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In vitro analysis of *Fusarium* head blight resistance in ancient Syrian wheats (*Triticum* sp.)

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ABSTRACT

Assessment of *Fusarium* head blight (FHB) resistance in ancient wheat (*Triticum* sp.) cultivars is crucial for disease management. To update our knowledge, *in vitro* resistance in two ancient Syrian bread and durum wheat cultivars with known resistance to four Fusarium species was investigated at Plant Protection Lab., Damascus under Atomic Energy Commission of Syria during 2019. Three criteria involved in a Petri-dish test were compared on wheat plants. Cultivar differences at seedlings stage after inoculation with a set of 16 Fusarium isolates relative to the controls were detected. Standardized area under disease progress curve ($AUDPC_{standard}$) did differentiate the two wheat cultivars; however, seed germination rate reduction and coleoptile length reduction did not. Inter- and intra-specific differences were observed in pathogenicity of four Fusarium species toward wheat plants. Less $AUDPC_{standard}$ was related to greater FHB disease-type I and -type II resistance previously generated under controlled conditions. *In vitro* data confirmed artificial head and floral inoculations in which the bread wheat cultivar was less affected to FHB infection than durum wheat. The ancient Syrian wheat plants may be introduced into wheat breeding programs because of their resistance to FHB.

Key words: $AUDPC_{standard}$, FHB species, Pathogenicity, Type I and II resistance

Wheat (*Triticum* sp.) is of particular interest in the Fertile Crescent where wheat was first domesticated (Peng *et al.* 2011). In Syria, wheat is one of the most important crops occupying up to 1.7 million ha with annual production of more than 4 million tonnes (FAO/WFP 2015). Thereby, ancient Syrian wheat cultivars constitute a valuable genetic resource, including excellent grain quality and acceptable-level of resistance to abiotic and biotic constraints (Bishawa *et al.* 2015). Seed infection by several species of Fusarium is a great risk for wheat cultivation (Dahl and Wilson 2018). Fusarium species are widespread and cause *Fusarium* head blight (FHB). FHB causes significant yield losses and quality reduction due to contamination of the harvest with mycotoxins (Dahl and Wilson, 2018). *F. graminearum* is reported as the most prominent FHB species, other species are less frequently isolated (Debona *et al.* 2017). Development of resistant cultivars is the most practical method for FHB management (Steiner *et al.* 2017). Wheat exhibits two primary kinds of quantitative resistance which are termed as type I and type II (Mesterhazy 1995). *In vitro* methods predicting resistance at early plant stage have been evaluated with satisfactory results (Browne 2009, Shin *et al.* 2014, Soresi *et al.* 2015). Recently, Sakr (2018c) used a Petri-dish assay (Purahong *et al.* 2012) to quantify aggressiveness in *F. graminearum*, to assess barely resistance to FHB infection.

Wide epidemics of FHB have been recorded after the spread of modern wheats with excellent agronomical characteristics because of losing plant resistance during breeding process (Steiner *et al.* 2017, Sakr 2018a). So, ancient wheats are very important for breeding resistance and managing the disease in traditional systems (Xie and Nevo 2008). A good example of this is Sumai 3, registered in the 1970s, which is considered the most effective source of resistance (Debona *et al.* 2017). FHB resistance in ancient Syrian wheat cultivars has not been reported yet. In this context, the aim of this study was to investigate *in vitro* Petri-dish resistance in two ancient Syrian wheats to FHB and compare this with previous artificial inoculations assays.

MATERIALS AND METHODS

Ancient Syrian durum wheat Acsad65 (released in 1984) and ancient Syrian bread wheat Cham4 (released in 1986), with desirable agronomical characteristics and resistance to fungal diseases, are cultivated so far in Syria (FWD 2007) were used in this study. Cham4 is less affected to FHB infection than Acsad65 detected using head and floret inoculations under controlled conditions (values of disease incidence determined by head inoculation for Type I and disease severity detected by floral inoculation for Type

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II on Cham4 were (45.0% and 41.6%, respectively) and on Acsad65 were (55.6% and 50.3%, respectively) in a previous study (Sakr 2019).

Sixteen 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 collected (2015) from naturally infected wheat spikes exhibiting FHB symptoms from Ghab Plain, one of the principal Syrian wheat production areas. Although *F. graminearum* is considered the major causative of FHB complex worldwide (Debona *et al.* 2017), this species was not found in the surveyed region as observed in other studies investigating the composition of FHB complex species in Ghab Plain during the 2005 growing season (Alazem 2007) and through spring of three seasons (2008-2010) (Al-Chaabi *et al.* 2018). Morphological identification of fungal isolates was carried out using methods described by Leslie and Summerell (2006). For long term preservation, fungal cultures were maintained in sterile distilled water at 4°C and freezing at -16°C (Sakr 2018b).

Three quantitative resistance criteria: Seed germination rate reduction, standardized area under disease progress curve, and coleoptile length reduction involved in a Petri-dish test (Purahong *et al.* 2012) were used to assess resistance on Acsad65 and Cham4 at Plant Protection laboratory, Damascus, Syria under Atomic Energy Commission during 2019. Sterilized wheat seeds were inoculated with a suspension of conidia at 1×10^6 conidia per ml for the 16 FHB isolates, or in sterile distilled water for control treatment into a Petri-dish with sterile double-layer filter paper. The seeds were submerged under the fungal inoculum in the slanting Petri-dish then immediately aligned on the filter with the embryo turned upwards. Infected and control treatments were incubated in an incubator at 22°C in the dark. Three replicates of each cultivar/isolate were set up, and the experiment was repeated. Seed 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 (very resistant) to 1 (not resistant), and it was calculated from the percentage of healthy coleoptiles as a function of time (from 2–6 days after inoculation).

Data were performed using StatView, 4.57® Abacus Concepts, Berkley, Canada. Before statistical analysis, the percentages were transformed using the Arcsines function. ANOVA incorporating the Fisher's LSD test at $P\leq0.05$ was used to differentiate the means.

RESULTS AND DISCUSSION

All 16 Fusarium isolates analyzed with the *in vitro* Petri-

Table 1 Disease responses on two ancient Syrian wheat cultivars (Cham4, bread and Acsad65, durum) infected with *16* fungal isolates of four Fusarium species detected in an *in vitro* Petri-dish assay

Fungal isolate	Seed germination rate reduction (%)			$AUDPC_{standard}$			Coleoptile length eduction (%)		
	Cham4	Acsad65	Mean	Cham4	Acsad65	Mean	Cham4	Acsad65	Mean
F1^{Fc}	19	19	18.6a	0.42	0.62	0.52abc	58	57	57.5a
F2^{Fc}	17	19	17.8a	0.47	0.41	0.44bcdef	60	57	58.5a
F3^{Fc}	21	22	21.6a	0.42	0.50	0.46bcdef	62	52	57.2a
F28^{Fc}	19	22	21.1a	0.37	0.58	0.48bcde	58	58	58.0a
F30^{Fc}	17	22	19.5a	0.28	0.58	0.43bcdef	57	57	57.2a
F7 ^{Fs}	23	23	23.5a	0.46	0.52	0.49bcd	55	58	54.8a
F20 ^{Fs}	23	23	23.5a	0.52	0.52	0.52ab	55	56	55.5a
F26 ^{Fs}	23	23	23.2a	0.46	0.47	0.47bcdef	56	56	56.3a
F29 ^{Fs}	21	23	22.1a	0.33	0.52	0.43cdef	58	57	57.6
F31^{Fs}	20	22	21.5a	0.52	0.42	0.47bcdef	56	55	55.5a
F35 ^{Fs}	23	23	23.7a	0.66	0.52	0.59a	59	55	56.6a
F15 ^{FV}	19	18	18.6a	0.36	0.40	0.38f	58	59	58.7a
F16 ^{FV}	19	19	18.8a	0.36	0.47	0.41def	59	58	58.5a
F21 ^{FV}	18	18	18.1a	0.40	0.44	0.42def	58	58	58.2a
F27 ^{FV}	17	18	17.7a	0.45	0.43	0.39ef	59	55	57.1a
F43^{Fe}	17	19	17.8a	0.49	0.41	0.45bcdef	57	50	53.4a
Mean	17a	19a		0.44b	0.48a		58a	56a	
Enter isolates	F isolates=1.007 ns; P=0.460			F isolates=2.535; P=0.0052			F isolates=0.474 ns; P=0.9456		
Enter cultivars	F cultivars=1.169 ns; P=0.2838			F cultivars=8.147; P=0.0058			F cultivars=2.702 ns; P=0.1051		

Fungal identification: ^{Fc} *Fusarium culmorum*, ^{Fs} *F. solani*, ^{Fv} *F. verticillioides*, ^{Fe} *F. equiseti*. According to the Fisher's LSD test, means followed by the same letter are not significantly different at $P\leq0.05$, ns= not significant, F tests ($P\leq0.05$) (F), Probability (P). In the current study, isolates F2, F35, F27 and F43 reanalyzed for disease response test on Cham4 and Acsad65, however, disease response for the four isolates was analyzed previously and presented by Sakr (2017b).

dish assay fulfilled the capacity to cause FHB disease, thus they were pathogenic (Table 1). No significant differences for seed germination rate reduction and coleoptile length reduction (P=0.460, P=0.9456, respectively) among the 16 tested isolates were detected (Table 1). Our results are in accordance with *in vitro* analyses in which these two criteria did not identify FHB isolates on wheat and barley plants (Sakr 2017b, 2018a,c). Significant differences among the 16 isolates for $AUDPC_{standard}$ (P=0.0052) were detected (Table 1). Inter- and intra-specific differences were observed in pathognicity revealed by $AUDPC_{standard}$ among the four Fusarium species toward the two tested wheat cultivars. Our results agree with previous *in vitro* $AUDPC_{standard}$ analyses in which this criterion did distinguish FHB isolates on wheat and barley plants (Sakr 2017b, 2018a,c). Mutation, genetic recombination or selection may play crucial roles in pathogenesis. In nine Fusarium species recovered from naturally infected wheat spikes in Ghab Plain, high pathogenic variations within and among species were detected (Alazem 2007).

Overall, exposure of treatments for the three quantitative resistance criteria on two tested ancient wheat cultivars to 16 Fusarium isolates reduced mean values of the infected treatments relative to the water controls (Table 1), suggesting a strong effect of different Fusarium isolates on the growth of these two cultivars. All the 16 isolates tested caused brown spots on the coleoptiles, and/or mycelium that completely covered the seeds of the two tested wheat cultivars, whereas the control plants did not show any disease symptoms (Fig 1). $AUDPC_{standard}$ rating for the two wheat cultivars reflects the ability of the same isolate of the pathogen to differentiate several levels of resistance as observed for the same pathosystem (Alazem 2007). The two tested cultivars which can resist high pathogenic isolates form a certain species can also resist another pathogenic isolates from another species, the results here are consummative to the ideas of Sakr (2019).

The reductions in seed germination rate and coleoptile length were not proper methods to distinguish the two tested wheat cultivars (Table 1). ANOVA detected non-significant differences for seed germination rate reduction and coleoptile length reduction between Cham4 and Acsad65 (P=0.2838, P=0.1051, respectively). Mean seed germination rate reduction on Cham4 was 17% and on Acsad65 was 19%. Diseased coleoptiles were only one half of mean lengths of healthy coleoptiles that reached 11.5 mm and 10.2 mm on Cham4 and Acsad65, respectively whatever was the FHB isolate. Mean coleoptile length reduction on Cham4 was 58% and on Acsad65 was 56%. Our results are in accordance with those previously obtained *in vitro*; those criteria did not differentiate six wheat cultivars (Sakr 2017b). Seed germination rate reduction and coleoptile length reduction did not distinguish between Cham4 and Acsad65 showing different levels of type I and II quantitative resistance. However, seed germination and coleoptile length methods are two assays routinely used to selection for FHB resistance. Higher germination rates were highly correlated with the degree of FHB type II resistance in adult plants (Browne 2009). Soresi *et al.* (2015) found that coleoptile length was related with head blight resistance. Contrary, Shin *et al.* (2014) noticed that reductions in germination rate were not correlated with FHB types I and II resistance.

$AUDPC_{standard}$ is distinctive of the resistance or susceptibility levels in wheat to FHB infection at early stages. $AUDPC_{standard}$ of resistant cultivar, Cham4, was less by 8.33% than in the susceptible cultivar, Acsad65 (Table 1). ANOVA detected significant differences for $AUDPC_{standard}$ (P=0.0058) between the two tested cultivars. There were substantial differences in $AUDPC_{standard}$ between cultivars, with rates ranging from 0.44 on Cham4 and 0.48 on Acsad65. Thus, less $AUDPC_{standard}$ has related to greater FHB disease-type I and -type II resistance previously generated under controlled conditions (Sakr 2019). $AUDPC_{standard}$ was calculated from the decreasing number of healthy wheat seedlings after fungal inoculation of the seeds (Purahong *et al.* 2012). The slower the reduction of the number of healthy seedlings, the more resistant is the cultivar (Purahong *et al.* 2012). Our results are in accordance with *in vitro* previous

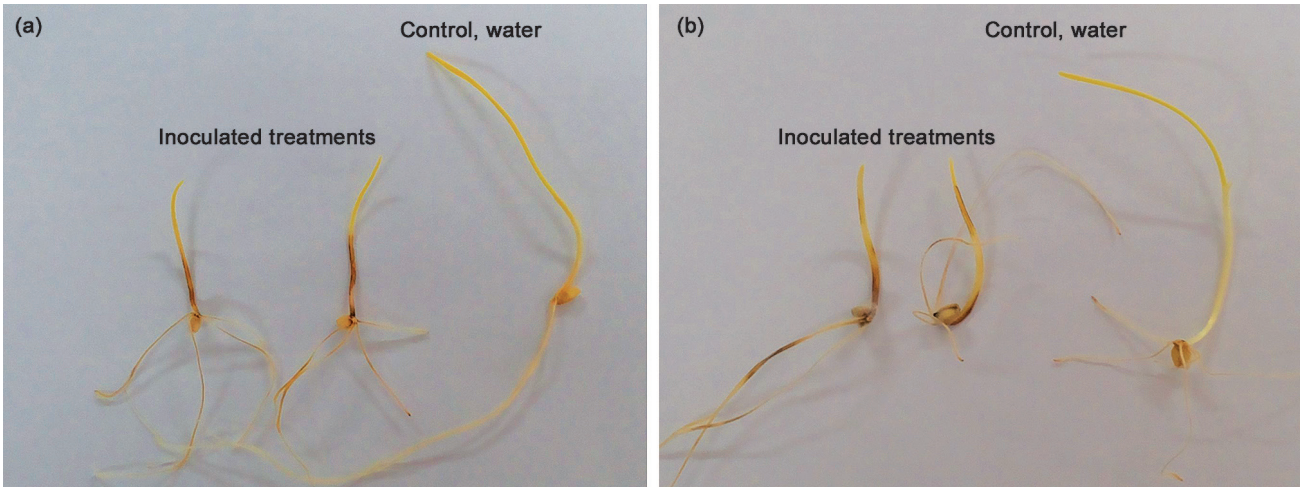

Fig 1 Fusarium symptoms on seedlings of two ancient Syrian wheat cultivar Cham4 (bread): (a) and Acsad65 (durum), (b) inoculated with isolate F1 (*Fusarium culmorum*) compare with control (water) at 6 day after inoculation

analysis in which this criterion did distinguish six wheat cultivars (Sakr, 2017b). *In vitro* $AUDPC_{standard}$ data were highly significantly correlated with floret inoculation data obtained using adult plants in the growth chamber (Types I and II) (Purahong *et al.* 2012, Sakr 2017a).

$AUDPC_{standard}$ could reflect aspects of resistance reaction at early stages of plant growth by promoting the interaction between wheat tissues and fungi. The situation in the *in vitro* Petri-dish assay was similar to artificial inoculation under controlled conditions because FHB species need to overcome the morphology of the head spike and they could directly penetrate and infect germinating seeds (Sakr 2018c). This *in vitro* criterion could be of potential use in evaluating the quantitative resistance in adult wheat plants under controlled conditions to FHB infection.

The two tested ancient Syrian cultivars were shown to exhibit moderate to high resistance levels measured by $AUDPC_{standard}$ evaluations to FHB infection (Table 1). Also, the acceptable resistance level in the two wheat cultivars made it possible to detect differences in pathogenicity among the 16 tested isolates. Thus, *in vitro* Petri-dish data confirmed artificial head and floral inoculations in which Cham4 was less affected to FHB infection than Acsad65. Our results agree with resistance hypothesis in FHB-wheat pathosystem in which bread wheat is less affected to FHB infection than durum wheat (Steiner *et al.* 2017). Although the most FHB resistant wheat cultivars exhibit poor agronomical characteristics (Debona *et al.* 2017), the variability of resistance for Cham4 and Acsad65, with favorable agronomical characteristics under Syrian conditions than those of the Far Eastern cultivars used worldwide (FWD 2007), is promising resistance sources to FHB in wheat breeding. Since only two ancient wheat cultivars were tested here, further research using a large sample of available Syrian ancient and modern wheat cultivars is needed to validate our results *in vitro*, under controlled and field conditions.

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