Indian Phytopathology (2020) 73:483–491
https://doi.org/10.1007/s42360-020-00234-x

RESEARCH ARTICLE

Aggressiveness of *Fusarium* species causing head blight on wheat plants determined in detached leaf and seedling in vitro assays

Nachaat Sakr[1]

Received: 19 December 2019 / Revised: 13 April 2020 / Accepted: 29 April 2020 / Published online: 14 May 2020

Abstract

Fusarium head blight (FHB) is a serious disease of wheat. In vitro assays for reliable prediction of the head blight reaction in whole plant was investigated. The capacity of 16 fungal isolates of four FHB species to cause disease on individual plant organs was evaluated in vitro. Six quantitative components related to detached leaf and seedling assays were analyzed in six durum and bread wheat cultivars with known resistance. Differences in inoculated treatment were observed on young plant parts relative to water controls, indicating that these FHB species were found to be suitable for the differential expression of all tested quantitative components. There was a wide variation in aggressiveness among the 16 FHB isolates for latent period (LP) in detached leaf assay. Nevertheless, the other five components: incubation period and lesion length of detached leaf assay, percentage of infected seedlings (of foliar-spraying and pin-point inoculations) and lesion length (of clip-dipping inoculation) did not distinguish isolates. Significant correlation was found for the aggressiveness measured by LP of 16 FHB isolates and the previous pathogenic component ratings generated in vitro and in growth chamber and field study. These results suggest that LP predicts aggressiveness occurring at the earliest and latest wheat development stages during FHB infection. To our best knowledge, this is the primary in-depth report combining six different quantitative components delivered from two distinct in vitro assays to quantify aggressiveness in FHB species complex. Our data also highlighted, for the first time, the utility of LP for rapid and early determination of aggressiveness in FHB-wheat pathosystem.

Keywords FHB species · Latent period · Pathogenic variability · Wheat resistance

Introduction

Fusarium head blight (FHB) is a devastating disease of wheat. FHB has received more attention because of the impact that infection may cause yield losses of up to 50–70% and quality reduction due to the accumulation of several mycotoxins [especially deoxynivalenol (DON)] in the grain that poses a significant risk to food and feed safety (Parry et al. 1995; Dahl and Wilson 2018). The disease complex is associated with at least seventeen *Fusarium* species and two *Microdochium* causal agents like *F. graminearum*, *F. culmorum* and *F. avenaceum* are the most pathogenic and widely distributed species in most infected areas worldwide. *F. equiseti*, *F. poae* and *F. cerealis* are less frequent, and *F. proliferatum*, *F. solani* and *F. verticillioides* are the least frequent species of FHB (Saharan and Sharma 2009).

Assessment of quantitative components related to pathogenicity is a fundamental aspect in the FHB-wheat pathosystem (Wu et al. 2005). The expression of aggressiveness is not only controlled by the broad range of *Fusarium* species associated and the host, but also by the environment and their interactions (Singh and Aujla 1994; Dweba et al. 2017). Aggressiveness describing the quantitative pathogenicity may vary among FHB species and even among different isolates of a given species (Xue et al. 2004; Xu et al. 2008; Saharan and Sharma 2009; Xu and Nicholson 2009). There are very limited data on the differences in aggressiveness of the less frequent FHB species compared to the most commonly distributed (Zhang et al. 2012). No complete resistance to FHB was detected and limited number of resistant wheat genotypes are available (Kharbikar et al. 2019), and two primary types of polygenic resistance in wheat to disease infection were recorded (Mesterhazy et al. 1999): type

✉ Nachaat Sakr
ascientific33@aec.org.sy

[1] Department of Agriculture, AEC of Syria, P.O. Box 6091, Damascus, Syria

1 (resistance to initial penetration of the pathogen) and Type II (resistance to spreading within a spike).

Traditionally, pathogenic reaction of wheat to FHB pathogen to quantify aggressiveness is commonly evaluated under controlled and field conditions at mid-anthesis stage in adult plants by determining the visual scoring of head symptoms. It has the disadvantage of influenced by environment and leads to experimental error (Wu et al. 2005; Pakdaman et al. 2006; Imathiu et al. 2014). Thus, the testing of highly aggressive isolates without the risk of escaping into wheat production areas is the major constraint in traditional method. During the last two decades, less costly in vitro assays permitting for simple, accurate and reliable prediction of the head blight reaction in whole plant have been studied.

Germination rate reduction, lesion length, coleoptile length reduction and area under disease progress curve (AUDPC) have been utilized effectively to examine aggressiveness of different FHB isolates and several fungal species (Brennan et al. 2003; Wu et al. 2005; Imathiu et al. 2009; Opoku et al. 2011; Purahong et al. 2012; Khaledi et al. 2017; Sakr 2017, 2018a, c, d). In addition, several reports have showed that disease reactions revealed in diverse in vitro assays on juvenile plants strongly correlated with pathogenicity determined on mature plants (Wu et al. 2005; Purahong et al. 2012; Khaledi et al. 2017; Sakr 2017, 2018a, c, d, 2019b, 2020). Sakr (2019c) reported that AUDPC and latent period differentiated FHB isolates on barley plants. Furthermore, these criteria were indicators of aggressiveness occurring in the adult plant during FHB infection (2019c). The novel method of choice to analyze responses to individual FHB species should account for their aggressiveness on individual tissues or parts and their capacity to induce significant disease ratings at specific growth stages under favorable experimental conditions. Incubation period and LP of detached leaf assay and percentage infection of seedling assay are components of quantitative wheat resistance to FHB infection (Browne and Cooke 2004; Browne 2007; Shin et al. 2014; Sakr 2019a). Also, these criteria are measured as indicators of aggressiveness in several pathosystems (Lannou 2012). Till recently, disease reaction as measured by more distinct in vitro methodologies, i.e., detached leaf and seedling assays, to assess aggressiveness in several FHB species on durum and bread cultivars has not been combined in one experiment.

An important challenge is to analyze more effective and accurate in vitro disease evaluation methods for detecting pathogen differences and investigating if aggressiveness observed in individual plant organs and earlier growth stages may relate to disease expression in the whole plant (Purahong et al. 2012; Sakr 2017, 2018a, c, d, 2019b, 2020). In this context, we report the evaluation of quantitative components related to pathogenicity in FHB species complex using in vitro detached leaf and seedling assays on six durum and bread wheat cultivars. We have also examined the relationships between the present data and the findings previously and currently generated using in vitro Petri-dish test and artificial inoculations under controlled and field conditions.

Materials and methods

Fungal isolates

Sixteen fungal isolates representing the four *Fusarium* species (*F. culmorum* (F1, F2, F3, F28 and F30), *F. solani* (F7, F20, F26, F29, F31 and F35), *F. verticillioides* (synonym *F. moniliforme*) (F15, F16, F21 and F27) and *F. equiseti* (F43)) were recovered from symptomatic wheat spikes originating from Ghab Plain, one of the principal Syrian wheat production regions, during the 2015 growing season. On potato-dextrose agar, single-spore derived cultures were identified to species by microscopic observations of sporulation shape and type (spores and phialides morphology), along with optical observations of the cultural characteristics, such as colony shape and morphology, pigmentation and density of aerial and medial parts (Leslie and Summerell 2006). The 16 fungal isolates were verified to be belonged to four FHB species (*F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti*) based on molecular identification (Sakr unpublished data). For long term storage, fungal cultures were stored in sterile distilled water at 4 °C and freezing at − 16 °C (Sakr 2018b). Conidia were dislodged and harvested by flooding the cultures with 10 ml of sterile distilled water and the suspensions of the 16 FHB isolates were filtered through two layers of sterile cheesecloth to remove mycelia. Concentrations of resulting spore suspensions were determined using a haemocytometer and adjusted to a desirable concentration.

Wheat cultivars

Six Syrian durum (Acsad65 released in 1984, Cham7 in 2004 and Cham9 in 2010) and bread (Cham4 released in 1986, Douma4 in 2007 and Bohoth10 in 2014) wheat cultivars were used for evaluating of the quantitative components. These cultivars with highest agronomic characteristics and resistance to biotic and abiotic stresses were selected because they are currently the most important wheat cultivars in Syria. Furthermore, pathogenic reactions in these cultivars were determined using in vitro component (AUDPC) of Petri-dish inoculation methodology (Sakr 2018a, c, d, 2020) and head and floret artificial inoculations under controlled conditions (Sakr 2019b). Therefore, we were able examine the relationships between the current findings with

the previous results of in vitro AUDPC, spraying and point inoculations under controlled conditions.

Quantitative component tests in vitro

The capacity of 16 FHB isolates of four *Fusarium* species to confer disease on young plant organs was evaluated using a detached leaf assay according to Browne and Cooke (2004) and a seedling assay described by Shin et al. (2014) (Fig. 1). These methodologies were used by Sakr (2019a, c) to assess quantitative components of resistance in wheat to FHB infection and quantify aggressiveness of FHB agents on barley plants. Three aggressiveness components were assessed in the detached leaf assay: incubation period (days from inoculation to first appearance on the leaf surface, of a dull gray-green water-soaked lesion), latent period (days from inoculation to sporulation), and lesion length (measured after 7 days as a visible chlorotic area). Three replicates of each isolate based on observations on 120 detached leaves were set up, and the experimentation was conducted twice. Regarding seedling inoculation, the percentage of infected seedlings was assessed using pin-point and foliar-spraying methods. Lesion length was assessed utilizing a clip-dipping method. For these three experiments, three replications of each isolate were set up, and the experiment was conducted twice.

Quantitative component tests under pot conditions

The 16 FHB isolates were separately inoculated on Acsad65, Cham7, Cham9, Cham4, Douma4 and Bohoth10 to measure disease incidence (DI) for aggressiveness as indicator of quantitative pathogenicity of the fungal isolates. The six wheat cultivars were cultivated at the Deir Al-Hajar Agricultural Experiment Station, located south east of Damascus, Syria (33°20′ N, 36°26′ E) at 617 m above sea level over the growing season 2018/19.

Wheat plants were grown in plastic 15-cm pots containing soil pasteurized at 5 k Gray of Gamma Ray (GR) with 60Co source using a gamma irradiator (ROBO, Russia). The soil used was a clay soil (57% clay, 39% loam and 2% sand) collected from Sojji research station (located east of

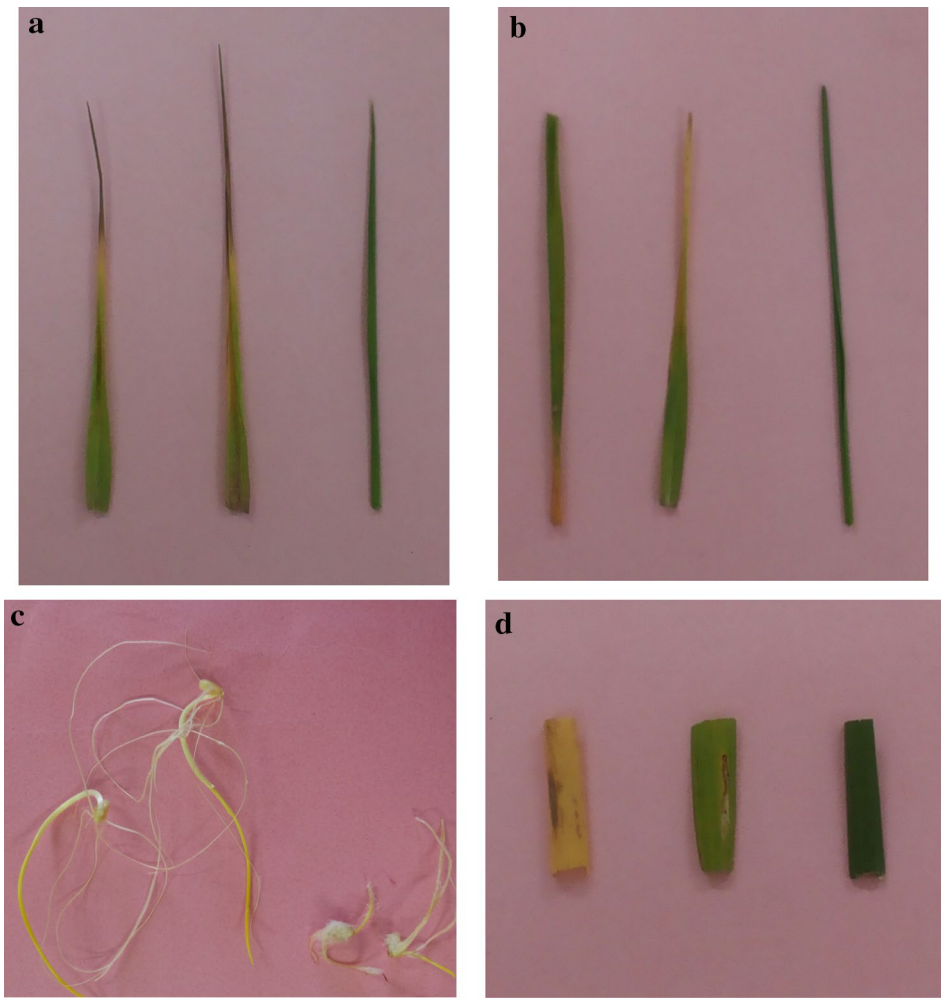

Fig. 1 in vitro inoculation techniques used to assess Fusarium head blight aggressiveness on plant materials of Syrian durum wheat cultivar Acsad65 infected with isolate F29 (*F. solani*), **a** foliar-spraying assay, **c** clip-dipping assay, **d** pin-point assay and **e** detached-leaf assay

Damascus, Syria, 3330′54.4″N 36°07′33.2″E) with the following characteristics: pH = 7.8; P = 13.4 ppm; K, Na, Ca, Mg = 1.81, 2.99, 33.1, 14 mg/100 g soil respectively, and organic matter = 1.25%. Each plastic pot was filled with 2 kg of air-dried, sieved (2 mm) soil. The experimental layout was a randomized complete design with three replicates (pots). Three pots per replicate were left non-inoculated as control treatment. Following emergence, plants were thinned and nitrogen fertilizer was applied twice at two dates: thinning and tillering. The plants were watered when needed. At mid-anthesis stage, plants of a pot were sprayed with a spore suspension at 5×10^4 spores/ml of 16 FHB isolates or sterile distilled water (control). Wheat heads were covered with clear plastic bags for 2 days to provide constant high humidity to promote primary infection. The experiment was repeated twice on Acsad65, Cham7, Cham9, Cham4, Douma4 and Bohoth10. Disease incidence (% symptomatic spike) for aggressiveness was estimated as the percentage of spikes showing pathogenic symptoms.

Statistical analyses

Data were subjected to analysis of variances (ANOVA) using StatView, 4.57® Abacus Concepts, Berkley, Canada. Prior statistical analysis, the percentages of infected seedlings were converted using the angular transformation to stabilize variances. Fisher's LSD test was used to compare the means at p = 0.05. The sample correlation coefficients (Pearson r) were calculated using overall values per isolates at p = 0.05.

Results and discussion

It has been adopted that under natural conditions, FHB occurs unpredictably and the disease in not equally spread across fields, hence artificial infection is the most suitable for FHB bioassay assessment (Dweba et al. 2017). Quantitative components in wheat cultivars to FHB complex depends on percentage of diseased spike in growth chamber and field, however, this visual scoring of head symptoms incorporate several constraints (Imathiu et al. 2014). Such in vitro tests making it possible to predict aggressiveness at earlier plant stages relative to disease scoring in whole plant have been developed (Wu et al. 2005; Purahong et al. 2012; Khaledi et al. 2017; Sakr 2017, 2018a, c, d, 2019b, 2020). For the first time, we showed that the latent period (LP) of detached-leaf in vitro assay is a reliable and rapid method to discriminate aggressiveness in FHB species in wheat in the laboratory in a short period of time. In addition, we investigated the potential use of LP in predicting FHB data generated under several experimental conditions.

During our experiment, differences in inoculated treatment on young plant organs relative to water controls were found (Table 1), indicating that the FHB species used in this research were found to be suitable for the differential expression of all tested quantitative components. Detached leaves and seedlings of six wheat cultivars growing in the presence of 16 *Fusarium* isolates showed typical in vitro FHB symptoms according to two tested inoculations, suggesting a strong effect of the four *Fusarium* species on the growth of these cultivars whereas the control plants did not show any head blight symptoms. This also refers that the two different tested in vitro detached leaf and seedling assays (varying in incoculum concentration, infection methods, growth conditions and target young plant parts) conducted on *Microdochium nivale*, *F. graminearum* and the four tested FHB species to screen quantitative wheat resistance (Browne and Cooke 2004; Shin et al. 2014; Sakr 2019a) can be used to assess pathogenicity components of FHB species: *F. culmorum*, *F. verticillioides*, *F. solani* and *F. equiseti* on several bread and durum wheat plants.

The four tested species were found to provide distinct and observable symptoms on detached wheat leaves (Fig. 1). All FHB isolates caused obvious lesions on detached leaves of six wheat cultivars by the sixth day after inoculation. Lesions, almost oval in shape, appeared as dark-brownish water-soaked patches and characterized later by more chlorosis and less necrosis on lesions developed on wheat leaves (Fig. 1). This observation is of great importance in FHB research since the *Microdochium* genus has been used to study quantitative components compared with other FHB complex species, it provides more obvious and observable symptoms on leaf segments (Browne and Cooke 2004). The type of chlorosis formation in our investigation was comparable to its observed type in the detached leaf assay conducted on the methodology described by Browne and Cooke (2005); lesions being accompanied by chlorosis of leaf tissues and later by less necrosis on lesions developed on wheat leaves. This may imply that delay of death of cells and living tissue occurs in wheat leaves irrespective of the 16 FHB isolates involved, as necrosis occurs as a result of cell death (Imathiu et al. 2009). Also, wheat seedlings inoculated with FHB conidia showed visible fungal head blight symptoms within a few days after inoculation (Fig. 1). Brown lesions started from the infected portions in sections in 3-day-old seedling coleoptiles and stems. Seven-day-old visually necrotic area exhibited varying degrees of necrosis as a consequence of the pathogen severity. Thus, the symptoms observed on wheat seedling in the present research were similar to its noted pattern caused by *F. graminearum* (Shin et al. 2014).

The two in vitro assays: detached leaf and seedling provide favorable conditions for FHB species to actively grow, thus ensure that their aggressiveness is stable and/or

Table 1 Mean aggressiveness components determined using in vitro detached leaf and seedling assays among 16 fungal isolates of four Fusarium head blight species measured on six wheat cultivars

Fungal isolates (identification)	Aggressiveness components					
	Incubation period (days)	Latent period (days)	Lesion length (mm)	Percentage of infected seedlings (%) of foliar-spraying infection	Percentage of infected seedlings (%) of pin-point infection	Lesion length (cm) of clip-dipping inoculation
F1 (*F. culmorum)*	2.1a	5.1cdef	7.9a	45.4a	42.7a	2.0a
F2 (*F. culmorum*)	2.1a	6.1ab	7.2a	44.0a	41.9a	2.2a
F3 (*F. culmorum*)	2.2a	5.7bc	7.4a	44.2a	40.1a	2.2a
F28 (*F. culmorum*)	2.2a	5.1cdef	8.1a	46.7a	40.5a	2.3a
F30 (*F. culmorum*)	2.0a	5.0defg	7.0a	44.8a	40.5a	1.9a
F7 (*F. solani*)	2.3a	4.9efgh	7.3a	41.5a	40.5a	2.0a
F20 (*F. solani*)	2.0a	5.5 cd	7.3a	44.7a	39.5a	2.1a
F26 (*F. solani*)	2.0a	4.4ghi	7.4a	42.5a	40.9a	2.1a
F29 (*F. solani*)	2.3a	4.3i	7.1a	45.7a	40.1a	2.1a
F31 (*F. solani*)	2.0a	4.9cde	7.1a	44.6a	39.8a	2.0a
F35 (*F. solani*)	2.1a	6.4a	7.8a	45.3a	40.2a	2.0a
F15 (*F. verticillioides*)	2.2a	4.8fghi	7.7a	44.0a	42.1a	2.1a
F16 (*F. verticillioides*)	2.0a	4.4hi	7.8a	41.5a	39.2a	2.0a
F21 (*F. verticillioides*)	2.0a	5.6bc	7.2a	45.3a	43.8a	1.9a
F27 (*F. verticillioides*)	2.1a	4.4hi	7.8a	41.4a	40.9a	2.0a
F43 (*F. equiesti*)	2.1a	5.1defg	7.7a	45.2a	40.0a	2.2a
	F isolates = 0.822 ns; P = 0.652 (incubation period)					
	F isolates = 9.698; P = 0.001 (latent period)					
	F isolates = 0.808 ns; P = 0.936 (lesion length)					
	F isolates = 0.495 ns; P = 0.941 (percentage of infected seedlings of foliar-spraying infection)					
	F isolates = 0.844 ns; P = 0.927 (percentage of infected seedlings of pin-point infection)					
	F isolates = 1.400 ns; P = 0.150 (lesion length of clip-dipping inoculation)					

According to the Fisher's LSD test, means followed by the same letter within a column are not significantly different at p = 0.05, ns = not significant, F tests (p = 0.05) (F), Probability (P). In the current study, all fungal isolates were reanalyzed for disease reaction on Cham7; however, response of Cham7–16 tested FHB isolates was analyzed previously and presented by Sakr (unpublished data)

correctly distinguished or assessed. All the fungal isolates treated with the two in vitro assays fulfilled the requirement to induce FHB disease, thus they are pathogenic. The four FHB species used in this experiment are known for mycotoxin production (Xu and Nicholson 2009). Thus, the capability of 16 fungal isolates to cause chlorosis and necrosis in varying amounts on wheat leaves and seedlings (Fig. 1) might be mainly the result of phytotoxic action of these metabolites. Furthermore, it is important to measure directly DON or other toxins characteristics to the given fungal species in the research relevance to pathogeneicity and disease development.

The significant reductions observed with incubation period (IP) and LP, long lesion length (LL) and high percentage infection are indications of aggressiveness (Lannou 2012). The five aggressiveness components: IP (P = 0.652) and LL (P = 0.936) of detached leaf assay, percentage of infected seedlings (PIS) (P = 0.941 and P = 0.927, respectively) (of foliar-spraying and pin-point inoculations) and lesion length (P = 0.150) of clip-dipping inoculation did not distinguish isolates within and among species (Table 1). Mean IP and LL ranged from 2.0 to 2.3 days and 7.2 to 8.1 mm, respectively. The PIS (of spraying inoculation) varied from 41.5 to 45.7%. The PIS (of pin-point inoculation) varied from 39.8 to 43.8%. The LL (of clip-dipping inoculation) varied from 1.9 to 2.2 cm. Our data are not in accordance with those found by Imathiu et al. (2009), Opoku et al. (2011) and Khaledi et al. (2017), they observed highly significant differences in the aggressiveness of *F. graminearum* and *F. langsethiae* as measured by LL of detached-leaf assay.

In our study, LP varied from one fungal isolate to another irrespective of origin in wheat cultivars (P = 0.001). F35 (*F. solani*), the least aggressive isolate, showed maximum LP (6.4 days), however, F29 (*F. solani*) (4.3 days) was significantly more aggressive (Table 1). Until now, LP applies more easily to pathogens with no specific biological features, i.e., fungi like *Fusarium* species and also for pathogens with specific biological features (Lannou 2012). Recently, inter and intraspecific differences in aggressiveness of the four tested FHB species were observed towards barley plants as

measured by LP (Sakr 2019c). Regarding in vitro standardized area under disease progress curve (AUDPC) component determined using a Petri-dish assay, variability in aggressiveness of the same isolates was also detected on the same wheat cultivars (Sakr 2018a, c, d, 2020). Mutation, genetic recombination or selection may play a basic role in pathogenesis (Imathiu et al. 2009). The five mentioned above aggressiveness components showed similar results for the various FHB isolates; while the individual aggressiveness component, LP, differentially influence aspects of FHB disease development in wheat plants at early stages.

The mean disease incidence (% symptomatic spike) (DI) values generated under field conditions of FHB fungi varied from 35.0% for the least aggressive isolate F27 (*F. verticillioides*) to 57.0% for the most aggressive isolate F20 (*F. solani*) on wheat cultivars as compared with 0% for the non-inoculated controls (P = 0.001) (Table 2). Inoculation of heads carried out to determine Type I FHB resistance showed substantial differences in the resistance of wheat cultivars. The mean portion of plants exhibiting FHB symptoms ranged from 38.7 to 53.4% (P = 0.001) (Table 2). Cham4 and Bohoth10 showed the lowest infection levels, with DI values of 38.7 and 40.5% respectively, whereas Acsad65 was the most affected cultivar, with DI value of 53.4%. Based on Type I resistance, Bohoth10 and Cham4 were moderately resistant cultivars, Douma4 was moderately susceptible, the two durums, Cham7 and Cham9, were susceptible to moderately susceptible, and Acsad65 was susceptible.

Although large differences among isolates of individual species for aggressiveness were detected, analysis of our results showed that the four FHB species did not vary in their comparative aggressiveness measured by LP on bread and durum wheat plants (Fig. 2) because of the relative homogeneity in pathogenic level among the 16 fungal isolates. Fernandez and Chen (2005) noted an obvious lack of a difference in aggressiveness between *F. graminearum* and *F. culmorum* on wheat. Similarly, Sakr (2018a, c, d, 2020) did not cluster the same fungal species on wheat cultivars using AUDPC. Our results did not support previous reports showing that FHB species varied in their aggressiveness (Bottalico and Perrone 2002; Xue et al. 2004; Saharan and Sharma 2009).

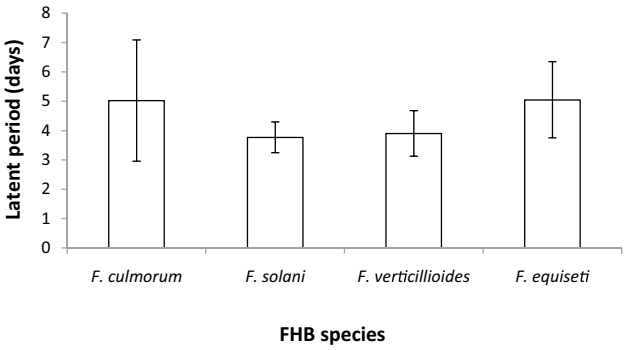

Fig. 2 Mean latent period (days) of four Fusarium head blight species on six bread and durum wheat cultivars detected in an in vitro detached leaf assay. Bars represent the standard errors of means

Table 2 Disease incidence (% symptomatic spike) (DI) determined using a head artificial inoculation under field conditions for six wheat cultivars infected with 16 fungal isolates of four Fusarium head blight species

Fungal isolates (identification)	DI						
	Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10	Mean
F1 (*F. culmorum)*	64	55	62	43	35	36	49.2bcd
F2 (*F. culmorum*)	45	57	44	61	55	49	51.9ab
F3 (*F. culmorum*)	60	33	51	41	57	44	47.6bcd
F28 (*F. culmorum*)	69	30	31	26	60	55	45.2cde
F30 (*F. culmorum*)	71	30	36	42	39	30	41.3ef
F7 (*F. solani*)	51	30	45	37	50	37	41.7ef
F20 (*F. solani*)	71	55	60	56	60	40	57.0a
F26 (*F. solani*)	50	50	45	43	55	45	48.0bcd
F29 (*F. solani*)	71	40	55	38	50	45	49.7bc
F31 (*F. solani*)	35	40	55	56	35	29	41.7ef
F35 (*F. solani*)	39	50	60	69	43	30	48.5bcd
F15 (*F. verticillioides*)	40	35	53	23	45	30	37.7fg
F16 (*F. verticillioides*)	55	28	35	51	40	40	41.5ef
F21 (*F. verticillioides*)	54	40	65	49	49	38	49.1bcd
F27 (*F. verticillioides*)	35	40	30	35	38	32	35.0g
F43 (*F. equiesti*)	44	35	60	46	40	40	44.2de
Mean	53.4a	40.5d	49.2b	44.8c	46.9bc	38.7d	

According to the Fisher's LSD test, means followed by the same letter within a column are not significantly different at p = 0.05

The differences in these data may be due to the contrasting fungal isolates and host wheat cultivars used in this study and previous work. Origin of the 16 FHB cultures may contribute to this relative pathogenic similarity (Sakr 2018a).

Variation in aggressiveness in FHB populations can lead to break down of host resistance (Xu and Nicholson 2009). Correlation values of LP component among the six wheat cultivars showed that all possible comparisons were not significantly correlated, suggesting that aggressiveness mechanisms and genes probably different to head blight disease caused by individual FHB species. So, results shown in this research indicated that a complex genotype interaction exists among bread and durum cultivars and pathogens for LP component. Our results agree with pervious in vitro AUDPC data showing the presence of a cultivar-specific pathogenicity in the same fungal isolates infected wheat cultivars, i.e., Acsad65, Cham7, Cham4, Douma4 (Sakr (2018a, c, d, 2020)). This type of specific interaction has previously been reported by Foroud et al. (2012), who noted that *F. graminearum* pathogenicity is host-dependent in wheat.

Parry et al. (1995) showed no strong evidence for specific aggressiveness interactions among fungal species implicated in the FHB complex and wheat plants. It seems that a minor gene–for–minor gene interaction may exist between six wheat cultivars and 16 fungal isolates, suggesting that the isolate-specific effectiveness may lead to erosion of wheat quantitative resistance to FHB invasion. However, further investigation is required in order to draw any final conclusions. The six aggressiveness components involved in detached leaf and seedling assays were found to be not correlated, indicating that these components are genetically different, and also reflecting into complex polygenic nature of aggressiveness in the interaction in the FHP-wheat system, which are not fully understood (Castiblanco et al. 2018).

Correlations were obtained between the data of LP, AUDPC, disease incidence and disease severity previously generated in vitro and under controlled and field conditions for the six tested wheat cultivars (Table 3). The repeatability and the stability of LP test over several experimental conditions were proved using different bread and durum cultivars by yielding correlations with the data from in vitro, head and floret inoculations. Current durum wheat cultivars are highly susceptible to FHB infection than bread cultivars (Mesterhazy et al. 1999), thus in our study we chose six wheat cultivars with different resistance levels under controlled and field conditions (Sakr 2019b, Table 2) (two moderately resistant, one moderately susceptible, two susceptible to moderately susceptible and one susceptible) that would be sufficient to investigate the stability and repeatability of LP test in vitro and under controlled and field conditions. In accordance with our data, a weak and negative correlation ($r = -0.47$) of wheat seed germination value caused by *M. majus* and FHB rating obtained by head inoculation of *F. graminearum* in the field was observed by Browne (2007). In parallel, Purahong et al. (2012) reported positive relationships of AUDPC estimations and FHB evaluations obtained by spray inoculation of *F. graminearum* across four durum wheat cultivars in the growth chamber and field. They found highly correlations between these two parameters (Purahong et al., 2012), which is similar to our results.

Table 3 Correlation coefficients between aggressiveness components generated under several experimental conditions on six wheat cultivars infected with 16 fungal isolates of four Fusarium head blight species determined by Pearson correlation coefficient

Wheat cultivar	LP×AUDPC	LP×DI	LP×DS	LP×DIFC
Acsad65	0.698**	0.522*	0.579*	0.510*
Cham4	0.707**	0.536*	0.543*	0.564*
Cham7	0.511*	0.493*	0.634**	0.534*
Douma4	0.688**	0.767**	0.537*	0.721**
Cham9	0.673**	0.507*	0. 503*	0.667**
Bohoth10	0.588*	0.501*	0.749***	0.623**

Latent period (LP) (days) component determined using in vitro detached leaf assay, area under disease progress curve (AUDPC) component determined using in vitro Petri-dish assay, disease incidence (DI) (%) component determined using head inoculation assay in growth chamber, disease severity (DS) (%) component determined using floret inoculation in growth chamber and disease incidence (DIFC) (%) component determined using head inoculation assay in field

*p = 0.05, **p = 0.01, ***p = 0.001

Thus, our findings showed that the LP test is repeatable and stable with a large diversity depending on the six used wheat cultivars. The situation in the detached leaf assay was similar to artificial head and floret inoculations because FHB species need to overcome the morphology of the head and spikelet and they could directly penetrate and infect leaves. Thus, disease development is manifested through appearance of symptoms such as discoloured, malformed, necrotic or chlorotic areas on the affected plant part (Browne and Cooke 2004). Therefore, the in vitro component, LP, predicts aggressiveness occurring at the earliest and latest wheat development stages during FHB infection. In parallel, Sakr (2019c) revealed the potential use of in vitro indicator, LP, in predicting FHB data generated under controlled and field conditions on barley. The biological clarification for an association between the in vitro and *in planta* responses to FHB infection remains largely speculative, but it can be hypothesized that similar genetic pathways become stimulated at both developmental stages (Sakr 2019a, c).

Screening for disease reaction of different FHB species on several wheat cultivars in the growth chamber and field has been found to be time-consuming and is influenced by climatic conditions under field conditions making interpretation of data difficult. LP assay can be applied for screening the most aggressive isolates of different FHB species

for FHB resistance breeding of wheat as LP has proved to be very useful tool in identifying quantitative resistance in wheat to FHB infection (Browne and Cooke 2004; Sakr 2019a). LP test has a high potential to simplify the advance of research into the wheat-FHB pathosystem, especially in Europe where genetically modified FHB species are not allowed to be sprayed in the field. On the other hand, LP test required only 21 days from seed sowing to obtaining results and the data correlates significantly with inoculations conducted under several experimental conditions.

Funding This work was funded by the AEC of Syria.

Compliance with ethical standards

Conflicts of interest The Author declares that he has no conflict of interest.

References

Brennan JM, Fagan B, van Maanen A, Cooke BM, Doohan FM (2003) Studies on in vitro growth and pathogenicity of European Fusarium fungi. Eur J Plant Pathol 109:577–587. https://doi.org/10.1023/A:1024712415326

Bottalico A, Perrone G (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol 108:611–624. https://doi.org/10.1023/A:1020635214971

Browne RA (2007) Components of resistance to fusarium head blight (FHB) in wheat detected in a seed-germination assay with *Microdochium majus* and the relationship to FHB disease development and mycotoxin accumulation from *Fusarium graminearum* infection. Plant Pathol 56:65–72. https://doi.org/10.1111/j.1365-3059.2006.01509.x

Browne RA, Cooke BM (2004) Development and evaluation of an in vitro detached leaf assay for pre-screening resistance to Fusarium head blight in wheat. Eur J Plant Pathol 110:91–102. https://doi.org/10.1023/B:EJPP.0000010143.20226.21

Browne RA, Cooke BM (2005) A comparative assessment of potential components of partial disease resistance to Fusarium head blight using a detached leaf assay of wheat, barley and oats. Eur J Plant Pathol 112:247–258. https://doi.org/10.1007/s10658-005-2077-z

Castiblanco V, Castillo HE, Miedaner T (2018) Candidate Genes for aggressiveness in a natural *Fusarium culmorum* population greatly differ between wheat and rye head blight. J Fungi (Basel) 4:14. https://doi.org/10.3390/jof4010014

Dahl B, Wilson WW (2018) Risk premiums due to Fusarium Head Blight (FHB) in wheat and barley. Agric Syst 162:145–153. https://doi.org/10.1016/j.agsy.2018.01.025

Dweba CC, Figlan S, Shimelis HA et al (2017) Fusarium head blight of wheat: pathogenesis and control strategies. Crop Prot 91:114–122. https://doi.org/10.1016/j.cropro.2016.10.002

Fernandez MR, Chen Y (2005) Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. Plant Dis 89:164–169. https://doi.org/10.1094/PD-89-0164

Foroud NA, McCormick SP, MacMillan T et al (2012) Greenhouse studies reveal increased aggressiveness of emergent Canadian *Fusarium graminearum* chemotypes in wheat. Plant Dis 96:1271–1279. https://doi.org/10.1094/PDIS-10-11-0863-RE

Imathiu SM, Ray RV, Back M et al (2009) *Fusarium langsethiae* pathogenicity and aggressiveness towards oats and wheat in wounded and unwounded in vitro detached leaf assays. Eur J Plant Pathol 124:117–126. https://doi.org/10.1007/s10658-008-9398-7

Imathiu S, Edwards S, Ray R et al (2014) Artificial inoculum and inoculation techniques commonly used in the investigation of Fusarium head blight in cereals. Acta Phytopathol et Entomol Hung 49:129–139. https://doi.org/10.1556/APhyt.49.2014.2.1

Khaledi N, Taheri P, Rastegar FM (2017) Identification, virulence factors characterization, pathogenicity and aggressiveness analysis of *Fusarium* spp., causing wheat head blight in Iran. Eur J Plant Pathol 147:897–918. https://doi.org/10.1007/s10658-016-1059-7

Kharbikar LL, Shanware AS, Saharan MS et al (2019) *Fusarium graminearum* microRNA-like RNAs and their interactions with wheat genome: a much-needed study. Indian Phytopathol. https://doi.org/10.1007/s42360-019-00139-4

Lannou C (2012) Variation and selection of quantitative components in plant pathogens. Annu Rev Phytopathol 50:319–338. https://doi.org/10.1146/annurev-phyto-081211-173031

Leslie JF, Summerell AB (2006) The *Fusarium* laboratory manual. Blackwell Publishing Professional, Ames

Mesterhazy A, Bartok T, Mirocha CG et al (1999) Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110. https://doi.org/10.1046/j.1439-0523.1999.118002097.x

Opoku N, Back M, Edwards S (2011) Aggressiveness of *Fusarium langsethiae* isolates towards wheat, barley and oats in an in vitro leaf assay. Plant Breed Seed Sci 64:55–63. https://doi.org/10.2478/v10129-011-0028-8

Pakdaman BS, Goltapeh EM, Alizadeh A (2006) Effect of Fusarium graminearum phytotoxins on germinating seeds and tissue of wheat in relation to Fusarium head blight resistance. Indian Phytopathol 59:27–31

Parry DW, Jekinson P, MCleod L (1995) Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathol 44:207–238. https://doi.org/10.1111/j.1365-3059.1995.tb02773.x

Purahong W, Alkadri D, Nipoti P et al (2012) Validation of a modified Petri-dish test to quantify aggressiveness of *Fusarium graminearum* in durum wheat. Eur J Plant Pathol 132:381–391. https://doi.org/10.1007/s10658-011-9883-2

Saharan MS, Sharma AK (2009) Studies on pathogenic variation among *Fusarium* spp./isolates causing head blight/head scab of wheat in India. Indian Phytopathol 62:402

Sakr N (2017) Aggressiveness of four Fusarium head blight species on wheat cultivars. Adv Hortic Sci 31:199–203. https://doi.org/10.13128/ahs-20585

Sakr N (2018a) Aggressiveness of Fusarium head blight species towards two modern Syrian wheat cultivars in an in vitro Petri-dish. Cereal Res Commun 46:480–489. https://doi.org/10.1556/0806.46.2018.031

Sakr N (2018b) Evaluation of two storage methods for fungal isolates of *Fusarium* sp. and *Cochliobolus sativus*. Acta Phytopathol et Entomol Hung 53:11–18. https://doi.org/10.1556/038.53.2018.003

Sakr N (2018c) Interaction between *Triticum aestivum* plants and four Fusarium head blight species on the level of pathogenicity: detected in an in vitro Petri-dish assay. Acta Phytopathol et Entomol Hung 53:171–179. https://doi.org/10.1556/038.53.2018.010

Sakr N (2018d) *Intra-* and *inter*-species variability of the aggressiveness in four Fusarium head blight species on durum wheat plants detected in an in vitro Petri-dish assay. Arch Phytopathol Plant Protect 51:814–823. https://doi.org/10.1080/03235408.2018.1495390

Sakr N (2019a) In vitro quantitative resistance components in wheat plants to Fusarium head blight. Open Agric J 13:9–18. https://doi.org/10.2174/1874331501913010009

Sakr N (2019b) Pathogenicity and quantitative resistance in Mediterranean durum and bread wheat cultivars of Syrian origin towards Fusarium head blight agents under controlled conditions. J Plant Protect Res 59:451–464. https://doi.org/10.24425/jppr.2019.131261

Sakr N (2019c) Variation in aggressiveness of Fusarium head blight species towards barley plants determined using three in vitro assays. Pak J Phytopath 31:19–33. https://doi.org/10.33866/phytopathol.031.01.0478

Sakr N (2020) In vitro analysis of Fusarium head blight resistance in ancient Syrian wheats (*Triticum* sp.). Indian J Agr Sci 90:283–286

Shin S, Kim KH, Kang CS et al (2014) Simple method for the assessment of Fusarium head blight resistance in Korean wheat seedlings inoculated with *Fusarium graminearum*. Plant Pathol J 30:25–32. https://doi.org/10.5423/PPJ.OA.06.2013.0059

Singh PJ, Aujla SS (1994) Effect of lodging on development of head scab of wheat. Indian Phytopathol 47:256–257

Wu AB, Li HP, Zhao CS, Liao YC (2005) Comparative pathogenicity of *Fusarium graminearum* isolates from China revealed by wheat coleoptile and floret inoculations. Mycopathologia 160:75–83. https://doi.org/10.1007/s11046-005-1153-4

Xu X, Nicholson P (2009) Community ecology of fungal pathogens causing wheat head blight. Annu Rev Phytopathol 47:83–103. https://doi.org/10.1146/annurev-phyto-080508-081737

Xu XM, Parry DW, Nicholson P et al (2008) Within field variability of Fusarium head blight pathogens and their associated mycotoxins. Eur J Plant Pathol 120:21–34. https://doi.org/10.1007/s10658-007-9189-6

Xue AG, Armstrong KC, Voldeng HD et al (2004) Comparative aggressiveness of isolates of *Fusarium* species causing head blight on wheat in Canada. Can J Plant Pathol 26:81–88. https://doi.org/10.1080/07060660409507117

Zhang H, Van der Lee T, Waalwijk C et al (2012) Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. PLoS One 7:e31722. https://doi.org/10.1371/journal.pone.0031722