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Aggressiveness Variation among and within Fusarium Head Blight Species on Barley *in vitro*

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Aggressiveness variation among 16 isolates of four *Fusarium* species (*F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti*) causing Fusarium head blight (FHB) was studied *in vitro*. Evaluation of three aggressiveness criteria involved in a Petri-dish test: germination rate reduction, standardized area under disease progress curve ($AUDPC_{standard}$), and coleoptile length reduction was carried out on the barley cultivar Arabi Aswad. Results showed differences between barley plants inoculated with FHB isolates and control for the three tested aggressiveness criteria. Regarding $AUDPC_{standard}$ and Petri-dish aggressiveness index which is calculated from the mean value of three aggressiveness criteria, within and among variation was detected. *Intra-* and *inter*-species variability was not distinguished for the other two aggressiveness criteria. However, pathogenic level observed among 16 isolates can not be differentiated within the four FHB species. Significant correlation was detected only between $AUDPC_{standard}$ and Petri-dish aggressiveness index. The results were comparable with those previously obtained using the same fungal isolates on wheat cultivar *in vitro*. It seems that FHB isolates recovered from wheat spikes and tested on wheat plants showed a similar range of aggressiveness on a barley cultivar, Arabi Aswad.

Keywords: Barley cultivar, FHB species, *in vitro* test, pathogenic variation.

Fusarium head blight (FHB) is one of the most important diseases of barley, wheat and other small grain crops worldwide. FHB infection occurs primarily during the flowering of the crop and shortly afterward when humid conditions and moderate temperatures prevail. Bleached spike appearance due to premature death of tissues is a typical symptom of FHB in infected plants. Diseased spikes are sterile or contain discolored and shriveled seeds (Parry et al., 1995). This disease known to impact significantly upon the yield causing dramatic economic losses of up to 50% (Parry et al., 1995; McMullen et al., 2012). Also, FHB reduces grain quality by producing mycotoxins during fungal colonization such as deoxynivalenol and nivalenol, which presents a major potential risk to both humans and animals (Maresca, 2013). Several functional parameters of grain related to malting and brewing quality were severely influenced by mycotoxin contamination (Nielsen et al., 2014).

FHB is caused by at least seventeen species within the *Fusarium* genus. *Fusarium graminearum* is reported as the most prominent species in barley. Also, other FHB causal agents are isolated frequently from FHB infected kernels (McCallum and Tekauz, 2002;

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Xue et al., 2005; Bilikova and Hudec, 2014; Ovsyankina et al., 2016). The pathogenic level revealed that *F. crookwellense*, *F. culmorum* and *F. graminearum* are highly pathogenic, *F. avenaceum* is moderately pathogenic, and *F. equiseti*, *F. poae* and *F. sporotrichioides* are weakly pathogenic (Xue et al., 2005). Environmental factors, agricultural practices and cultivar susceptibility play an important role in pathogenesis (Doohan et al., 2003).

Fundamental characterization of aggressiveness of a FHB species, the extent to which it can attack a susceptible host, explains the interaction between FHB populations and barley. Also, comprehension of the aggressiveness variability is crucial for disease management (Xue et al., 2005). Resistance of barley to FHB is a character controlled by a polygenic system (quantitative trait loci detected on all chromosomes), with no clear evidence for host by pathogen species interaction (Steffenson, 2003). For a given FHB species, aggressiveness of different isolates may vary, with the more aggressive ones affecting barley plants faster and/or more intensively. Several reports have underlined a significant variation for aggressiveness in FHB causal agents on barley plants (McCallum and Tekauz, 2002; Xue et al., 2005; Bilikova and Hudec, 2014; Ovsyankina et al., 2016). Floret inoculation in adult plants in a growth chamber and in the field represents the traditional evaluation of FHB aggressiveness on barley plants (Imathiu et al., 2014). However, *in vitro* assays for prescreening FHB aggressiveness has been gained little attention as compared with wheat such as: seedling test (Hestbjerg et al., 2002), and detached leaf assay (Opoku et al., 2011). Whether FHB isolates recovered from barley and wheat show similar agressiveness lack specialization in their hosts is not clear since most studies focus on single measurement of aggressiveness in a specific host. The pathogenicity of *Fusarium* spp. on barley may be or not the same as on wheat (McCallum and Tekauz, 2002; Xue et al., 2005).

In Syria, barley cultivation up to 1262000 hectares is distributed in marginal environments where severe drought, thermal stress and spatial and temporal variations in rainfall are prevailing. Barley is mostly used for livestock feed with less than one million tones in 2013. Syrian farmers predominantly grow one cultivar Arabi Aswad, it is largely adapted to drier areas (Bishawa et al., 2015). There are no reports about the presence of FHB on barley in Syria, but *Fusarium* species are present and frequently isolated in wheat cultivated areas (Alazem, 2007). Sakr (2017b) used an *in vitro* approach adapted by Purahong et al., (2012) to analyse pathogenic variation in FHB isolates recovered from wheat kernels, and he found significant differences between pathogen isolates and wheat cultivars. Also, floret inoculations data under controlled conditions were comparable with those obtained *in vitro* (Sakr, 2017a). In order to better understand the pathogenic variation in Syrian FHB species complex on barley plants, the objectives of the current research were to (i) investigate variability within four FHB species (*F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti*) *in vitro*, and (ii) determine whether or not the observed variability can be used to differentiate the FHB species.

Materials and Methods

Fungal isolates and barley cultivar

Sixteen 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. equiseti* (F43)) were recovered from wheat spikes showing FHB symptoms in 2015. Isolates were identified morphologically according to Nelson et al. (1983). The cultures were maintained in sterile distilled water at 4 °C and freezing at –16 °C until needed.

Barley cultivar Arabi Aswad, largely cultivated in Syria, is characterized with high agricultural criteria under sever climatic conditions, and resistance to fungal diseases. It was used to characterize aggressiveness of 16 FHB isolates.

Aggressiveness tests

Purahong et al. (2012) adapted a Petri-dish test to quantify aggressiveness in *F. graminearum*, and this method was used by Sakr (2017b) to analyse aggressiveness in other FHB species on wheat plants. It requires less time (only 6 days for the whole experiment), and *in vitro* data were comparable with the floret inoculations (Purahong et al., 2012; Sakr, 2017a). In the current study, this method was conducted to characterize aggressiveness of 16 FHB isolates.

Sterilized barley seeds for Arabi Aswad 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 aggressiveness 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 in an incubator at 22 °C in the dark. Germination rate reduction and coleoptile length reduction were determined by comparison with the control treatment at 6 days after inoculation. The value of $AUDPC_{standard}$ ranges from 0 (not aggressive) to 1 (very aggressive), and it is calculated from the percentage of healthy coleoptiles as a function of time (from 2 to 6 days after inoculation). Petri-dish index calculated from the mean value of three aggressiveness criteria linked with aggressiveness of fungal isolates.

Statistical analyses

Statistical analyses of aggressiveness data were performed using StatView, 4.57® Abacus Concepts, Berkley, Canada. Before statistical analysis, the percentages were transformed using the Arcsines function. A complete randomized design with one factor (*Fusarium* isolate) and 3 replications was used for aggressiveness analysis. Fisher's LSD test was used to compare the means at $P = 0.05$. The sample correlation coefficients (Pearson *r*) were calculated using overall mean values per isolates at $P = 0.05$ and $P = 0.01$.

Results and Discussion

Because of costly, time consuming, laborious work in the floret inoculation assay under controlled and field conditions (Imathiu et al., 2014), *in vitro* assay has a high potential to facilitate the analysis of aggressiveness in several phytopathogenic species of the genus *Fusarium.* With this in mind, the variation of quantitative component of pathogenicity was analysed for four Fusarium head blight (FHB) species using a Petri-dish test adapted by Purahong et al., (2012) on a barley cultivar Arabi Aswad widely cultivated in Syrian provinces. Indeed, Sakr (2017b) quantified the aggressiveness of four FHB isolates on six wheat cultivars and Sakr (unpublished data) analysed pathogenic variation in 16 fungal isolates on wheat cultivar Cham7 using a Petri-dish test adapted by Purahong et al. (2012). However, in the current study, a barley cultivar Arabi Aswad showing a high level of quantitative resistance was used to underline variation of aggressiveness of 16 fungal isolates of four FHB species (previously analysed by Sakr (unpublished data) on wheat).

Intra- and *inter-* differences in aggressiveness of four *Fusarium* species (*F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiseti*) are indicated when isolates vary in the amount of damage that they cause in barley plants. All the 16 isolates of four *Fusarium* species caused brown spots on the coleoptiles and/or by mycelium completely covering the seeds, typical *in vitro* FHB symptoms (Purahong et al., 2012, Sakr, 2017b), in the inoculated Arabi Aswad barley plants, whereas the control plants did not exhibit any symptoms (Fig. 1).

Differences between barley plants inoculated with fungal isolates and control were shown for three tested aggressiveness criteria: germination rate reduction, standardized area under disease progress curve ($AUDPC_{standard}$), and coleoptile length reduction (Table 1). This indicates that a modified Petri-dish test (Purahong et al., 2012) conducted on *F. graminearum* and four FHB species (Sakr, 2017b) can distinguish significant differences between control treatments and barley plants infected with FHB species (*F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiseti*). Although the number of germinated seeds reduced significantly in fungal treatments by a fifth compared with the control ranging from 19 to 23% (Table 1), and the diseased coleoptiles were only one half of mean lengths of healthy coleoptiles that reached 10.1 mm on Arabi Aswad whatever was the FHB isolate ranging from 55 to 58% (Table 1), there were no significant differences *intra-* and *inter-* the four FHB species (Table 1). Our results are in accordance with *in vitro* previous germination rate reduction and coleoptile length reduction analyses in which those two aggressiveness criteria did not distinguish FHB isolates (Sakr, 2017b). Also, our results regarding germination rate reduction correspond to those reported by Purahong et al. (2012) for *F. graminearum*, they observed that reductions in germination rate ranged from 0.17 to 0.38% were not significant to differentiate among fungal isolates. However, the reduction of the coleoptile length caused by *F. graminearum* reported by Brennan et al. (2003) has been related to aggressiveness. Purahong et al. (2012) found that this parameter distinguished *F. graminearum* isolates. The values of $AUDPC_{standard}$ (from 0.22 for 0.45) underlined a variation in aggressiveness within and among four FHB species (Table 1). Our results are comparable with those found by Purahong et al. (2012) and Sakr (2017b). $AUDPC_{standard}$ was calculated from the decreasing number of healthy wheat seed-

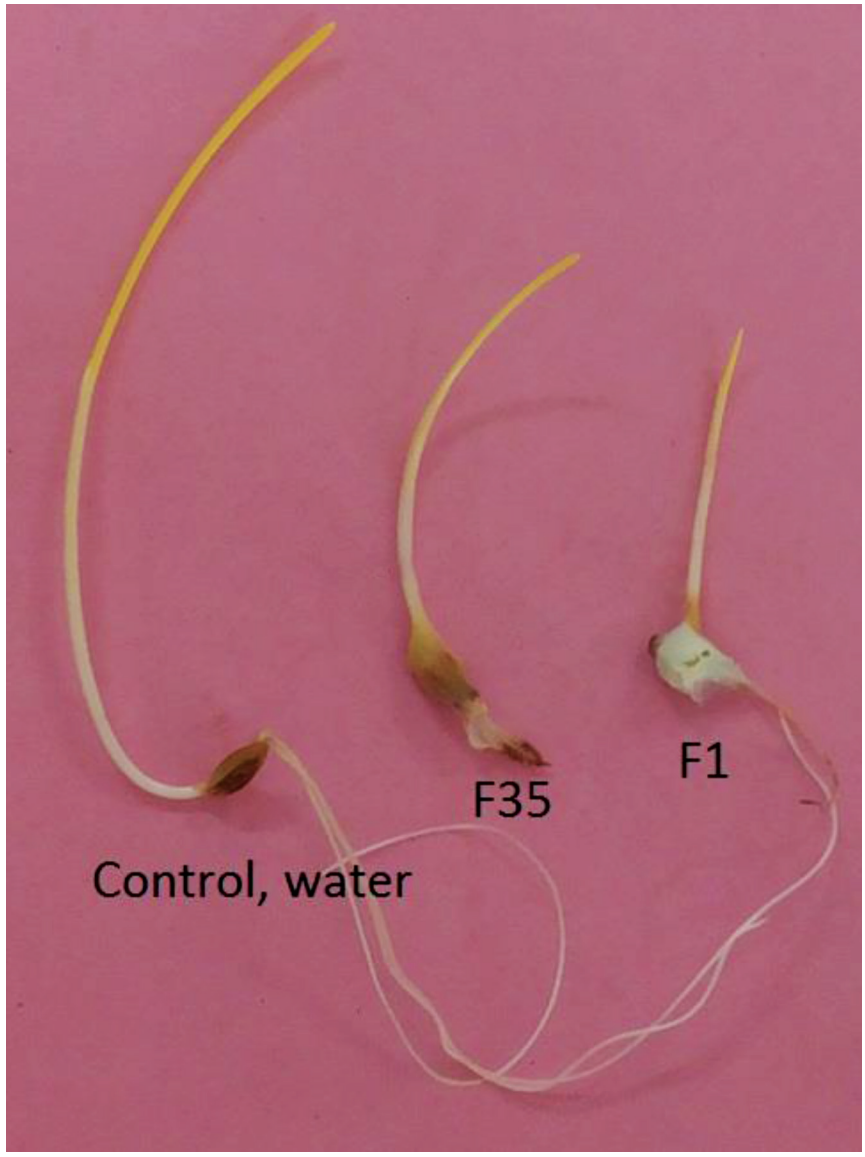

Fig. 1. Symptoms on seedlings of inoculated barley cultivar Arabi Aswad with isolate F1 (*Fusarium culmorum*) and F35 (*F. solani*) compare with control (water) at 6 days after inoculation

lings after fungal inoculation of the seeds (Purahong et al., 2012). The faster the reduction of the number of healthy seedlings, the more aggressive is the fungal isolate (Purahong et al., 2012). Our results indicated that the same fungal isolates tested previously on wheat cultivar Cham7 and barley cultivar Arabi Aswad behave similar pathogenic reaction regarding the three analysed aggressiveness criteria. Petri-dish aggressiveness index ranged

one fold within and among four FHB species (Table 1), and significantly differences in isolates were due to variability in $AUDPC_{standard}$ parameter. However, for 25 *F. graminearum* isolates, Purahong et al. (2012) found that Petri-dish aggressiveness index varied four folds, and for 16 FHB isolates, Sakr (unpublished data) found that it varied one fold.

Table 1

Aggressiveness within and among four Fusarium head blight species measured on barley cultivar Arabi Aswad

Fungal isolates (identification)	Germination rate reduction (%)	AUDPCstandard	Coleoptile length reduction (%)	Petri-dish aggressiveness index
F1 (*F. culmorum*)	22	0.22 b E	57	0.34 ABC
F2 (*F. culmorum*)	21	0.29 ab CDE	56	0.35 BC
F3 (*F. culmorum*)	20	0.39 a AB	58	0.39 AB
F28 (*F. culmorum*)	22	0.29 ab CDE	55	0.35 AB
F30 (*F. culmorum*)	23	0.34 ab BCD	56	0.38 ABC
	F = 0.232 ns	F = 2.300	F = 0.133 ns	F = 0.0803 ns
	P = 0.9143	P = 0.1302	P = 0.9668	P = 0.5503
F7 (*F. solani*)	20	0.45 a A	56	0.45 a A
F20 (*F. solani*)	23	0.40 ab AB	56	0.46 a AB
F26 (*F. solani*)	20	0.39 abc AB	57	0.42 ab AB
F29 (*F. solani*)	20	0.38 bc ABC	55	0.38 b ABC
F31 (*F. solani*)	20	0.33 c BCD	55	0.42 ab ABC
F35 (*F. solani*)	19	0.39 abc AB	55	0.41 ab ABC
	F = 0.176 ns	F = 2.904	F = 0.123	F = 2.295
	P = 0.9667	P = 0.604	P = 0.9844 ns	P = 0.1107
F15 (*F. verticillioides*)	21	0.22 b E	57	0.39 BC
F16 (*F. verticillioides*)	19	0.31 ab BCDE	55	0.41 ABC
F21 (*F. verticillioides*)	20	0.35 a BC	55	0.43 ABC
F27 (*F. verticillioides*)	19	0.25 ab DE	56	0.38 C
	F = 0.371 ns	F = 2.940	F = 0.175 ns	F = 1.976 ns
	P = 0.7762	P = 0.0989	P = 0.9104	P = 0.1963
F43 (*F. equiesti*)	21	0.40 AB	57	0.40 AB
Enter isolates	F = 0.416 ns	F = 4.440	F = 1.170	F = 1.266
	P = 0.9631	P = 0.0002	P = 0.3417	P = 0.2784

According to the Fisher's LSD test, means followed by the same letter are not significantly different at p = 0.05 (small letters refer to aggressiveness within species and capital letters to aggressiveness between isolates of species), ns = not significant, Probability (P), F-tests (F), Petri-dish aggressiveness index = (germination rate reduction (expressed by value% / 100) + $AUDPC\text{-}_{standard}$ + coleoptile length reduction (expressed by value% / 100)) /3 (Purahong et al., 2012).

Table 2

Correlation coefficients on barley cultivar Arabi Aswad among criteria of aggressiveness for 16 isolates of four Fusarium head blight species

	Germination rate reduction	$AUDPC_{standard}$	Coleoptile length reduction	Petri-dish aggressiveness index
Germination rate reduction	1.000			
$AUDPC_{standard}$	–0.129 ns	1.000		
Coleoptile length reduction	0.211 ns	–0.04	1.000	
Petri-dish aggressiveness index	–0.169 ns	0.680**	–0.100 ns	1.000

*P = 0.05, **P = 0.01, ns = no significant.

The three parameters obtained with the Petri dish test were not significantly correlated (Table 2). Significant correlation was detected only between $AUDPC_{standard}$ and Petri-dish aggressiveness index: r = 0.680**. It seems that mechanisms underlying these three aggressiveness criteria did not share the same genetic background. Our results correspond with those found by Sakr (unpublished data) in which the three aggressiveness parameters obtained with the Petri dish test were not significantly correlated. However, our results are not in accordance with those obtained by Purahong et al. (2012); they found significant correlations among the four aggressiveness parameters used in the present research: germination rate reduction, standardized area under disease progress curve ($AUDPC_{standard}$), coleoptile length reduction, Petri-dish aggressiveness index.

High germination rate reduction, high $AUDPC_{standard}$ values, and significant reduction in the length of the coleoptile represent high aggressiveness (Purahong et al., 2012). Results shown in Table 1 indicated that $AUDPC_{standard}$ parameter can underline within and among variation of aggressiveness in four FHB species. Within *F. culmorum* species, the lowest $AUDPC_{standard}$ value (0.22) occurred for F1, whereas the most aggressive isolate F3, with value (0.39). Within *F. solani* species, the least $AUDPC_{standard}$ value was for F31 (0.33), and the most aggressive isolate was F7 (0.45). Within *F. verticillioides* species, F15 gave the least $AUDPC_{standard}$ (0.22), while F21 (0.35) was significantly more aggressive. Our results are comparable with those found by Sakr (unpublished data) in which significant differences were detected *in vitro* on wheat plants within three FHB species *F. culmorum*, *F. solani*, and *F. verticillioides*. There were significant differences in the variability of aggressiveness among the isolates from the four FHB species, the isolate F7 (*F. solani*) showed the greatest aggressiveness, while F1 (*F. culmorum*) and F15 (*F. verticillioides*) were the least aggressive isolates (Table 1). It is clear that variations in Petri-dish aggressiveness index were due to variations in $AUDPC_{standard}$ parameter, and within and among variability of aggressiveness in four FHB species was nearly similar to $AUDPC_{standard}$ values, except for *intra- F. culmorum* and *F. verticillioides* species in

which Petri-dish aggressiveness index did not give significant differences. However, the $AUDPC_{standard}$ differences among 16 isolates can not be used to differentiate between the four FHB species, for example, isolates F3 and F30 (*F. culmorum*), F20, F26, and F35 (*F. solani*), F16 and F21 (*F. verticillioides*), and F43 (*F. equiseti*) were not significantly different (Table 1). Sakr (unpublished data) hypothesized that geographic origin of FHB isolates plays a crucial role in increasing the level of pathogenic similarity between fungal species. More interestingly, our results confirmed that FHB isolates recovered from wheat spikes and tested on wheat cultivar Cham7 (Sakr, unpublished data) showed similar range of aggressiveness on barley cultivar Arabi Aswad ($r = 0.584^*$). Similar range of aggressiveness in cross-species pathogenicity test was reported in association between FHB populations and wheat and maize host plants (Kuhnem et al., 2015).

It is widely accepted that the level of FHB resistance in wheat is less than in barley (Steffenson, 2003). Our results showed that wheat cultivar Cham7 exhibited a higher $AUDPC_{standard}$ (Sakr, unpublished data) than barley cultivar Arabi Aswad using the same fungal isolates. $AUDPC_{standard}$ on barley cultivar Arabi Aswad was 25.6% less than on wheat cultivar Cham7 (Sakr, unpublished data). Thus, the current study provides evidence that Arabi Aswad is more resistant than Cham7. High level of quantitative resistance in Arabi Aswad made it possible to detect *intra-* and *inter*-significant differences (Table 1). These results are in accordance with previous analysis on the good level of resistance for Arabi Aswad against common root rot (*Cochliobolus sativus*) (van Leur et al., 1997) and leaf blotch caused by the fungus *Rhynchosporium secalis* (Abang et al., 2006). Arabi Aswad could be considered as promising resistance sources to FHB for introgression in the adapted barley gene pool.

Conclusion

The current research provides interesting data regarding the *in vitro* pathogenic reaction between barley cultivar Arabi Aswad and 16 fungal isolates of four FHB species recovered from wheat kernels. The method described by Purahong et al. (2012) for *F. graminearum* on wheat could be conducted also in other FHB agents on barley plants. $AUDPC_{standard}$ parameter did distinguish variation of aggressiveness for isolates within and among species, and the other two parameters did not. Mechanisms underlying three aggressiveness criteria could not share the same genetic background. The high level of quantitative resistance in Arabi Aswad permitted to underline *intra-* and *inter*-species variability. It will be necessary to analyse the pathogenic reaction of the fungal isolates recovered from naturally infected barley plants on Syrian barley cultivars using *in vitro* techniques and floret inoculation assay under controlled and field conditions to screen levels of resistance in Syrian barley cultivars.

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