

Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online) http://www.pakps.com



THE USE OF *IN VITRO* AREA UNDER DISEASE PROGRESS CURVE TO PREDICT QUANTITATIVE TRAITS IN THE FUSARIUM HEAD BLIGHT-SMALL GRAIN CEREAL PATHOSYSTEM

Nachaat Sakr

Department of Agriculture, Syrian Atomic Energy Commission, Damascus, P.O. Box 6091, Syria.

ABSTRACT

Small grain cereals worldwide are seriously affected by Fusarium head blight (FHB) infection originated by various Fusarium pathogens. Traditional screening for disease reaction performed at the flowering period in the whole plant in the growth chamber and filed has been accompanied by a number of challenges. *In vitro* screenings allowing for simple, effective, and trustworthy indication of FHB response at the mature plant phase were undertaken to overcome the limitations of classical screening. Area under disease progress curve (AUDPC) is a useful in vitro quantitative measurement to quantify disease progress over time in several pathosystems. AUDPC criterion has been used in recent research successfully predicting aggressiveness and quantitative resistance in FHB-small grain cereal pathosystem including four FHB species and several widely cultivated Syrian wheat and barley cultivars. However, no overall study involving all experimental data was conducted to distinguish pathogenic levels in a set of 16 FHB isolates and susceptibility to disease infection of eight cereal cultivars. The applied method allowed to easily and effectively predicting the response of plants infected in the adult stage of development. The effectiveness of AUDPC lies in determining the species composition of the pathogen and determining the aggressiveness of various isolates of species of fungi of the genus Fusarium In addition, using AUDPC in the laboratory, it is possible to conduct a presowing assessment of seed material for the susceptibility or resistance to the most common isolates of Fusarium in the region. Cultivar resistance screening identified Arabi Aswad and Bohoth10 as agronomically favorable and potential participants in the Syria's cereal breeding programs as donors of FHB resistance.

Keywords: cereal quantitative resistance, FHB species, pathogenic variation, Petri-dish assay.

INTRODUCTION

Bread wheat (*Triticum aestivum*), durum wheat (*T. durum*) and barley (*Hordeum vulgare*) are the major Syrian strategically important crops, with a yearly whole output of 4 and one million tones at the growing season 2011, respectively (FAO/WFP, 2015). So, examinations on this major collection of wheat and barley materials are claimed for the enhancement of commercially beneficial properties in cereal screening plans since Syrian genetically diverse genotypes may maintain gene combinations for quality features and resistance to biotic

Submitted: August 05, 2021 Revised: November 11, 2021 Accepted for Publication: December 11, 2021 * Corresponding Author: Email: ascientific@aec.org.sy © 2017 Pak. J. Phytopathol. All rights reserved. and abiotic impediment (Ceccarelli and Grando, 2000; Bishawa *et al.*, 2015). Barley along with wheat, and other small- cereal crops (rye, oat, triticale and corn), can be ponderously devastated by aggressive *Fusarium* pathogens originating by Fusarium head blight (FHB).

Globally, FHB rapidly has become one of the most serious diseases in wheat and barley (Parry *et al.*, 1995). During periods with periodic rainfall and elevated moisture within anthesis, and continuing until soft dough and maturation stages, FHB produces blanching of the flowers creating in infertility or producing of distorted, contractile, pallid and discolored grains (red, brown, pink, tan or orange) dispersed in every part of the spike. Serious FHB infestation reduce essentially yield and quality because the aggregation of grave mycotoxins, deoxynivalenol (DON), subsequently, DON makes grains disadvantageous for human and animal chains and malting and brewing industries (Dweba *et al.*, 2017). More than 17 *Fusarium* species favored by different weather conditions with various levels of pathogenicity (Xu and Nicholson, 2009) have been recovered from naturally infected small-grain cereal spikes. While *F. graminearum* species complex (FGSC) and *F. culmorum* are the principal causative organisms and the most pathogenic species worldwide, the other *Fusarium* causative agents have been isolated frequently from cereal crops (Bottalico and Perrone, 2002).

Although host resistance is the maximum cost efficient and environmentally sound method of reducing the disease, breeding for FHB resistance has proven to be difficult due to that resistance to FHB is under polygenic inheritance and the strong cultivar-by-environment interaction (Dweba et al., 2017). Differences in disease incidence (DI) determined to assess Type I resistance have been reported for FHB isolates recovered from different world regions, countries or states and even individual fields (Parry et al., 1995; Xu and Nicholson, 2009), suggesting that the extreme level of pathogenic diversity observed within FHB populations for wheat and barley should be accounted in development of breeding policies. Nevertheless, few findings are obtainable on the aggressiveness of relative other pathogens correlated with head blight on barley and wheat comparing to FGSC (Bottalico and Perrone, 2002; Malihipour et al., 2012; Garmendia et al., 2018; Xue et al., 2019). The achievement of a tolerant genotype principally relies on the wide framework of head blight pathogens involved, meteorological factors and the interrelationship between these two factors in a given locality (Xu and Nicholson, 2009). So, the search for production commercial barley and wheat genotypes with advantageous agronomical characteristics and robust resistance persists for the extremely varying FHB agent existent in various developing countries (Dweba et al., 2017). In the growth chamber and filed, traditional screening for disease reaction performed at the flowering period in the whole plant has been accompanied by a number of challenges (Wu et al., 2005; Imathiu et al., 2014). In vitro screenings allowing for simple, effective and trustworthy indication of FHB response at the mature plant phase were undertaken to overcome the limitations of classical screening (Wu *et al.,* 2005; Browne, 2007; Purahong *et al.,* 2012; Kumar *et al.,* 2011; Bedawy *et al.,* 2018).

The area under the disease progress curve (AUDPC) is a useful in vitro quantitative measurement to quantify disease progress over time (Simko and Piepho, 2012). Calculation of AUDPC as a measure of traits related to pathogenicity and resistance entailing repeated disease assessments during pathogen progression has been reported in several pathosystems (Jeger and Viljanen-Rollinson, 2001; Meena et al., 2011; Alves et al., 2017). Recently, AUDPC criterion has been analyzed in studies predicting successfully aggressiveness and quantitative resistance in FHB-small grain cereal pathosystem including four FHB species and several widely cultivated Syrian wheat and barley cultivars (Sakr, 2019a,b, 2020a,b; Sakr and Al-Attar, 2021; Sakr and Shoaib, 2021). However, no overall report involving all experimental data was undertaken to differentiate pathogenic levels in a set of 16 FHB isolates and susceptibility to disease infection of eight cereal cultivars. In this context, the objective of the current research was to combine the in vitro AUDPC and artificial inoculation in growth chamber and field data for better comprehension of the structure of aggressiveness in several FHB species and availability of new resistant donors with favorable agronomical traits in FHB-wheat and barley breeding programs.

MATERIAL AND METHODS

Plant materials, fungal isolates and inoculum preparation: In the current research, we analyzed disease responses revealed by AUDPC criterion in eight Syrian cereal cultivars including six largely planted plentiful bread (Cham4, CH4 released in 1986, Douma4, DO4 in 2007 and Bohoth10, BO10 in 2014) and durum (Acsad65, AC65 released in 1984, Cham7, CH7 in 2004 and Cham9, CH9 in 2010) wheat cultivars, and two barley landraces: Arabi Aswad (AS) and Arabi Abiad (AB) with the most desirable agronomic features and highest tolerance to biotic and abiotic stresses (Ceccarelli and Grando, 2000; Bishawa et al., 2015). B010 and AS (moderately resistant), AB, CH4 and D04 (moderately susceptible), CH7 and CH9 (susceptible to moderately susceptible), and AC65 (susceptible) were chosen from previous growth chamber and field experiments to represent a range of quantitative resistance types to head blight (Sakr, 2019a,b, 2020a,b; Sakr and Al-Attar, 2021; Sakr and Shoaib, 2021). Irrespective of the botanical source, wheat cultivars, i.e., Acsad65 and Cham4 released earlier than 2000 were assessed as ancient sources, and the four remaining cultivars as novel materials. Thus, we were capable to examine the disease responses between the wheat and barley cultivars, breads and durums as well as the novel and ancient bread and durum wheat cultivars.

Fungal isolates collected from diseased spikelets during the 2015 growing season originating from 9 different localities of Ghab Plain with a history of head blight epidemics, one of the major Syrian wheat output regions, morphologically analyzed and genetically identified by RAPD (Sakr and Shoaib, 2021) were chosen for their varying aggressiveness (established on previous different exploratory observances (Sakr, 2019a,b, 2020a,b; Sakr and Al-Attar, 2021; Sakr and Shoaib, 2021). In total, 16 single-spore derived cultures of four head blight pathogens, i.e., (F. culmorum (5 isolates), F. solani (6 isolates), F. verticillioides, synonym F. moniliforme (4 isolates) and F. equiseti (1 isolate)) were used in this study. Isolates were preserved in cold sterile distilled water (SDW) and frozen fungal cultures till needed (Sakr, 2020c).

Fungal inoculum for the *in vitro* experiments was prepared as follows: FHB suspension or 4 to 6 agar plugs out of each stocked isolate were placed above the surface of potato dextrose agar (PDA) in Petri plates and incubated for 10 days at 22°C in the dark to permit mycelial development and sporulation. Later, cultures were immerged with 10 ml of SDW and spores were removed. FHB suspensions were filtered with two layers of sterilized cheesecloth to take off the segments of agar and mycelia and immediately assessed under an optic microscope with a Neubauer chamber and diluted to 5×10^4 spores/ml.

Quantitative trait tests under in vitro conditions: Procedures for AUDPC experiment were conducted as described earlier by Purahong *et al.* (2012) to analyze aggressiveness components under laboratory conditions. Surface-sterilized seeds of the eight tested wheat and barley cultivars were inoculated with a conidial suspension for FHB isolates or SDW in the non-infected treatment in plates with sterilized double-layer filter paper. Inoculated and control treatments were placed at an incubator in the dark at 22°C for 6 days. The aggressiveness criterion of an isolate (AUDPC) was assessed as disease progression for 6 days post inoculation (dpi) and its score was varied from 0 (not pathogenic) to 1 (completely pathogenic). The score of AUDPC varied from 0 (completely resistant) to 1 (not resistant), and it was estimated from the proportion of not-diseased coleoptiles as a function of period (from 2 to 6 dpi). Three replications of each isolate were installed in which the plates were organized in a randomized block design, and the experiment was repeated a second time.

Repeatability and stability of the Petri-dish test in different wheat and barley cultivars: To confirm the repeatability and stability of the Petridish test among several cereal cultivars, eight durum and bread wheat cultivars and two barley cultivars with contrasted resistance levels for FHB were inoculated with the 16 FHB isolates with different aggressiveness levels. Three replications were set up and this assay was repeated twice. The repeatability and stability among the different cereal cultivars was assessed by correlation analyses between AUDPC of wheat and barley cultivars and disease incidence (DI) detected for Type I following artificial inoculation of spikes under controlled and field conditions. DI (% diseased head) for aggressiveness was quantified as the proportion of heads displaying head blight symptoms.

STATISTICAL ANALYSIS

Data were treated with analyses of variance (ANOVA) utilizing DSAASTAT add-in version 2011. To compare the means, Fisher's LSD test was used at P>0.05.The sample correlation coefficients (Pearson r) were calculated using overall values per isolates and cultivars at P>0.05.

RESULTS AND DISCUSSION

F-tests cores from analyses of variance for AUDPC were presented in Table 1 and showed statistically significant variations in aggressiveness among the 16 fungal isolates and susceptibility among eight cereal cultivars.

	Itivals								
Fungal cultures					AUDPC				
(identification)	AC65	CH4	CH7	D04	CH9	B010	AS	AB	Mean
F1 (F. culmorum)	0.62	0.42	0.47	0.34	0.39	0.31	0.22	0.35	0.39de
F2 (F. culmorum)	0.41	0.47	0.36	0.49	0.49	0.59	0.29	0.26	0.42cde
F3 (<i>F. culmorum</i>)	0.50	0.42	0.58	0.59	0.52	0.36	0.39	0.58	0.49a
F28 (F. culmorum)	0.58	0.37	0.42	0.44	0.49	0.54	0.29	0.45	0.45abc
F30 (F. culmorum)	0.58	0.28	0.40	0.44	0.43	0.39	0.34	0.70	0.45bc
F7 (F. solani)	0.52	0.46	0.55	0.45	0.48	0.34	0.45	0.67	0.49a
F20 (F. solani)	0.52	0.52	0.49	0.45	0.52	0.42	0.40	0.40	0.46ab
F26 (F. solani)	0.47	0.46	0.51	0.50	0.41	0.29	0.39	0.40	0.43bcde
F29 (F. solani)	0.52	0.33	0.41	0.28	0.39	0.35	0.38	0.60	0.41cde
F31 (F. solani)	0.42	0.52	0.51	0.45	0.43	0.34	0.33	0.30	0.41cde
F35 (<i>F. solani</i>)	0.52	0.66	0.46	0.56	0.51	0.46	0.39	0.38	0.49a
F15 (F. verticillioides)	0.40	0.36	0.44	0.25	0.45	0.36	0.22	0.25	0.34f
F16 (F. verticillioides)	0.47	0.36	0.41	0.49	0.36	0.25	0.31	0.41	0.38ef
F21 (F. verticillioides)	0.44	0.40	0.50	0.37	0.41	0.45	0.35	0.38	0.41cde
F27 (F. verticillioides)	0.34	0.45	0.33	0.41	0.36	0.33	0.25	0.22	0.34f
F43 (F. equiesti)	0.41	0.49	0.48	0.48	0.41	0.45	0.40	0.33	0.43bcd
Mean	0.48a	0.44bc	0.46ab	0.44bc	0.44bc	0.39d	0.34e	0.42cd	
F isolates=10.4 at <i>P</i> >0.01									
F cultivars=18.3 at <i>P</i> > 0.01									
F interactions=3.8 at P>0.01									
				7) DO	4 (D 4				10) 1 1

Table 1. Scores of AUDPC in a set of 16 head blight isolates of four *Fusarium* pathogens assessed on eight Syrian wheat and barley cultivars

Abbreviation: AC65 (Acsad65), CH4 (Cham4), CH7 (Cham7), DO4 (Douma4), CH9 (Cham9), BO10 (Bohoth10), Arabi Aswad (AS) and Arabi Abiad (AB).

Mean values of AUDPC succeed by the identical letter are not significantly different by the Fisher's LSD test at *P*>0.05. Values of AUDPC for all tested fungal isolates on the eight wheat and barley cultivars were quantified earlier and showed by Sakr (2019a,b, 2020a,b,c).

Variation of aggressiveness among FHB species on wheat and barley cultivars: There was a broad difference in aggressiveness among the 16 head blight single-spore derived cultures of the four head blight pathogens, as a consequence of the pathogen severity. The isolates F35 and F7 (F. solani) and F3 (F. culmorum) exhibited the highest pathogenicity, whereas F15 and F27 (F. verticillioides) were the minimum pathogenic cultures. Variations within and among species were highlighted in the pathogenicity of diverse head blight pathogens across barley and wheat entries (Bottalico and Perrone, 2002; Malihipour et al., 2012; Garmendia et al., 2018; Xue et al., 2019). In spite of considerable variations were recognized among cultures of a given pathogen for aggressiveness, data shown in Figure 1 exhibited that the four head blight pathogens did not differ in their relative pathogenicity assessed by AUDPC on barley and wheat cultivars due to the comparative homogeneity in aggressiveness scale among the 16 fungal cultures. An apparent lack of a variation in pathogenicity was observed between F. culmorum and F.

graminearum on wheat (Fernandez and Chen, 2005). Our data did not accord with earlier studies exhibiting that head blight pathogens differed in their pathogenicity (Bottalico and Perrone, 2002; Malihipour *et al.*, 2012; Garmendia *et al.*, 2018; Xue *et al.*, 2019).

The variations in these findings may be due to the differing FHB cultures and host barley and wheat cultivars utilized in the current research and earlier studies. Source of the 16 *Fusarium* isolates may participate to the observed comparative pathogenic uniformity. Data of the current work insert to our extended knowing that the pathogenicity of the four analyzed pathogens is not geographically constructed while the 16 fungal cultures with minimum, moderate and elevated grades of pathogenicity create the population in a given locality, which agree with previous report (Xu and Nicholson, 2009).

Complex FHB-wheat and barley interactions: Diversity in pathogenicity in head blight populations can allow host resistance being overcome (Miedaner *et al.*, 2008). Correlation scores of AUDPC among the eight barley and

wheat cultivars exhibited that 5 of the 28 probable compares were significantly linked (Table 2). So, findings presented in the current research highlighted that a complex host relationship may or may not present among barley, durum and bread cultivars and pathogens for AUDPC criterion. This kind of particular biological reaction has been earlier observed in wheat by Foroud *et al.* (2012), who reported that *F. graminearum* aggressiveness is host-dependent. Parry *et al.*, (1995) reported no powerful confirmation for specified aggressiveness links among *Fusarium* pathogens involved in the head blight complex and cereals. It appears that a minor gene–for–minor gene relationship may present between eight barley and wheat cultivars and 16 fungal isolates, indicating that the isolate-specific efficiency may allow to erosion of barley and wheat quantitative resistance to head blight infection. However, additional examination is needs to conclude.

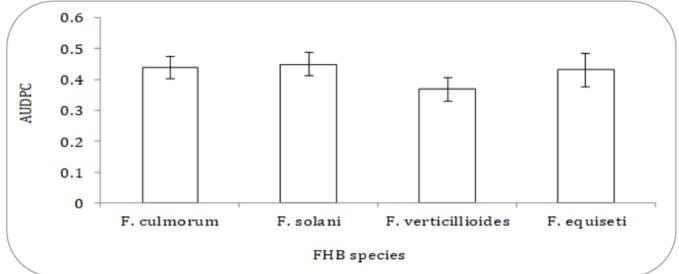


Figure 1. Mean AUDPC of four *Fusarium* pathogens causing head blight on eight Syrian wheat and barley cultivars detected in an *in vitro* Petri-dish assay. Mean AUDPC values were calculated from *F. culmorum* (5 isolates), *F. solani* (6 isolates), *F. verticillioides* (4 isolates) and *F. equiseti* (1 isolate). Bars represent the standard errors of means

Table 2. Correlation coefficients of AUDPC scores on eight Syrian wheat and barley cultivars infected with a set of 16head blight isolates of four *Fusarium* pathogens

11	ieuu biigiit isoit	ites of four 1 us	arram pathoger	15				
	AC65	CH4	CH7	D04	CH9	BO10	AS	AB
AC65	1.000							
CH4	-0.182ns	1.000						
CH7	0.232ns	0.286ns	1.000					
D04	0.019ns	0.504*	0.293ns	1.000				
CH9	0.264ns	0.413ns	0.384ns	0.421ns	1.000			
B010	-0.002ns	0.217ns	-0.218ns	0.156ns	0.559*	1.000		
AS	0.148ns	0.320ns	0.589*	0.483ns	0.350ns	0.007ns	1.000	
AB	0.631**	-0.399ns	0.340ns	0.120ns	0.187ns	-0.167ns	0.575*	1.000
						(C) 0) DO1		

Abbreviation: AC65 (Acsad65), CH4 (Cham4), CH7 (Cham7), DO4 (Douma4), CH9 (Cham9), BO10 (Bohoth10), Arabi Aswad (AS) and Arabi Abiad (AB).

*P>0.05, **P>0.01, ns = no significant. Values of AUDPC for all tested fungal isolates on the eight wheat and barley cultivars were quantified earlier and showed by Sakr (2019a,b, 2020a,b,c).

Repeatability and the stability of AUDPC aggressiveness test: Significant correlation coefficients were achieved between the findings of AUDPC and DI obtained earlier in growth chamber and in the field for the eight analyzed barley and wheat cultivars (Table 3). The repeatability and the stability of AUDPC test across

different experimental situations were confirmed utilizing several barley, durum and bread cultivars by obtaining links with the results from spike inoculation. When analyzed jointly, these independent aggressiveness works show the utility of AUDPC for FHB estimation regarding both the fungus and the host.

16 fungal isolates of four Fus	arium head blight species	
Cereal cultivars	AUDPC × DI (CC)	AUDPC × DI (FC)
AC65	0.620*	0.780***
CH4	0.604*	0.535*
CH7	0.631**	0.511*
D04	0.657**	0.538*
CH9	0.531*	0.640**
B010	0.519*	0.682**
AS	0.533*	0.750***
AB	0.887***	0.839***

Table 3. Correlation coefficients of AUDPC and disease incidence (DI) following head inoculation of spikes under controlled (CC) and field conditions (FC) among eight Syrian wheat and barley cultivars infected with a set of 16 fungal isolates of four Fusarium head blight species

Abbreviation: AC65 (Acsad65), CH4 (Cham4), CH7 (Cham7), DO4 (Douma4), CH9 (Cham9), BO10 (Bohoth10), Arabi Aswad (AS) and Arabi Abiad (AB).

P*>0.05, *P*>0.01, ****P*>0.001. Values of AUDPC for all tested fungal isolates on the eight wheat and barley cultivars were quantified earlier and showed by Sakr (2019a,b, 2020a,b,c). DI (% diseased head) for aggressiveness was quantified as the proportion of heads displaying head blight symptoms.

Compared to FHB resistance in barley, there is no elevated FHB resistance in wheat, furthermore, current durum wheat cultivars are highly susceptible to head blight than bread cultivars (Mesterhazy et al., 2011), so in our work we selected eight barley and wheat cultivars with four susceptibilities (one susceptible, two susceptible to moderately susceptible, three moderately susceptible and two moderately resistant) that would be appropriate to explore the stability and repeatability of AUDPC assay in the growth chamber and field. In parallel, a low and negative link (r=-0.47) of wheat seed germination scores provoked by Microdochium majus and head blight evaluating generated by spike infection of F. graminearum under field conditions was reported by Browne (2007). In accordance, Purahong et al., (2012) observed positive correlations of AUDPC evaluations and head blight rates obtained by head infection of F. graminearum in four durum wheat cultivars under controlled and field conditions. They reported high links between these three criteria (Purahong et al., 2012), which is comparable to the current findings. Thus, the present results showed that the AUDPC assay is repeatable and constant with different wheat and barley cultivars with a high variation relying on the eight used cereals. Consequently, the in vitro criterion, AUDPC, predicts aggressiveness occurring at the youngest and adult wheat and barley development phases through head blight infection.

The condition in an *in vitro* experiment was identical to artificial infection due to *Fusarium* pathogens want to defeat the head morphology and they could immediately enter and infect germinating grains (Purahong *et al.,* 2012). The biological explanation for a link between the

in vitro and *in planta* reactions to head blight infection remains broadly suppositive, but it can be concluded that identical genetic pathways become modified at both developmental stages (Xu and Nicholson, 2009).

Differences among wheat and barley cultivars: Effects of AUDPC did seem to be principal features of quantitative resistance in Syrian wheat and barley cultivars to head blight. Substantial differences were detected between cultivars (Table 1), suggesting differences in capability to resist pathogen effects at early growth stages. The mean AUDPC scores varied from 0.34 to 0.48. AS exhibited the lowest infection levels and AC65 was the most affected cereal. In the current investigation, quantitative resistant cereal cultivars are distinguished by weak AUDPC evaluations of FHB fungi comparing with the susceptible ones. Regarding resistance as measured by AUDPC,AS and BO10 were moderately resistant cultivars, AB, DO4, CH4, CH7 and CH9 susceptible to moderately susceptible, and AC65 was susceptible (Figure 2). As shown in Table 1, resistance of a particular analyzed cultivar is not linked to a specific head blight pathogen. Also, the eight barley and wheat cultivars which can resist strongly aggressive cultures of a specific pathogen can resist other aggressive cultures from another pathogen (Table 1). The findings in the current research are in accordance with the conclusions of Xue et al. (2019). The existence/loss of specific quantitative trait loci (QTLs) may clarify these changes in the resistance reactions of the eight cereals following infection with head blight agents since some QTLs have linked to most efficient resistance to FHB (Buerstmayr et al., 2009).

Repeatability and the stability of AUDPC resistance test: Barley is more resistant to head blight infection than wheat, and durum wheat is also more susceptible to *Fusarium* infection than bread, since there is no elevated infection resistance (Mesterhazy *et al.*, 2011). As expected, previous growth chamber and field findings highlighted current *in vitro* data that `AC65, old durum` was susceptible and AS and `BO10, modern bread` were moderately resistant (Figure 2).

The repeatability of this cultivar arrangement was fulfilled by the significant correlations between the AUDPC and FHB Type I generated in the growth chamber and field ($r=0.947^{***}$ and $r=0.832^{*}$) (Figure 3). More principally, spike infections assays made it possible to separate the collection which encompassed five lasting cultivars categorized as susceptible to moderately susceptible in vitro into two distinguished sub-groups as the two modern durums `CH7 and CH9` assessed as susceptible to moderately susceptible and the two breads `CH4, old and DO4, modern` and AB quantified as moderately susceptible (Figure 2). In general, cereal plants with the smallest scores for AUDPC were those having the elevated scores of FHB Type I resistance ratings (Figure 3). Overall, barley and bread wheat cultivars exhibited lower infection head scales than durum cultivars in spite of the time of cultivar release, showing that old and modern breads provided wide, still incomplete, resistance to the four head blight pathogens analyzed compared to old and modern durums (Figure 2). Our data suggest that the quantification of resistance rating is repeatable and constant under diverse experimental conditions.

Syrian cereal cultivars in breeding programs: In spite of the variations in response to the four *Fusarium* species causing head blight were generally identical to growth chamber and field studies of the eight wheat and barley cultivars in FHB resistance, significant cultivar × isolate interactions were indicated in this work, which corresponds with an earlier study on wheat (Xue *et al.,* 2019). Taking into mind that there were broad genetic diversities among some of the analyzed cereals, i.e., AS and AB, CH4 and CH7 (Ceccarelli and Grando, 2000; Bishawa *et al.,* 2015), selection and progress of FHB resistant cultivars must be conducted by phenotypic selection under epidemic conditions as described by Buerstmayr *et al.* (2009).

The in vitro, growth chamber and field findings generated present that all analyzed cereals differed in their head blight resistance and susceptibility behavior (Table 1), in which the decrease of the number of notdiseased seedlings and primary head blight infection (Type I) were linked to cultivar resistance (Xu and Nicholson, 2009). Overall, the eight wheat and barley cultivars exhibited favorable resistance scales to initial fungal infection (Figure 2). Our findings reinforce the judgment that Syrian barley and wheat cultivars can be hopeful sources of head blight resistance due to the deficiency of 100% resistance to Fusarium pathogens in the present commercial cultivars (Mesterhazy et al., 2011). AS may be a novel plant material for domestic animals and AB for resistance breeding in both converting into malt and business of producing beer.

1		0	•	0	•	0
	Petri-dish assay		Cultivars	н	ead infection in the gro chamber and filed	wth
-	Moderately resistant		Arabi Aswasd Bohoth10		Moderately resistant	
	Susceptible to moderately susceptible		Arabi Abiad Cham4 Douma4		Moderately susceptible	
			Cham9 Cham7		Susceptible to moderately susceptible	
	Susceptible		Acsad65		Susceptible	/

Figure 2. Ranking of eight Syrian wheat and barley cultivars based on AUDPC in a set of 16 head blight isolates of four *Fusarium* pathogens-mediated *in vitro* Petri-dish assay and on FHB incidence following head infection of heads under controlled and field conditions

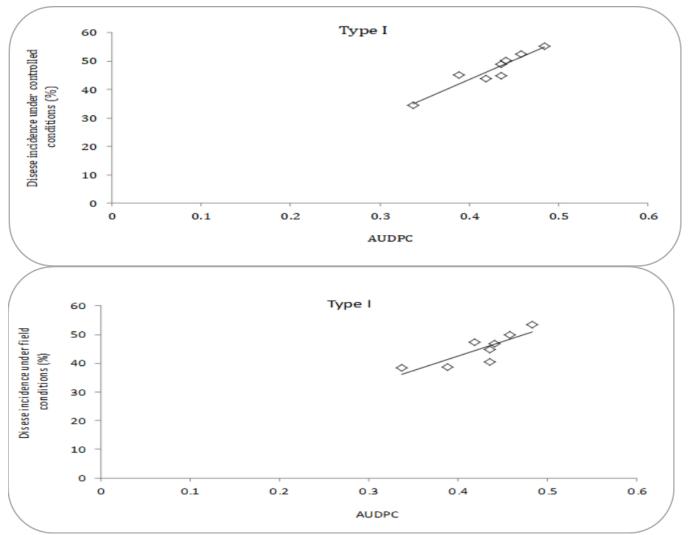


Figure 3. Correlations between the resistance measured by AUDPC of Petri-dish inoculation test and spraying infection (Type I) under controlled and field conditions on eight Syrian wheat and barley cultivars infected with a set of 16 head blight isolates of four *Fusarium* pathogens detected by Pearson correlation coefficient, r=0.947*** (Type I) and r=0.832* (Type I)

CONCLUSION

Favorable *in vitro* environments were detected for the Petri-dish experiment to elevate variations in AUDPC among head blight pathogens and eight barley and wheat cereals. Diversities in aggressiveness and susceptibility among barley and wheat cultivars were reported, suggesting the necessity to select the fungal cultures that best represent the pathogen population, also necessities continual updating when screening breeding genotypes for resistance. AUDPC test has en elevated link with spike infection and is stable in various wheat and barley cultivars under controlled and field conditions. So, the predictive capability of AUDPC seems to be pivotal in analyzing aggressiveness and susceptibility in mature barley and wheat plants under controlled and filed conditions. The AUDPC experiment has an elevated possibility to facilitate the improvement of study into the FHB-small grain cereal pathosystem since it proposes a true potential of easy, quick and trustworthy prediction of susceptibility behavior in barley and wheat cultivars and aggressiveness of head blight pathogens. Arabi Aswad and Bohoth10 with favorable agronomical traits may be introduced into cereal breeding programs due to their resistance to FHB. The main value of this study lies in the data proving that the AUDPC method can indeed be successfully applied to assess adult plants in the field and the correct choice of cereal varieties when breeding for FHB resistance and cultivating them on an industrial scale. Additional analyzes on QTLs linked with aggressiveness in *Fusarium* pathogens and susceptibility in wheat and barley plants should be undertaken to better realize fungus-host interactions on molecular level obtained in the expression of head blight aggressiveness and resistance.

ACKNOWLEDGEMENTS

The author would like to thank the AEC of Syria for supporting this study. The unknown Reviewers are also thanked for useful comments on this manuscript.

REFERENCES

- Alves, D. P., R. S. Tomaz, B. S. Laurindo, R. D. F. Laurindo, F. F. Silva, C. D. Cruz, C. Nick and D. J. H. Silva. 2017. Artificial neural network for prediction of the area under the disease progress curve of tomato late blight. Scientia Agricola, 74: 51-59.
- Bedawy, I. M. A., H. W. Dehne, J. Leon and A. A. Naz. 2018. Mining the global diversity of barley for *Fusarium* resistance using leaf and spike inoculations. Euphytica, 214: 18.
- Bishawaa, Z., P. C. Struikb and A. J. G. van Gastelc. 2015. Wheat and barley seed system in Syria: How diverse are wheat and barley varieties and landraces from farmer's fields? International Journal of Plant Production, 9: 117–150.
- Bottalico, A and G. Perrone. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology, 108: 611-624.
- Browne, R. A. 2007. Components of resistance to fusarium head blight (FHB) in wheat detected in a seedgermination assay with *Microdochium majus*and the relationship to FHB disease development and mycotoxin accumulation from *Fusarium graminearum* infection. Plant Pathology, 56: 65–72.
- Buerstmayr, H., T. Ban and J. A. Anderson. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. Plant Breeding, 128: 1-26.
- Ceccarelli, S. and S. Grando. 2000. In: BrushSB, Ed. Barley landraces from the Fertile Crescent: a lesson for plant breeders. Genes in the field: on-farm conservation of crop, Diversity International Development Research Centre, Ottawa, 51-76.
- Dweba, C. C., S. Figlan, H. A. Shimelis, T. E. Motaung, S. Sydenham, L. Mwadzingeni and T. J. Tsilo. 2017.Fusarium head blight of wheat: pathogenesis and control strategies. Crop Protection, 91: 114-122.

- FAO/WFP. 2015. Crop and food security assessment mission to the Syrian Arab Republic. [cited 2015 July 23] Available from: http://www.wfp.org/foodsecurity/reports/CFSAM.
- Foroud, N. A., S. P. McCormick, T. MacMillan, A. Badea, D. F. Kendra, B. E. Ellis and F. Eudes. 2012. Greenhouse studies reveal increased aggressiveness of emergent Canadian *Fusarium graminearum* chemotypes in wheat. Plant Disease, 96: 1271–1279.
- Fernandez, M. R. and Y. Chen. 2005. Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. Plant Disease, 89: 164–169.
- Garmendia G., L. Pattarino, C. Negrin, A. Martínez-Silveira, S. Pereyra, T. J. Ward, and S. Vero. 2018. Species composition, toxigenic potential and aggressiveness of Fusarium isolates causing head blight of barley in Uruguay. Food Microbiology, 76: 426-433.
- Imathiu, S., S. Edwards, R. Ray, and M. Back. 2014. Artificial inoculum and inoculation techniques commonly used in the investigation of Fusarium head blight in cereals. Acta Phytopathologica et Entomologica Hungarica, 49: 129-139.
- Jeger, M. and S. Viljanen-Rollinson. 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics, 102: 32-40.
- Kumar, K., K.Xi,K. T. Turkington, A. J. H. Helm, and J. P. Tewari. 2011. Evaluation of a detached leaf assay to measure fusarium head blight resistance components in barley. Canadian Journal of Plant Pathology, 33: 364-374.
- Malihipour, A., J. Gilbert, M. Piercey-Normore, and S.Cloutier. 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of *Fusarium* isolates causing head blight in wheat. Plant Disease, 96: 1016-1025.
- Meena, P. D., C. Chattopadhyay, S. S. Meena, and A. Kumar.2011. Area under disease progress curve and apparent infection rate of *Alternaria* blight disease of Indian mustard (*Brassica juncea*) at different plant age. Archives of Phytopathology and Plant Protection, 44:684-693.
- Mesterhazy, A., B. Toth, M. Varga, T. Bartok, A. Szabo-Hever, L. Farady, and S. Lehoczki-Krsjak. 2011. Role of fungicides, application of nozzle types, and the resistance level of wheat varieties in the control of

Fusarium head blight and deoxynivalenol. Toxins, 3: 1453–1483.

- Miedaner, T., C. J. R. Cumagun and S. Chakraborty. 2008. Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. Journal of Phytopathology, 156: 129–139.
- Parry, D.W., P. Jekinson, and L. MCleod. 1995. Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathology, 44: 207-238.
- Purahong, W., D. Alkadri, P. Nipoti, A. Pisi, M. Lemmens and A. Prodi. 2012. Validation of a modified Petri-dish test to quantify aggressiveness of *Fusarium graminearum* in durum wheat. European Journal of Plant Pathology, 132: 381–391.
- Sakr, N. 2019a. Pathogenicity and quantitative resistance in Mediterranean durum and bread wheat cultivars of Syrian origin towards Fusarium head blight agents under controlled conditions. Journal of Plant Protection Research, 59: 451-464.
- Sakr, N. 2019b. Variation in aggressiveness of Fusarium head blight species towards barley plants determined using three *in vitro* assays. Pakistan Journal of Phytopathology, 31: 19-33
- Sakr, N. 2020a. Aggressiveness of *Fusarium* species causing head blight in barley landraces grown under Fertile Crescent conditions. Pakistan Journal of Phytopathology, 32: 41-52.
- Sakr N. 2020b. Aggressiveness of *Fusarium* species causing head blight on wheat plants determined in detached

:

Contribution of Authors:

Nachaat Sakr

leaf and seedling *in vitro* assays. Indian Phytopathology, 73: 483-491.

- Sakr, N. 2020c. Conservation of cereal fungi following different methods of preservation for long terms. Pakistan Journal of Phytopathology, 32: 159-168.
- Sakr, N. and A. Shoaib. 2021. Pathogenic and molecular variation of *Fusarium* species causing head blight on barley landraces. Acta Phytopathologica et Entomologica Hungarica, 56: 5–23.
- Sakr, N. and J. Al-Attar. 2021. Screening for wheat resistance and pathogenicity of Fusarium head blight species using an *in vitro* detached head test. Archives of Phytopathology and Plant Protection, https://doi.org/10.1080/03235408.2021.1984747
- Simko, I. and H. P. Piepho. 2012. The area under the disease progress stairs: calculation, advantage, and application. Phytopathology, 102: 381-389.
- Wu, A. B., H. P. Li, C. S. Zhao and Y. C. Liao. 2005. Comparative pathogenicity of *Fusarium graminearum* isolates from China revealed by wheat coleoptile and floret inoculations. Mycopathologia, 160: 75-83.
- Xu, X. and P. Nicholson 2009. Community ecology of fungal pathogens causing wheat head blight. Annual Review of Phytopathology, 47: 83-103.
- Xue, A. G., Y. Chen, K. Seifert, W. Guo, B. A. Blackwell, L. J. Harris and D. P. Overy. 2019. Prevalence of *Fusarium* species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. Canadian Journal of Plant Pathology, 41: 392–402.

Design experiment, conduct research and writing manuscript