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

NACHAAT SAKR

Department of Agriculture, Atomic Energy Commission of Syria, Damascus, Syria

Received: 17 September 2018; Accepted: 19 July 2019

**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 (AUDPCstandard) 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 AUDPCstandard 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:** AUDPCstandard, 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 (Brown 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
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 et al., 2012) 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).

### RESULTS AND DISCUSSION

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

| Fungal isolate | Seed germination rate reduction (%) | AUDPCstandard | Coleoptile length reduction (%) |
|----------------|-------------------------------------|---------------|---------------------------------|
|                | Cham4 | Acsad65 | Mean | Cham4 | Acsad65 | Mean | Cham4 | Acsad65 | Mean |
| F1Fc           | 19    | 19      | 18.6a | 0.42   | 0.62   | 0.52abc | 58    | 57      | 57.5a |
| F2Fc           | 17    | 19      | 17.8a | 0.47   | 0.41   | 0.44bcd ef | 60    | 57      | 58.5a |
| F3Fc           | 21    | 22      | 21.6a | 0.42   | 0.50   | 0.46bcd ef | 62    | 52      | 57.2a |
| F28Fc          | 19    | 22      | 21.1a | 0.37   | 0.58   | 0.48bcde | 58    | 58      | 58.0a |
| F30Fc          | 17    | 22      | 19.5a | 0.28   | 0.58   | 0.43bcd ef | 57    | 57      | 57.2a |
| F7Fs           | 23    | 23      | 23.5a | 0.46   | 0.52   | 0.49bcd | 55    | 58      | 54.8a |
| F20Fs          | 23    | 23      | 23.5a | 0.52   | 0.52   | 0.52ab | 55    | 56      | 55.5a |
| F26Fs          | 23    | 23      | 23.2a | 0.46   | 0.47   | 0.47bcd ef | 56    | 56      | 56.3a |
| F29Fs          | 21    | 23      | 22.1a | 0.33   | 0.52   | 0.43cd ef | 58    | 57      | 57.6 |
| F31Fs          | 20    | 22      | 21.5a | 0.52   | 0.42   | 0.47bcd ef | 56    | 55      | 55.5a |
| F35Fs          | 23    | 23      | 23.7a | 0.66   | 0.52   | 0.59a | 59    | 55      | 56.6a |
| F15Fv          | 19    | 18      | 18.6a | 0.36   | 0.40   | 0.38f | 58    | 59      | 58.7a |
| F16Fv          | 19    | 19      | 18.8a | 0.36   | 0.47   | 0.41def | 59    | 58      | 58.5a |
| F21Fv          | 18    | 18      | 18.1a | 0.40   | 0.44   | 0.42def | 58    | 58      | 58.2a |
| F27Fv          | 17    | 18      | 17.7a | 0.45   | 0.43   | 0.39ef | 59    | 55      | 57.1a |
| F43Fe          | 17    | 19      | 17.8a | 0.49   | 0.41   | 0.45bcd ef | 57    | 50      | 53.4a |
| Mean           | 17a   | 19a     | 17.4a | 0.44b  | 0.48a  |

Enter isolates: *F* isolates=1.007 ns; *P*=0.460  
Enter cultivars: *F* cultivars=1.169 ns; *P*=0.2838

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*≤0.05, ns= not significant, *F* test (*P*≤0.05) (*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\textsubscript{standard} (P=0.0052) were detected (Table 1). Inter- and intra-specific differences were observed in pathogenicity revealed by AUDPC\textsubscript{standard} among the four Fusarium species toward the two tested wheat cultivars. Our results agree with previous in vitro AUDPC\textsubscript{standard} analyses in which this criterion did distinguish FHB isolates on wheat and barley plants (Sakr 2017b, 2018a,c). Significant differences among the 16 isolates for AUDPC\textsubscript{standard} were detected (P=0.0052) (Table 1). Inter- and intra-specific differences were observed in pathogenicity revealed by AUDPC\textsubscript{standard} among the four Fusarium species toward the two tested wheat cultivars. Our results agree with previous in vitro AUDPC\textsubscript{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\textsubscript{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\textsubscript{standard} is distinctive of the resistance or susceptibility levels in wheat to FHB infection at early stages. AUDPC\textsubscript{standard} of resistant cultivar, Cham4, was less by 8.33% than in the susceptible cultivar, Acsad65 (Table 1). ANOVA detected significant differences for AUDPC\textsubscript{standard} (P=0.0058) between the two tested cultivars. There were substantial differences in AUDPC\textsubscript{standard} between cultivars, with rates ranging from 0.44 on Cham4 and 0.48 on Acsad65. Thus, less AUDPC\textsubscript{standard} has related to greater FHB disease-type I and -type II resistance previously generated under controlled conditions (Sakr 2019). AUDPC\textsubscript{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
analysis in which this criterion did distinguish six wheat cultivars (Sakr, 2017b). In vitro AUDPC\textsubscript{standard} data were highly significantly correlated with floret inoculation data obtained using adult plants in the growth chamber (Types I and II) (Purahong \textit{et al.} 2012, Sakr 2017a).

AUDPC\textsubscript{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 \textit{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 \textit{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\textsubscript{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, \textit{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 \textit{et al.} 2017). Although the most FHB resistant wheat cultivars exhibit poor agronomical characteristics (Debona \textit{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 \textit{in vitro}, under controlled and field conditions.

**ACKNOWLEDGEMENTS**

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

**REFERENCES**

Alazem M. 2007. ‘Evaluating genetic variation of Fusarium head blight by molecular markers’. M Sc thesis, University of Damascus, Damascus, Syria.

Al-Chaibi S, Al-Masri S, Nehlawi A, Al-Matroud L and Abu-Fadel T. 2018. Monitoring of \textit{Fusarium} wheat head blight distribution, its causal agents, and pathogenicity variation in Al-Ghab plain, Syria. \textit{Arab Journal of Plant Protection} 36(2): 98–113.

Bishawa Z, Struik P C and van Gastel C J G. 2015. Wheat and barley seed system in Syria: How diverse are wheat and barley varieties and landraces from farmer’s fields? \textit{International Journal of Plant Production} 9(1): 117–50.

Browne R A. 2009. Investigation into components of partial disease resistance, determined \textit{in vitro}, and the concept of types of resistance to \textit{Fusarium} head blight (FHB) in wheat. \textit{European Journal of Plant Pathology} 123(2): 229–34.

Dahl B and Wilson W W. 2018. Risk premiums due to Fusarium Head Blight (FHB) in wheat and barley. \textit{Agricultural Systems} 162: 145–53.

Dweba C C, Figlan S, Shimelis H A, Motaung T E, Sydenham S, Mwadzenge L and Tsilo T J. 2017. Fusarium head blight of wheat: Pathogenesis and control strategies. \textit{Crop Protection} 91: 114–22.

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.

FWD. 2007. Field Wheat Directory. [cited 2007 August 10] Available from: http://gcsar.gov.sy/ar/wp-content/uploads/wheatguide.pdf.

Leslie J F and Summerrell A B. 2006. \textit{The Fusarium Laboratory Manual}. Blackwell Publishing Professional, Ames, USA.

Mesterhazy A. 1995. Types and components of resistance to \textit{Fusarium} head blight of wheat. \textit{Plant Breeding} 114(5): 577–86.

Peng J H, Sun D and Nevo E. 2011. Domestication evolution, genetics and genomics in wheat. \textit{Molecular Breeding} 28: 281–301.

Purahong W, Alkadri D, Nipoti P, Pisi A, Lemmens M and Prodi A. 2012. Validation of a modified Petri-dish test to quantify aggressiveness of \textit{Fusarium graminearum} in durum wheat. \textit{European Journal of Plant Pathology} 132(3): 381–91.

Sakr N. 2017a. Aggressiveness of four Fusarium head blight species on wheat cultivars. \textit{Advances in Horticultural Science} 31(3): 199–203.

Sakr N. 2017b. \textit{In vitro} assessment of Fusarium head blight spp. on wheat cultivars. \textit{Archives of Phytopathology and Plant Protection} 50(5-6): 254–61.

Sakr N. 2018a. Aggressiveness of Fusarium head blight species towards two modern Syrian wheat cultivars in an \textit{in vitro} Petri-dish. \textit{Cereal Research Communications} 46(3): 480–9.

Sakr N. 2018b. Evaluation of two storage methods for fungal isolates of \textit{Fusarium} sp. and \textit{Cochliobolus sativus}. \textit{Acta Phytopathologica et Entomologica Hungarica} 53(1): 11–18.

Sakr N. 2018c. Components of quantitative resistance in barley plants to Fusarium head blight infection determined using three \textit{in vitro} assays. \textit{Journal of Plant Protection Research} 58(2): 176–83.

Sakr N. 2019. Pathogenicity and quantitative resistance in Mediterranean durum and bread wheat cultivars of Syrian origin towards Fusarium head blight agents under controlled conditions. \textit{Journal of Plant Protection Research} https://doi.org/10.24425/jppr.2019.131261.

Shin S, Kim K H, Kang C S, Cho K M, Park C S, Okagaki R and Park J C. 2014. Simple method for the assessment of Fusarium head blight resistance in Korean wheat seedlings inoculated with \textit{Fusarium graminearum}. \textit{Plant Pathology Journal} 30(1): 25–32.

Soresi D, Zappacosta D, Garayalde A, Miranda R and Carrera A. 2017. Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. \textit{Tropical Plant Pathology} 42(3): 165–74.

Xie W and Nevo E. 2008. Wild emmer: genetic resources, gene mapping and potential for wheat improvement. \textit{Euphytica} 164(3): 603–14.