et al.* 2011). Also, environmental factors such as temperature and humidity affect field screening of FHB resistance and complicate phenotyping and breeding efforts (Soresi *et al.* 2015). Simple, rapid and reliable *in vitro* tests have been proposed as alternative methods for resistance screening in wheat such as detached leaf assay (Browne and Cooke 2004, 2005), seedling resistance assay (Shin *et al.* 2014), seed germination assay (Browne 2007; Shin *et al.* 2014), coleoptiles assay (Soresi *et al.* 2015) and response to the FHB mycotoxin (Mesterhazy 2002). Comparing with wheat, there are some reports about *in vitro* assays for screening FHB resistance in barely (Browne and Cooke 2005; Kumar *et al.* 2011; Bedawy *et al.* 2018).

The most FHB resistant barley cultivars exhibit undesirable agronomic attributes, susceptibility to other diseases, and poor grain quality, highlighting the necessity of identification and utilization of new resistance sources from the locally adaptive derivatives (He *et al.* 2015). In Syria, the domestication of barley took place prior to 7000 B.C. Barley cultivation covers up to one million hectares with less than one million tons in 2011. Syrian farmers predominantly grow two old cultivars: Arabi Aswad and Arabi Abiad. Thereby, these landraces may constitute a valuable genetic resource since they possess various desirable agronomic traits, including acceptable levels of resistance to FHB. To date, there are no reports about the presence of FHB on barley in Syria. However, FHB species are frequently isolated in wheat cultivated areas (Alazem 2007). Recently, an *in vitro* modified Petri-dish assay (Purahong *et al.* 2012) to analyze pathogenic variation in Syrian FHB isolates recovered from wheat on barley plants was used by Sakr (2018a). Purahong *et al.* (2012) analyzed three aggressiveness criteria largely used in FHB resistance breeding in wheat. The aim of the current study was to investigate the utility of nine criteria for three *in vitro* assays (detached leaf, modified Petri-dish and seedling), widely used in screening wheat resistance, for identification of the components of quantitative resistance in two barley cultivars (Arabi Aswad and Arabi Abiad) to FHB infection.

Materials and Methods

Syrian barley cultivars and fungal isolates

To characterize quantitative resistance components of barley plants infected by FHB isolates, two barley cultivars with the highest agronomic traits and resistance to fungal diseases and well adapted to arid growing conditions in different Syrian locations were used: Arabi Aswad (AS) and Arabi Abiad (AB).

The 16 fungal isolates of four Fusarium species: *F. culmorum* (F1, F2, F3, F28 and F30), *F. verticillioides* (F15, F16, F21 and F27), *F. solani* (F7, F20, F26, F29, F31 and F35), and *F. equiesti* (F43) used in this study were recovered from wheat spikes showing FHB symptoms in 2015. Isolates were identified morphologically according to Nelson *et al.* (1983). These FHB isolates showed a similar range of aggressiveness on Arabi Aswad and durum wheat plants *in vitro* (Sakr 2018a). The cultures were maintained in sterile distilled water at 4°C and by freezing at –16°C until needed (Sakr 2018b).

Measurement of quantitative resistance components

Methods for the detached-leaf assay were reported by Browne and Cooke (2004, 2005). The barley plants were grown in a growth chamber at 20°C during day and night with a 16-h photoperiod. After 14 days, segments 4 cm in length from the midsection of the first expanding seedling leaf were harvested and placed adaxial surface up on the surface of Petri dishes containing artificial media (four leaves per Petri dish). Leaf segments were inoculated at the center of the adaxial surface with 10 µl inoculum suspension of 1×10^6 conidia per ml. Sterile distilled water was applied on the control leaves. Petri dishes were incubated at 25°C with a 12-h photoperiod. Evaluations of symptom appearance and sporulation were carried out daily under a light microscope (magnification ×40). The components of quantitative disease resistance measured were: incubation period (period in days from inoculation to first appearance on the leaf surface, a dull gray-green water-soaked lesion), latent period (period in days from inoculation to sporulation), and lesion length (measured after 7 days as a visible necrotic area). Three replicates of each isolate based on observations on 120 detached leaves were set up, and the experiment was repeated.

Methods for the modified Petri-dish assay were presented by Purahong *et al.* (2012). Sterilized barley seeds were inoculated with a suspension of conidia at 1×10^6 conidia per ml (or sterile distilled water in the control treatment) for 16 fungal isolates in Petri dishes

with sterile double-layer filter paper. Three quantitative resistance criteria: germination rate reduction, standardized area under disease progress curve ($AUDPC_{standard}$), and coleoptile length reduction were evaluated. Three replicates of each isolate were set up, and the experiment was repeated. Infected and control treatments were incubated at 22°C in the dark. Germination rate reduction and coleoptile length reduction were determined by comparison with the control treatment 6 days after inoculation. The value of $AUDPC_{standard}$ ranged from 0 (very resistant) to 1 (not resistant). It was calculated from the percentage of healthy coleoptiles as a function of time (from 2 to 6 days after inoculation).

Methods for the seedling assay were described by Shin *et al.* (2014). In pin-point experiments, 3-day barley seedling stems were inoculated by pin-point wounding with 10 µl of a suspension of conidia at 4×10^4 conidia per ml, and sterile distilled water in the control treatment. Twenty seedlings were grown in an incubator at 20°C during day and night with a 12-h photoperiod. The percentage of infected seedlings with visible necrotic lesion and/or sporulation of fungal disease symptoms was measured 7 days after inoculation. In foliar-spraying experiments, 10-day barley seedling stems were sprayed on both sides of leaves with a conidial suspension at 4×10^4 conidia per ml using an atomizer. Twenty seedlings were grown in an incubator operated with relative humidity (RH) of 100% at 25°C for 3 days and then returned to the growth chamber for disease evaluation. Sterile distilled water was applied on the control seedlings. The percentage of infected seedlings was measured 7 days after inoculation. In clip-dipping experiments, the tips of 10-day barley seedling stems were cutoff and then dipped three times in 20 ml of a suspension of conidia at 4×10^4 conidia per ml. Inoculated seedlings were kept covered for 3 days using polythene bags to ensure 100% of RH and then moved to a growth chamber for disease evaluation. Controls were dipped with sterile distilled water only. Lesions on the inoculated leaves were measured 7 days after inoculation. For these three experiments, three replicates of each isolate were set up, and the experiment was repeated.

Statistical analysis

Statistical analysis of the quantitative resistance data were performed using StatView, 4.57® Abacus Concepts, Berkley, Canada. Prior to analysis, the percentages were transformed using the angular transformation to stabilize variances. A complete randomized design with two factors (Fusarium isolate and barley cultivar) and three replications were used for quantitative resistance analysis. Fisher's LSD test was used to compare the means at $p \leq 0.05$. The sample correlation coefficients (Pearson r) were calculated using overall mean values per isolates at $p \leq 0.001$.

Results

Compared with water control, the mean values of inoculated treatments for the nine quantitative resistance components on two barley cultivars: Arabi Abiad and Arabi Aswad were reduced, suggesting a strong effect of the FHB species complex on the growth of these landraces (Tables 1–3). Seedlings of two barley cultivars grown in the presence of 16 tested fungal isolates showed typical *in vitro* FHB symptoms according to three analyzed inoculation techniques, whereas the control plants did not show any disease symptoms.

Results for detached-leaf assay are presented in Table 1. There were no differences ($p \leq 0.4767$) in the incubation period of the two tested cultivars. Mean incubation period for AB was 2.07 days and for AS, it was 2.15 days. Mean latent period of AB (5.78 days) was significantly ($p \leq 0.0001$) shorter than of AS (6.78 days). Latent period of AB was 14.89% less than AS. Mean lesion length on AB (7.82 mm) was significantly ($p \leq 0.0012$) greater than that of AS (7.35 mm). Lesion length of AS was 6.01% less than AB. No significant differences for incubation period and lesion length were found between the tested fungal isolates. The latent period values underlined a variation in aggressiveness between the FHB isolates.

Results for modified Petri-dish assays are presented in Table 2. There were no differences ($p \leq 0.2010$) in germination rate reduction between the two tested cultivars. Mean germination rate reduction for AB was 21% and for AS, it was 20%. Mean $AUDPC_{standard}$ of AB (0.42) was significantly ($p \leq 0.0001$) greater than that of AS (0.34). $AUDPC_{standard}$ of AS was 17.07% less than AB. Diseased coleoptiles were only one half of mean lengths of healthy coleoptiles that reached 10.3 mm and 10.1 mm for AB and AS, respectively regardless of the FHB isolate. There were no differences ($p \leq 0.4528$) in coleoptile length reduction for the two tested cultivars. Mean coleoptile length reduction of AB was 57% and of AS it was 56%. No significant differences in germination rate reduction and coleoptile length reduction were found between the FHB isolates. The values of $AUDPC_{standard}$ underlined a variation in aggressiveness among the tested fungal isolates.

Results for seedling assay are presented in Table 3. There were no differences ($p \leq 0.4850$) in percentages of infected seedlings with foliar-spraying inoculation of the two tested cultivars. Mean percentage of infected seedlings of AB was 41% and of AS it was 43%. Mean

Table 1. Disease reactions of detached leaf assay in two Syrian barley cultivars inoculated with 16 fungal isolates of four Fusarium head blight species

Fungal isolates (identification)	Incubation period [days]		Latent period [days]		Lesion length [mm]	
	Arabi Abiad	Arabi Aswad	Arabi Abiad	Arabi Aswad	Arabi Abiad	Arabi Aswad
F1 (*Fusarium culmorum*)	2.27	2.23	8.10	7.71	8.92	7.43
F2 (*F. culmorum*)	2.08	2.23	3.59	5.79	7.58	7.20
F3 (*F. culmorum*)	1.98	2.27	4.94	4.41	7.43	7.51
F28 (*F. culmorum*)	2.23	2.20	6.28	5.78	8.49	8.10
F30 (*F. culmorum*)	2.27	1.98	8.45	7.55	7.51	6.77
F7 (*F. solani*)	2.03	2.35	9.45	9.00	7.62	7.31
F20 (*F. solani*)	2.35	1.97	5.58	7.97	7.12	7.31
F26 (*F. solani*)	2.04	2.35	5.65	7.85	7.12	7.62
F29 (*F. solani*)	2.03	2.35	8.45	7.55	7.31	7.12
F31 (*F. solani*)	2.03	2.00	4.15	6.52	8.02	7.12
F35 (*F. solani*)	1.95	2.10	5.30	7.73	9.28	6.82
F15 (*F. verticillioides*)	2.10	1.93	3.49	4.41	8.28	7.72
F16 (*F. verticillioides*)	2.04	1.95	3.13	4.96	7.21	7.86
F21 (*F. verticillioides*)	1.73	2.23	5.39	7.07	7.59	7.12
F27 (*F. verticillioides*)	1.97	2.04	5.80	6.28	7.86	7.17
F43 (*F. equiesti*)	2.08	2.23	4.66	7.97	7.84	7.45
Mean	2.07 a	2.15 a	5.78 b	6.78 a	7.82 a	7.35 b
	F isolates = 0.176 ns; p = 0.9997		F isolates = 13.321; p = 0.0001		F isolates = 1.623 ns; p = 0.0923	
	F cultivars = 0.512 ns; p = 0.4767		F cultivars = 23.816; p = 0.0001		F cultivars = 11.518; p = 0.0012	
	F interactions = 0.312 ns; p = 0.9925		F interactions = 3.047; p = 0.0010		F interactions = 1.808; p = 0.0528	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at $p \leq 0.05$, ns = not significant, F tests ($p \leq 0.05$) (F), probability (p)

percentage of infected seedlings of pin-point inoculation of AB (42%) was significantly ($p \leq 0.0205$) greater than that of AS (40%). Percentage of infected seedlings of AS was 4.87% less than AB. There were no differences ($p \leq 0.4501$) in lesion length of clip-dipping inoculation of the two tested cultivars. Mean lesion length on AB was 2.07 cm and on AS it was 2.16 cm. No significant differences were observed among the tested fungal isolates for the three criteria involved in this assay.

The repeatability and stability of *in vitro* tests were demonstrated with the highly significant correlation coefficients between the nine components (from two experiments): $r = 0.881$, $p \leq 0.001$ for incubation period, $r = 0.865$, $p \leq 0.001$ for lesion length of detached leaf inoculation, $r = 0.939$, $p \leq 0.001$ for latent period, $r = 0.874$, $p \leq 0.001$ for germination rate reduction, $r = 0.890$, $p \leq 0.001$ for coleoptile length reduction, $r = 0.854$, $p \leq 0.001$ for $AUDPC_{standard}$, $r = 0.805$, $p \leq 0.001$ for percentage of infected seedlings of foliar-spraying inoculation, $r = 0.887$, $p \leq 0.001$ for percentage of infected seedlings of pin-point inoculation and $r = 0.811$, $p \leq 0.001$ for lesion length of clip-dipping inoculation.

For all criteria studied (except for latent period, lesion length of detached-leaf inoculation and $AUDPC_{standard}$), there was no significant interaction between the two factors: FHB isolate and barley cultivar (Tables 1–3).

Discussion

Identifying new resistance sources for Fusarium head blight is the most practical and sound way to minimize economic losses from this disease (Chrpova *et al.* 2011; He *et al.* 2015; Lenc 2015; Lenc *et al.* 2015; Khaledi *et al.* 2018). Field and greenhouse screening has limitations e.g. approximately 2.5 months are required to get the spike and about 21–30 days are needed for disease evaluation after inoculations (Chrpova *et al.* 2011; He *et al.* 2015). Faster, easier and fewer experimental procedures are required as well as accurate methods to identify FHB resistant varieties for barley breeding. In this study, we evaluated nine quantitative resistance components involved in three *in vitro* assays for rapid

Table 2. Disease reactions of Petri-dish assay in two Syrian barley cultivars inoculated with 16 fungal isolates of four Fusarium head blight species

Fungal isolates (identification)	Germination rate reduction [%]		$AUDPC_{standard}$		Coleoptile length reduction [%]	
	Arabi Abiad	Arabi Aswad	Arabi Abiad	Arabi Aswad	Arabi Abiad	Arabi Aswad
F1 (*Fusarium culmorum*)	23	23	0.35	0.22	58	57
F2 (*F. culmorum*)	19	21	0.26	0.29	58	52
F3 (*F. culmorum*)	20	19	0.58	0.39	58	58
F28 (*F. culmorum*)	24	22	0.45	0.29	59	62
F30 (*F. culmorum*)	23	23	0.70	0.34	58	52
F7 (*F. solani*)	20	20	0.67	0.45	59	56
F20 (*F. solani*)	23	23	0.40	0.40	53	56
F26 (*F. solani*)	20	18	0.40	0.39	59	59
F29 (*F. solani*)	20	20	0.60	0.38	58	55
F31 (*F. solani*)	23	20	0.30	0.33	55	55
F35 (*F. solani*)	23	20	0.38	0.39	56	48
F15 (*F. verticillioides*)	22	19	0.25	0.22	54	59
F16 (*F. verticillioides*)	19	19	0.41	0.31	55	60
F21 (*F. verticillioides*)	20	17	0.38	0.35	55	55
F27 (*F. verticillioides*)	21	17	0.22	0.25	58	51
F43 (*F. equiesti*)	23	21	0.33	0.40	56	57
Mean	21 a	20 a	0.42 a	0.34 b	57 a	56 a
	F isolates = 0.692 ns; p = 0.7825		F isolates = 12.769; p = 0.0001		F isolates = 1.031 ns; p = 0.4364	
	F cultivars = 1.669 ns; p = 0.2010		F cultivars = 35.666; p = 0.0001		F cultivars = 0.571 ns; p = 0.4528	
	F interactions = 0.178 ns; p = 0.9997		F interactions = 5.002; p = 0.0001		F interactions = 1.006 ns; p = 0.4604	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at $p \leq 0.05$, ns = not significant, F tests ($p \leq 0.05$) (F), probability (p). In the current study, all fungal isolates were reanalyzed for disease reaction on Arabi Aswad, however, response of Arabi Aswad to 6 tested FHB isolates was analyzed previously and presented by Sakr (2018a)

screening of two barley cultivars for resistance to FHB. The two tested barley cultivars are two-row lines which are supposed to exhibit better resistance to FHB progression as described by He *et al.* (2015).

In FHB-wheat *in vitro* research, the relationships between detached leaf, modified Petri-dish and seedling-determined quantitative resistance components and FHB resistance in adult plant spikes using spraying and point inoculation assays for Type I and Type II resistance have been reported and yielded satisfactory results (Browne and Cooke 2004; Browne 2007, 2009; Purahong *et al.* 2012; Shin *et al.* 2014; Soresi *et al.* 2015). Due to these advantages, these three assays were selected in this current research. The repeatability and stability of the three tested *in vitro* assays were verified with the highly significant correlation coefficients between the nine components during our investigation.

Disease development by FHB fungi is thought to be different as a result of experimental conditions involved in the three *in vitro* assays: detached leaf, modified Petri dish and seedling. This ensures that their pathogenicity is constant and/or correctly characterized or measured. All 16 fungal isolates analyzed with the three *in vitro* assays fulfilled the requirement of pathogenicity (ability to induce FHB disease), thus they are pathogenic.

The seven criteria: incubation period and lesion length of detached leaf test, germination rate reduction and coleoptile length reduction of modified Petri-dish assay, percentage of infected seedlings (of foliar-spraying and pin-point inoculations) and lesion length of seedling test did not identify the tested fungal isolates. Our results are in accordance with *in vitro* previous germination rate reduction and coleoptile length reduction analyses in which these two criteria did not identify FHB isolates on barley and wheat plants (Sakr 2017b, 2018a). In contrast to our data, the variation, measured by lesion length of detached-leaf assay, was detected of different *F. langsethiae* isolates (Opoku *et al.* 2011). The values of latent period and $AUDPC_{standard}$ underlined the variability among the FHB isolates. Our results are in accordance with

Table 3. Disease reactions of seedling assay in two Syrian barley cultivars inoculated with 16 fungal isolates of four Fusarium head blight species

Fungal isolates (identification)	Spraying [%]		Pin-point [%]		Clip-dipping [cm]	
	Arabi Abiad	Arabi Aswad	Arabi Abiad	Arabi Aswad	Arabi Abiad	Arabi Aswad
F1 (*Fusarium culmorum*)	45	45	46	41	2.23	2.27
F2 (*F. culmorum*)	45	42	40	41	2.23	2.27
F3 (*F. culmorum*)	45	44	41	40	2.27	2.20
F28 (*F. culmorum*)	44	47	43	36	2.20	2.35
F30 (*F. culmorum*)	34	45	39	40	1.70	2.55
F7 (*F. solani*)	47	41	41	41	2.35	2.03
F20 (*F. solani*)	39	47	41	38	1.97	2.35
F26 (*F. solani*)	47	41	39	38	2.35	2.03
F29 (*F. solani*)	47	47	45	39	2.35	2.35
F31 (*F. solani*)	35	35	43	34	1.76	1.76
F35 (*F. solani*)	35	41	39	41	1.76	2.03
F15 (*F. verticillioides*)	37	51	44	41	1.83	1.93
F16 (*F. verticillioides*)	35	41	41	39	1.73	2.03
F21 (*F. verticillioides*)	45	39	45	41	2.23	1.93
F27 (*F. verticillioides*)	39	41	40	42	1.93	2.20
F43 (*F. equiesti*)	45	45	38	42	2.23	2.27
Mean	41 a	43 a	42 a	40 b	2.07 a	2.16 a
	F isolates = 0.480 ns; p = 0.9423		F isolates = 0.825 ns; p = 0.6465		F isolates = 0.551 ns; p = 0.9004	
	F cultivars = 0.493 ns; p = 0.4850		F cultivars = 5.647; p = 0.0205		F cultivars = 0.577 ns; p = 0.4501	
	F interactions = 0.390 ns; p = 0.9772		F interactions = 1.313 ns; p = 0.2208		F interactions = 0.402 ns; p = 0.9737	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at $p \leq 0.05$, ns = not significant, F tests ($p \leq 0.05$) (F), probability (p)

previous *in vitro* latent periods and $AUDPC_{standard}$ analyses in which these criteria distinguished FHB isolates on barley and wheat plants (Sakr 2017b, 2018a, unpublished data). Mutation, genetic recombination or selection may play crucial roles in these differences. In three FHB species: *F. culmorum*, *F. verticillioides*, *F. solani* collected from wheat kernels with FHB symptoms in different Syrian locations in 2007, high genotypic and pathogenic variances among FHB isolates were detected (Alazem 2007).

The collective effects of each or some of the quantitative components are particularly important. The differences between AB and AS were 6.01% in lesion length and 14.89% in latent period. Incubation period was not an important factor of quantitative resistance in barley plants. However, Browne and Cooke (2005) found significant differences in incubation period, lesion length and latent period for 15 winter barley cultivars with variable levels of resistance. Our results are comparable with those reported by Kumar *et al.* (2011). They noted that the latent period was correlated to field barley ratings and this measurement may be useful in identifying genotypes highly susceptible or resistant to FHB. Three quantitative trait loci (QTL) were common for leaf and spike disease scoring carried out on barley plants via artificial inoculations under control conditions. This indicates a partial genetic relatedness of these resistances in barley (Bedawy *et al.* 2018). Our observations indicated that lesions on barley were not visibly chlorotic when placed under a light microscope until sporulation occurred; this was in accordance with the findings of Browne and Cooke (2005) and Opoku *et al.* (2011) for *Microdochium nivale and F. langsethiae*. The pattern of delayed chlorosis of the infected leaf tissue and longer latent periods indicate that resistances are expressed in barley after the incubation period is observed, and that these temporarily arrest the development of mycelium and sporulation (Browne and Cooke 2005; Opoku *et al.* 2011). Also, Browne (2009) found that shorter incubation periods, longer latent periods and shorter lesions were related to an important component of whole-wheat FHB resistance measured by single point inoculation (type II).

Reductions in germination rate and coleoptile length did not appear to significantly affect quantitative resistance to FHB in barley. Our results are comparable with previous *in vitro* analysis in which those criteria did not differ between the wheat cultivars (Sakr 2017b). However, seed germination and coleoptile length assays are two methods commonly used for the assessment of resistant wheat cultivars. Higher germination rates were related to greater FHB type II resistance (Browne 2007, 2009). Soresi *et al.* (2015) found that coleoptile length was correlated with FHB type II resistance. In contrast, Shin *et al.* (2014) noted that reductions in germination rate were poorly correlated with the degree of resistance in adult plants. $AUDPC_{standard}$ of AS was 17.07% less than AB, and this value was the highest in this study. $AUDPC_{standard}$ was calculated from the decreasing number of healthy wheat seedlings after fungal inoculation of the seeds (Purahong *et al.* 2012). The slower reduction of the number of healthy seedlings, the more resistant is the cultivar (Purahong *et al.* 2012). Our results are in accordance with previous *in vitro* analysis in which this criterion distinguished between the wheat cultivars (Sakr 2017b). Sakr (2018a; unpublished data) showed that eight durum and bread Syrian wheat cultivars exhibited a higher $AUDPC_{standard}$ than AS using the same fungal isolates. Thus, Sakr (2018a; unpublished data) provided evidence that AS is more resistant than Syrian wheat cultivars using $AUDPC_{standard}$ criterion. Therefore, our data showed that $AUDPC_{standard}$ was the most important criterion in differentiating between the two tested barley cultivars. *In vitro* $AUDPC_{standard}$ data were highly significantly correlated with artificial inoculation data obtained using adult plants under controlled and field conditions (types I and II) (Purahong *et al.* 2012; Sakr 2017a).

The two quantitative components: percentage of infected seedlings of foliar-spraying inoculation and lesion length of clip-dipping inoculation did not differ between AB and AS. Indeed, the value (4.87%) for the percentage of infected seedlings of pin-point inoculation recorded the lowest value in differentiation between the two tested barley cultivars. However, lesion length was correlated with FHB type II resistance (Shin *et al.* 2014). Also, the percentage of infected seedlings of other seedling inoculations described above was not correlated with adult FHB resistance (Shin *et al.* 2014).

Quantitative resistant barley cultivars are identified by long latent periods, short lesion lengths, smaller percentages of infected seedlings and less $AUDPC_{standard}$ of the fungus compared with the susceptible one. Our results showed that AS was more resistant to FHB infection than AB. This observation suggests that in AS, the development of the FHB pathogens was slowed down, and might be due to resistance mechanisms expressed by different responses conferred by QTL during FHB infection in barley plants (Chrpova *et al.* 2011). These results are in accordance with a previous analysis in which AS was more resistant than AB against common root rot (*Cochliobolus sativus*) (van Leur *et al.* 1997) and leaf blotch caused by the fungus *Rhynchosporium secalis* (Abang *et al.* 2006). It seems that quantitative trait loci for resistance to these diseases may share the same genetic background. Although the most FHB resistant barley cultivars exhibit poor agronomical characteristics (Chrpova *et al.* 2011), the variability of resistance for AS and AB, with the highest agronomic traits, is interesting and promising for improving resistance of barley cultivars.

The *in vitro* components evaluated were not equally informative for FHB resistance in barley. The present study showed differences in lesion length and percentage of infected seedlings between AS and AB, with low values (6.01% and 4.87%, respectively) and did not present important criteria. However, these differences ranged from 14.89% in the latent period to 17.07% in $AUDPC_{standard}$. The latent period and $AUDPC_{standard}$ recorded the highest values compared with the lowest values for lesion length and percentage of infected seedlings. The *in vitro* evaluation of quantitative resistance components against FHB indicates the potential of the latent period and $AUDPC_{standard}$ assays to distinguish between specific sources/mechanisms of FHB resistance. Although based on two barley cultivars, our results indicate that the level of resistance or susceptibility to FHB isolates can be recognized at the early stages of plant growth. These *in vitro* assays can promote interaction between barley tissues and fungi. The situation in the detached leaf and modified Petri-dish assays was identical to head inoculation. FHB inoculum was put directly on the barley seeds and they could directly penetrate and infect germinating seeds as well as leaves. Thus, disease development is manifested through the appearance of symptoms such as brown spots on the coleoptiles and/or by mycelium completely covering the seeds, and discolored, malformed, necrotic or chlorotic areas on the affected plant part. It seems that measuring the latent period and $AUDPC_{standard}$ may be useful in identifying barley cultivars highly susceptible or resistant to FHB at early stages. Since only two barley cultivars were tested here, further research using a large sample of available Syrian barley cultivars is needed to validate our results *in vitro*, under controlled and field conditions. Also, it will be necessary to analyze the relationship between *in vitro*-determined quantitative resistance components and FHB resistance using spray inoculation, termed Type I resistance to better understand the utility of *in vitro* components involved in the expression of FHB resistance.

Acknowledgements

The author would like to thank the Director General of AECS and the Head of the Agriculture Department for their financial support.

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